



Ministério da Educação

Universidade Federal do Rio Grande

Programa de Pós-Graduação em Ciências da Saúde



**RESVERATROL E SUAS FORMAS GLICOSILADAS COMO
ALTERNATIVAS TERAPÊUTICAS PARA O TRATAMENTO DA
DOENÇA DE PARKINSON**

Lucia Emanuelli Schimith

Rio Grande, 2025



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Tese apresentada ao Programa
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como requisito parcial à obtenção do
título de Doutor em Ciências da Saúde.

Orientador(a): Profa. Dra. Mariana Appel Hort
Coorientador(a): Profa. Dra. Ana Luiza Muccillo-Baisch

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ATA DA SESSÃO DE DEFESA ABERTA DE TESE DE DOUTORADO EM CIÊNCIAS DA SAÚDE

A banca examinadora, designada pela Portaria nº 1321/2025 de oito de abril de dois mil e vinte e cinco, em sessão presidida e registrada pelas orientadoras interna e externa respectivamente, Profa. Dra. Mariana Appel Hort e a Profa. Dra. Corinne Miral, reuniu-se no dia dezessete de abril de dois mil e vinte e cinco, às 08h30min, por meio de videoconferência (<https://meet.google.com/xnf-jchw-ydj>), para avaliar a Tese de Doutorado do Programa de Pós-Graduação em Ciências da Saúde, intitulada: “Resveratrol e suas formas glicosiladas como alternativas terapêuticas para o tratamento da Doença de Parkinson” do(a) doutorando(a) Lucia Emanuelli Schimith. Para o início dos trabalhos, o Senhor Presidente procedeu à abertura oficial da sessão, com a apresentação dos membros da banca examinadora. A seguir, prestou esclarecimentos sobre a dinâmica de funcionamento da sessão, concedendo o tempo de até 50 (cinquenta) minutos para a apresentação da tese pela doutoranda(o), que iniciou às 8 horas e 40 minutos e terminou às 9 horas e 30 minutos. Após a apresentação, passou a palavra aos membros da banca examinadora, para que procedessem à arguição e apresentassem suas críticas e sugestões. Ao término dessa etapa de avaliação, de acordo com os membros da banca examinadora, a tese de doutorado avaliada foi APROVADA.

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RESUMO

A Doença de Parkinson (PD) é um distúrbio neurodegenerativo progressivo caracterizado pela perda seletiva de neurônios dopaminérgicos na substância negra, levando a déficits motores e sintomas não-motores. O resveratrol (RV), um polifenol natural com propriedades antioxidantes e anti-inflamatórias, tem sido amplamente estudado devido ao seu potencial neuroprotetor. No entanto, sua baixa solubilidade em água e rápida metabolização limitam sua biodisponibilidade e eficácia terapêutica. A glicosilação surge como uma estratégia promissora para melhorar essas limitações, modificando características farmacocinéticas do RV e podendo potencializar suas atividades biológicas. Neste estudo, investigamos os efeitos neuroprotetores de dois derivados glicosilados do RV, a polidatina (resveratrol-3-*O*- β -D-glicosídeo) e o resveratrol-3- α -glicosídeo (resveratrol-3-*O*- α -D-glicosídeo), usando abordagens *in silico* e *in vivo*. Exploramos ferramentas de bioinformática para a predição das propriedades farmacocinéticas dos derivados glicosilados do RV e na investigação da interação com alvos moleculares-chave na patogênese da DP. Além disso, investigamos o impacto do tipo de ligação glicosídica (alfa ou beta) sobre essas interações moleculares. A glicosilação pode melhorar a afinidade, estabilidade e as interações da polidatina e do resveratrol-3- α -glicosídeo com alvos da DP, como TNF- α , PPARG e ERBB2, potencialmente intensificando mecanismos neuroprotetores. O modelo de peixe-zebra (*Danio rerio*) foi utilizado na avaliação da potencial toxicidade da polidatina e na investigação dos efeitos neuroprotetores dos derivados glicosilados do RV *in vivo* no modelo de parkinsonismo induzido pela neurotoxina MPTP. A polidatina apresentou um perfil não tóxico em concentrações até 435 μ M, quando avaliadas a sobrevivência, morfologia, taxa de eclosão, função cardíaca e atividade locomotora do peixe-zebra. A polidatina e o resveratrol-3- α -glicosídeo foram capazes de proteger as larvas de peixe-zebra do déficit locomotor causado pelo parkinsonismo, sendo o resveratrol-3- α -glicosídeo particularmente eficaz na resposta a estímulos. Esses achados sugerem que a glicosilação pode ser uma abordagem viável para otimizar derivados do RV no contexto da neuroproteção. A elucidação dos mecanismos envolvidos pode contribuir para o desenvolvimento de novas estratégias terapêuticas para PD, explorando compostos naturais otimizados por modificações estruturais.

Palavras-chave: Neurodegeneração, glicosilação, bioinformática, neuroproteção, zebrafish.

ABSTRACT

Parkinson's Disease (PD) is a progressive neurodegenerative disorder characterized by the selective loss of dopaminergic neurons in the substantia nigra, leading to motor deficits and non-motor symptoms. Resveratrol (RV), a natural polyphenol with antioxidant and anti-inflammatory properties, has been extensively studied due to its neuroprotective potential. However, its low water solubility and rapid metabolism limit its bioavailability and therapeutic efficacy. Glycosylation emerges as a promising strategy to overcome these limitations by modifying the pharmacokinetic properties of RV and potentially enhancing its biological activities. In this study, we investigated the neuroprotective effects of two glycosylated derivatives of RV, polydatin (resveratrol-3-O- β -D-glucoside) and resveratrol-3- α -glucoside (resveratrol-3-O- α -D-glucoside), using both *in silico* and *in vivo* approaches. We explored bioinformatics tools to predict the pharmacokinetic properties of the glycosylated RV derivatives and investigate their interaction with key molecular targets in PD pathogenesis. Furthermore, we examined the impact of the glycosidic linkage type (alpha or beta) on these molecular interactions. Glycosylation may improve the affinity, stability, and interactions of polydatin and resveratrol-3- α -glucoside with PD targets, such as TNF- α , PPARG, and ERBB2, potentially enhancing neuroprotective mechanisms. The zebrafish model (*Danio rerio*) was used to assess the potential toxicity of polydatin and investigate the neuroprotective effects of glycosylated RV derivatives *in vivo* in an MPTP-induced parkinsonism model. Polydatin exhibited a non-toxic profile at concentrations up to 435 μ M, as assessed by survival, morphology, hatchability, cardiac function, and locomotor activity in zebrafish. Polydatin and resveratrol-3- α -glucoside were able to protect zebrafish larvae from locomotor deficits caused by parkinsonism, with resveratrol-3- α -glucoside being particularly effective in responding to sensory stimuli. These findings suggest that glycosylation may be a viable approach to optimize RV derivatives for neuroprotection. Elucidating the underlying mechanisms could contribute to the development of new therapeutic strategies for PD, exploring structurally optimized natural compounds.

Keywords: Neurodegeneration, glycosylation, bioinformatics, neuroprotection, zebrafish.

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LISTA DE ABREVIATURAS

AMPK = Proteína quinase ativada por adenosina monofosfato

CONCEA = Conselho Nacional de Controle de Experimentação Animal

DP = Doença de Parkinson

Dpf = Dias pós-fertilização

ERN = Espécies reativas de nitrogênio

ERO = Espécies reativas de oxigênio

HPf = Horas pós-fertilização

IL-1 β = Interleucina 1-beta

IL-6 = Interleucina 6

L-DOPA = Levodopa

MAPK = Proteína quinase ativada por mitógenos

MPP⁺ = 1-metil-4-fenilpiridínio

MPTP = 1-metil-4-fenil-1,2,3,6-tetrahidropiridina

NF- κ B = Fator nuclear kappa B

NO = Óxido nítrico

Nrf2 = Fator nuclear eritroide 2

OMS = Organização Mundial da Saúde

RV = Resveratrol

SIRT1 = Sirtuína 1

SNC = Sistema Nervoso Central

TNF- α = Fator de necrose tumoral alfa

Manuscritos

ACE = Angiotensin-converting enzyme

ADME = Absorption, Distribution, Metabolism, and Excretion

ALB = Albumin

B.O.D = Biochemical Oxygen Demand

BaSP = *Bifidobacterium adolescentis*

BBB = Blood-brain barrier

BP = Biological process

CASP3 = Caspase 3

CC = Cell component

COX-2 = Cyclooxygenase-2

CTD = Comparative Toxicogenomics Database

CYP450 = Cytochrome P450

DMSO = Dimethyl sulfoxide

dpf = Days post-fertilization

EGFR = Epidermal growth factor receptor

ERBB2 = Human epidermal growth factor receptor 2

ESR1 = Estrogen receptor 1

GBD = Global Burden of Diseases, Injuries, and Risk Factors

GHS = Globally Harmonized System of Classification of Labelling of Chemicals

GO = Gene Ontology

hpf = Hours post-fertilization

IL-6 = interleukin 6

KEGG = Kyoto Encyclopedia of Genes and Genomes

LD50 = Lethal Dose 50

MAP2K1 = Mitogen-activated protein kinase kinase 1

MF = Molecular function

MPP⁺ = 1-methyl-4-phenylpyridinium ion

MPTP = 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine

NF- κ B = Nuclear Factor kappa-light-chain-enhancer of activated B cells

Nrf2 = Nuclear factor erythroid 2-related factor 2

PD = Parkinson's disease

PDB = Protein Data Bank

P-gp = P-glycoprotein

PPAR γ = Peroxisome Proliferator-activated receptor gamma

PPI = Protein-protein interaction

PTGS2 = Prostaglandin-endoperoxide synthase 2

ROS = Reactive oxygen species

SRC = Proto-oncogene tyrosine-protein kinase

TNF- α = Tumor necrosis factor-alpha

VD = Volume of distribution

1. INTRODUÇÃO

A Doença de Parkinson (DP) é um distúrbio neurodegenerativo de caráter progressivo que afeta milhões de pessoas mundialmente, sendo a segunda doença neurodegenerativa mais frequente, ficando atrás apenas da Doença de Alzheimer (de Lau; Breteler, 2006; Tysnes; Storstein, 2017; Wirdefeldt *et al.*, 2011). A Organização Mundial da Saúde (OMS) estimou que a prevalência da DP dobrou nos últimos anos, representando um aumento de aproximadamente 145% no número de casos da doença (Feigin *et al.*, 2019). Patologicamente, a DP caracteriza-se pela degeneração de neurônios dopaminérgicos em áreas específicas da substância negra, levando a redução significativa dos níveis do neurotransmissor dopamina no estriado (Kalia; Lang, 2015). Clinicamente, a DP se manifesta através de sintomas motores, como bradicinesia, tremores em repouso, rigidez muscular e instabilidade postural, além de sintomas não motores, como alterações neuropsiquiátricas e sensoriais, distúrbios do sono e comprometimento cognitivo (Váradi, 2020).

A fisiopatologia da DP envolve múltiplos mecanismos, incluindo estresse oxidativo, disfunção mitocondrial, neuroinflamação e agregação anormal da proteína alfa-sinucleína (α -sinucleína), formando inclusões citoplasmáticas conhecidas como corpos de Lewy (Dickson, 2018). Atualmente, o tratamento farmacológico da doença é baseado principalmente na reposição da dopamina, através do uso de precursores do neurotransmissor, inibidores da degradação de dopamina e agonistas dos receptores dopaminérgicos (Connolly; Lang, 2014). Mesmo que tais fármacos possam oferecer melhoras significativas, nenhuma alternativa terapêutica disponível atualmente é capaz de interromper a progressão da doença. Esse cenário reforça a necessidade do desenvolvimento de novos fármacos com potencial neuroprotetor ou capazes de retardar a progressão da doença (AlDakheel; Kalia; Lang, 2014; Murakami *et al.*, 2023).

Compostos naturais vêm ganhando destaque sendo amplamente investigados como potenciais agentes neuroprotetores devido, principalmente, às suas propriedades antioxidantes e anti-inflamatórias (Pohl; Kong Thoo Lin, 2018). A ingestão de antioxidantes, através da dieta ou suplementação, pode melhorar a saúde do paciente e reduzir sintomas de diferentes doenças crônicas, como a DP (Ciulla *et al.*, 2019). Diferentes estudos têm demonstrado que compostos naturais, fitoquímicos, vitaminas e minerais, tanto de forma isolada como em associação às alternativas de tratamento

existentes, podem prevenir, retardar e aliviar os sintomas clínicos da DP (Alizadeh; Kheirouri; Keramati, 2023; Bhusal *et al.*, 2023a; Liu *et al.*, 2019; Rahman *et al.*, 2022).

Dentre os compostos naturais alvos de pesquisas encontra-se o resveratrol (3,4',5-*trans*-trihidroxiestilbeno) (RV), um polifenol encontrado em uvas, amendoim e frutas vermelhas, que tem se mostrado promissor devido às suas diversas atividades biológicas, incluindo ação antioxidante, anti-inflamatória e neuroprotetora (Baur; Sinclair, 2006; Gülçin, 2010). Apesar do seu potencial terapêutico, a aplicação clínica do RV pode ser limitada como consequência a sua baixa biodisponibilidade, decorrente da rápida metabolização e eliminação (Amri *et al.*, 2012; Szymkowiak; Kucinska; Murias, 2023). Desse modo, estratégias de otimização estrutural podem aprimorar as propriedades farmacológicas desta molécula, principalmente através da produção de derivados glicosilados (Salla *et al.*, 2024). A conjugação do RV com resíduos de açúcares pode melhorar sua solubilidade e biodisponibilidade, tornando essa modificação uma alternativa viável que pode representar um avanço significativo no desenvolvimento de novas terapias para a DP (Arbo *et al.*, 2020).

2. REVISÃO BIBLIOGRÁFICA

2.1. Doença de Parkinson (DP)

2.1.1. Epidemiologia e impactos socioeconômicos

Os distúrbios neurológicos possuem grande impacto sobre a sociedade e os sistemas de saúde, representando uma das principais causas de incapacidade e mortalidade no mundo (Feigin *et al.*, 2019). Dentre as neuropatologias, destacam-se as doenças neurodegenerativas, tais como a Doença de Alzheimer, DP, esclerose lateral amiotrófica, esclerose múltipla, Doença de Huntington e a atrofia de múltiplos sistemas. Essas doenças são resultantes da perda progressiva de neurônios e conexões neuronais que regularmente levam à disfunção motora e cognitiva (Gao; Hong, 2008; Jellinger, 2009).

A DP é considerada o segundo distúrbio neurodegenerativo progressivo mais frequente no mundo, com prevalência de 1% em indivíduos acima dos 60 anos, ultrapassando os 4% em indivíduos com 85 anos ou mais (de Lau; Breteler, 2006; Pringsheim *et al.*, 2014). Segundo o *Global Burden of Diseases, Injuries, and Risk Factors Study* (GBD), estudo que avalia a morbidade e mortalidade de diferentes enfermidades em níveis globais, nacionais e regionais, o número de indivíduos diagnosticados com DP mais do que dobrou nas últimas décadas, passando de 2,5 milhões em 1990 para 11,7 milhões em 2021 (Luo *et al.*, 2025). A OMS considerou a DP como principal fator de incapacidade entre os distúrbios neurológicos, afetando predominantemente homens (Organização Mundial da Saúde (OMS), 2025).

A degeneração neuronal está diretamente associada aos distúrbios motores característicos observados em pacientes com DP, como bradicinesia, rigidez muscular, tremores em repouso e instabilidade postural. Ainda que permaneça o fato de que os sintomas motores sejam os principais critérios para o diagnóstico clínico da doença, os sintomas não-motores, incluindo distúrbios do sono, hiposmia, disfunção gastrointestinal e manifestações neuropsiquiátricas como ansiedade e depressão, podem se tornar proeminentes antes, durante o início, e a progressão da DP (Chaudhuri; Schapira, 2009; Lees; Hardy; Revesz, 2009; Rodriguez-Oroz *et al.*, 2009).

O distúrbio de movimento e a diminuição da capacidade cognitiva contribuem para a redução significativa na qualidade de vida e independência funcional dos

indivíduos acometidos pela DP (Schapira; Chaudhuri; Jenner, 2017). À medida que a doença progride, o agravamento dos sintomas motores e não motores aumenta a necessidade de assistência especializada e cuidados, sendo esses frequentemente arcados financeiramente, ou ainda prestados, por membros da família do paciente, gerando impactos emocionais e financeiros (Hermanowicz; Jones; Hauser, 2019; Macchi *et al.*, 2020; Zhong *et al.*, 2016). Sendo assim, a sobrecarga emocional e econômica da DP, tanto para os pacientes como seus cuidadores e sistemas de saúde, representa um desafio socioeconômico expressivo (Chaudhuri *et al.*, 2024). Estratégias que possam reduzir a progressão da doença, visando um controle precoce e extensivo da patologia, podem impactar positivamente os indivíduos com a doença, melhorando a qualidade de vida, diminuindo os custos médicos e a sobrecarga dos pacientes, seus cuidadores e da sociedade (Borodziuk *et al.*, 2024; Scheife *et al.*, 2000).

2.1.2. Etiologia

Embora a etiologia exata da DP ainda não seja totalmente compreendida, tendo a maioria dos casos origem multifatorial, acredita-se que fatores genéticos e ambientais podem estar envolvidos no desenvolvimento da doença. Aproximadamente 10-15% dos casos têm origem genética, sendo frequentemente associados a indivíduos jovens que possuem predisposição genética. Mutações específicas nos genes GBA, LRRK2, PRKN, PINK1, DJ-1 e SNCA estão relacionadas ao aumento do risco de DP (Bonifati *et al.*, 2003; Kitada *et al.*, 1998; Paisán-Ruiz *et al.*, 2004; Polymeropoulos *et al.*, 1997; Sidransky; Lopez, 2012; Valente *et al.*, 2004). Fatores ambientais também desempenham papel relevante, a exposição a substâncias químicas como toxinas ambientais, pesticidas, herbicidas, metais pesados e a poluição do ar também tem sido associada ao risco de desenvolvimento da DP (Barbeau *et al.*, 1987; Breckenridge *et al.*, 2016; Schneider Medeiros *et al.*, 2020; van der Mark *et al.*, 2012). Tal associação ficou em evidência na década de 1980 após a ocorrência de parkinsonismo permanente em usuários de uma nova heroína sintética contaminada pelo composto 1-metil-4-fenil-1,2,3,6-tetrahidropiridina (MPTP), uma neurotoxina capaz de induzir seletivamente a morte de neurônios dopaminérgicos da substância negra (Langston *et al.*, 1983).

O envelhecimento também possui papel crítico na incidência da DP, sendo a idade o principal fator de risco para o desenvolvimento da doença (Kouli; Torsney; Kuan, 2018). Mesmo que a relação causal ainda não esteja completamente elucidada, se a morte celular seria um resultado inevitável do envelhecimento biológico ou se seria resultante

da exposição crônica a toxinas ambientais, o processo neurodegenerativo pode ser a consequência de interações complexas entre alterações celulares associadas à senescência, processos pré-determinados geneticamente e exposições ambientais (Costa *et al.*, 2023). Com o envelhecimento da população e o aumento da expectativa de vida, o número de pessoas com DP tende a aumentar em mais de 50% nos próximos anos, resultando em implicações importantes para a saúde pública (Dorsey *et al.*, 2007).

2.1.3. Fisiopatologia

O processo patológico da DP é caracterizado principalmente pela perda progressiva dos neurônios dopaminérgicos e pelo acúmulo intracelular da proteína α -sinucleína, formando inclusões citoplasmáticas conhecidas como corpos de Lewy (Dickson *et al.*, 2009; Halliday *et al.*, 2011). A perda marcante desses neurônios localizados na região da substância negra pars compacta leva à redução significativa dos níveis do neurotransmissor dopamina no estriado e à disfunção dos núcleos da base, estruturas responsáveis pelo controle e execução do movimento, desencadeando a manifestação dos sintomas clínicos característicos da doença (Poewe *et al.*, 2017) (Figura 1A). Estudos farmacológicos, genéticos, clínicos e estudos *post-mortem* em cérebros de pacientes com DP, apontam que a ocorrência de níveis anormais de agregados da proteína α -sinucleína, a disfunção mitocondrial, por meio do dano ao complexo I da cadeia respiratória mitocondrial, o aumento das espécies reativas de oxigênio (ERO), além da indução de um quadro de resposta inflamatória exacerbada, desempenham papel central na fisiopatologia da DP (Jenner; Olanow, 1996; Kalia; Lang, 2015; Olanow; Schapira; Agid, 2003; Phani; Loike; Przedborski, 2012) (Figura 1B).

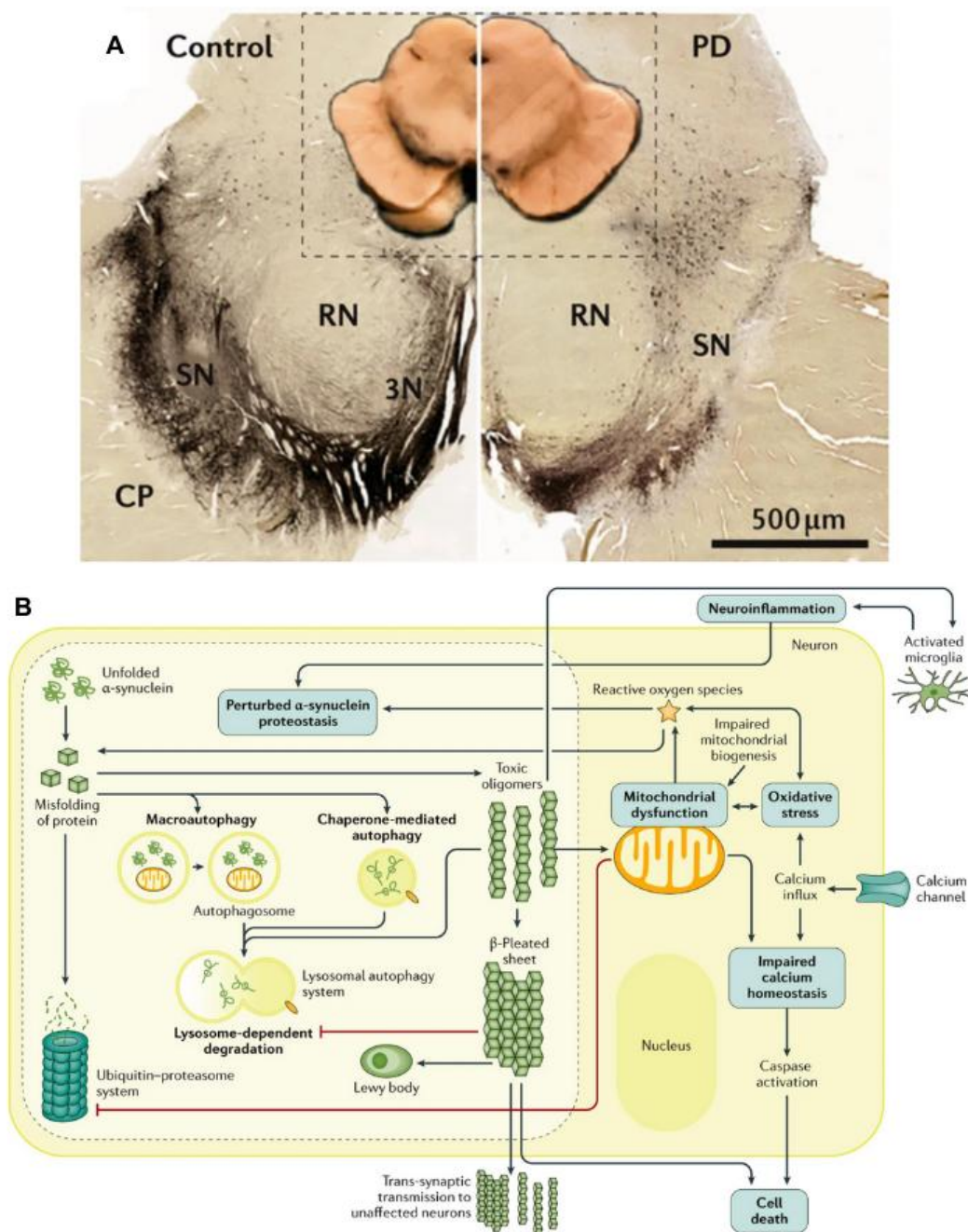


Figura 1. A) Cortes macroscópicas e transversais do mesencéfalo após marcação imuno-histoquímica para tirosina hidroxilase, enzima limitadora da síntese de dopamina. Despigmentação da região da substância negra de um paciente com a DP no painel da direita, comparado ao cérebro de um indivíduo saudável, painel da esquerda. PD: Doença de Parkinson; 3N: fibras do terceiro nervo; CP: pedúnculo cerebral; RN: núcleo vermelho. B) Diagrama sobre as interações entre as principais vias moleculares que estão implicadas na patogênese da doença de Parkinson (Fonte: Poewe *et al.*, 2017).

A α -sinucleína é uma proteína constituída por 140 aminoácidos, que em sua forma nativa pode ser encontrada nos terminais pré-sinápticos associada a vesículas, no núcleo de células neuronais (Maroteaux; Campanelli; Scheller, 1988), mitocôndrias (Li *et al.*, 2007), retículo endoplasmático (Hoozemans *et al.*, 2007), complexo de Golgi (Gosavi *et al.*, 2002) e no sistema endolisossomal (Lee *et al.*, 2004). Sua atividade

biológica em cada um dos compartimentos celulares ainda não é totalmente caracterizada, porém, acredita-se que sua função esteja relacionada principalmente à liberação e recaptação de neurotransmissores (Burré *et al.*, 2010). Alterações no processo de dobramento da estrutura da proteína tornam-na insolúvel e podem levar à sua agregação. Os agregados proteicos gerados podem apresentar tipos e tamanhos diferentes, variando de pequenas estruturas como monômeros ou oligômeros a filamentos maiores, podendo formar ainda os corpos de Lewy, caracterizados por inclusões maiores da α -sinucleína. Estudos exploraram a relação entre estrutura-toxicidade dos tipos de agregados e demonstraram que a formação de oligômeros pode ser altamente citotóxica quando comparada a neurotoxicidade apresentada pelas inclusões citoplasmáticas maiores de α -sinucleína (Fink, 2006; Ross; Poirier, 2005; Wood *et al.*, 1999). Também podem ocorrer agregações neurofibrilares de outros tipos de proteínas como a proteína tau e β -amiloide, características da Doença de Alzheimer, que podem atuar de forma sinérgica com os agregados de α -sinucleína e contribuir para as manifestações clínicas da DP (Irwin; Lee; Trojanowski, 2013; Lim *et al.*, 2019).

As mitocôndrias, principais fontes de energia celular, produzem naturalmente espécies reativas durante o processo de fosforilação oxidativa, mas também são alvos dos danos causados por essas moléculas (Spinazzi *et al.*, 2012; Turens, 2003). Na DP, a presença de corpos de Lewy e o aumento do metabolismo da dopamina provocam lesões diretas às mitocôndrias, reduzindo a atividade do complexo I da cadeia respiratória mitocondrial. Como consequência, há um aumento excessivo dos níveis de ERO, ultrapassando a capacidade celular de neutralização dessas substâncias, o que compromete o metabolismo energético mitocondrial e reduz o aporte energético necessário para a sobrevivência neuronal. Esse desequilíbrio leva, progressivamente, à morte dos neurônios dopaminérgicos (Bose; Beal, 2016; Franco-Iborra; Vila; Perier, 2016; Henchcliffe; Beal, 2008; Henrich *et al.*, 2023). Além disso, a proteína α -sinucleína também pode desencadear a ativação de vias pró-inflamatórias em resposta ao estresse oxidativo, agravando os danos às populações neuronais vulneráveis (Perfeito *et al.*, 2014).

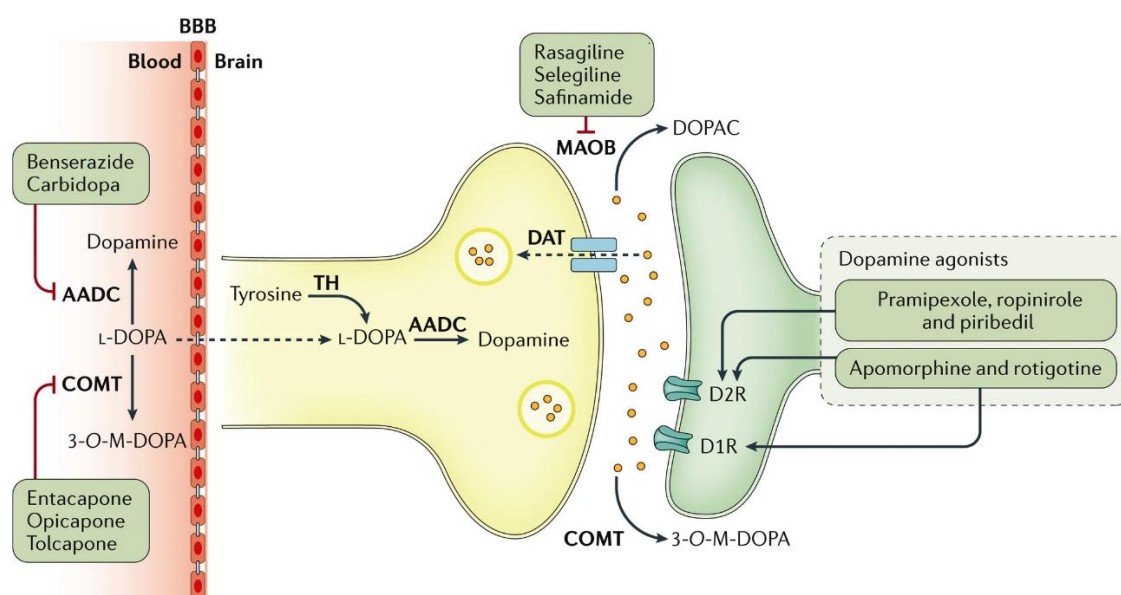
A resposta inflamatória também desempenha papel crítico na patologia da DP, contribuindo para a degeneração progressiva dos neurônios dopaminérgicos. Evidências crescentes sugerem que, embora a microglia em estado de repouso produza fatores neurotróficos e anti-inflamatórios, sua ativação exacerbada em condições de estresse pode promover um ambiente neurotóxico (Li; Barres, 2018). Esse efeito ocorre

principalmente através da expressão do fenótipo M1, caracterizado pela liberação de citocinas pró-inflamatórias como fator de necrose tumoral alfa (TNF- α), interleucina 1-beta (IL-1 β) e interleucina 6 (IL-6), além da produção de ERO e espécies reativas de nitrogênio (ERN) (Isik *et al.*, 2023; Moehle; West, 2015; Williams-Gray *et al.*, 2016). Um estado crônico de neuroinflamação pode desencadear um ciclo de dano neuronal que se retroalimenta, no qual a liberação contínua de mediadores inflamatórios intensifica a disfunção mitocondrial, o estresse oxidativo e a apoptose neuronal. Além disso, a deposição de α -sinucleína mal dobrada pode atuar como um estímulo adicional para a ativação da microglia, exacerbando a resposta inflamatória e contribuindo para a progressão da doença (Jellinger, 2010; Su *et al.*, 2008). Estudos indicam que a interação entre neurônios, microglia e astrócitos desempenha um papel chave na modulação desse processo inflamatório. Os astrócitos reativos, por sua vez, também contribuem para a neuroinflamação, liberando moléculas pró-inflamatórias e reduzindo sua função de suporte neuronal (Kam *et al.*, 2020; Troncoso-Escudero *et al.*, 2018).

2.1.4. Tratamentos disponíveis e suas limitações

Atualmente, não existem alternativas terapêuticas capazes de curar a DP. A terapia farmacológica existente está focada no alívio e controle dos sintomas (Connolly; Lang, 2014). O documento “Protocolo Clínico e Diretrizes Terapêuticas da Doença de Parkinson” do Ministério da Saúde, estabelece o protocolo padrão de tratamento para a DP, visando num primeiro momento a redução da progressão da doença (neuroproteção), quando possível, e o controle dos sintomas (tratamento sintomático) (Brasil, 2017). O protocolo de tratamento medicamentoso é ajustado a cada paciente, de acordo com o estágio da doença, a gravidade dos sintomas e os efeitos adversos (AlDakheel; Kalia; Lang, 2014). Dentre as intervenções farmacológicas existentes estão os precursores de dopamina, como a levodopa (L-DOPA), inibidores da degradação de dopamina, agonistas dos receptores de dopamina bem como fármacos não-dopaminérgicos, como os agentes anticolinérgicos e glutamatérgicos (Golan *et al.*, 2012) (Figura 2). De modo geral, o efeito clínico da L-DOPA é notado rapidamente, principalmente nos estágios iniciais da doença. Porém, à medida que a doença progride, o efeito do fármaco diminui drasticamente e o indivíduo passa a apresentar efeitos adversos como flutuações rápidas na função motora (Goldenber, 2008). Uma das alternativas para a redução desses efeitos indesejados são as associações a outros fármacos, como inibidores da enzima monoamina oxidase B ou da catecol-O-metiltransferase, agonistas dopaminérgicos e anticolinérgicos. Usualmente,

essas opções terapêuticas alternativas são indicadas como terapia inicial nos casos de diagnóstico precoce, especialmente nos casos onde o tremor é a manifestação predominante, retardando o uso da L-DOPA, ou ainda como adjunto na terapia com L-DOPA, diminuindo seus efeitos adversos (Cenci, 2014). Apesar de proporcionarem melhoras significativas, esses fármacos podem não ser plenamente eficazes no controle de todas as manifestações da DP. Além disso, nenhuma das opções farmacológicas disponíveis é capaz de reduzir ou interromper a progressão da doença (Zahoor; Shafi; Haq, 2018). Desse modo, torna-se necessária a busca por substâncias neuroprotetoras e neurorestauradoras para o tratamento da DP.



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Figura 2. Mecanismos de ação das principais opções farmacológicas dopaminérgicas utilizadas no tratamento da DP. AADC: descarboxilase de aminoácidos aromáticos; COMT: catecol-O-metiltransferase; MAOB: monoamina oxidase tipo B; BBB: barreira hematoencefálica; DAT: transportador de dopamina; D1R: receptor de dopamina D1; 3-O-M-DOPA: 3-O-metil-DOPA; DOPAC: ácido 3,4-dioxifenilacético; TH: tirosina hidroxilase (Fonte: Poewe *et al.*, 2017).

As pesquisas recentes têm tido como alvo para os potenciais novos fármacos a redução dos agregados de alfa-sinucleína, da ativação da neuroglia — e consequente neuroinflamação, além da redução da disfunção mitocondrial e potencial neuroproteção (Kispotta; Das; Prusty, 2024; Murakami *et al.*, 2023; Pajares *et al.*, 2020; Rouaud *et al.*, 2021). Como as doenças neurodegenerativas encontram-se diretamente ligadas ao estresse oxidativo, substâncias antioxidantes podem vir a desempenhar papel importante na prevenção e tratamento de tais enfermidades, especialmente na DP. Nos últimos anos, muitos estudos vêm investigando o potencial de diferentes compostos naturais antioxidantes no tratamento da DP.

2.2. Compostos naturais como fonte de novos fármacos

Compostos naturais já são conhecidos historicamente como fontes importantes de biomoléculas com potenciais atividades terapêuticas, seja através do descobrimento de compostos isolados ou explorando-os na forma de extratos. Metabólitos secundários de plantas têm sido investigados como uma plataforma no processo de desenvolvimento de novos fármacos, uma vez que são produzidos em grandes quantidades e apresentam uma ampla diversidade estrutural (Dar *et al.*, 2017; Samuelsson, 2004; Wahid *et al.*, 2020).

Uma das principais classes de compostos naturais originários de plantas são os polifenóis, onde já foram identificadas mais de 8.000 estruturas. Os polifenóis são responsáveis por diversas funções fisiológicas, incluindo a regulação de processos do crescimento, desenvolvimento, sinalização e comunicação, além de participar na defesa contra estresses bióticos e abióticos (Dai; Mumper, 2010). Podem ser encontrados amplamente em frutas, vegetais, frutas secas, oleaginosas, sementes e raízes, além de quantidades suficientes em chás, café e vinhos (Adebooye; Alashi; Aluko, 2018).

Sua estrutura química é caracterizada pela presença de múltiplas unidades estruturais fenólicas, com um ou mais grupos hidroxila (-OH) ligados a um átomo de carbono que constituem os anéis aromáticos. Esses anéis aromáticos podem dar origem a diferentes interações entre grupos funcionais, criando estruturas fenólicas ainda mais complexas gerando uma diversidade de polifenóis (Hanuka Katz *et al.*, 2020). Os polifenóis podem ser classificados ainda com base nas diferenças entre suas estruturas químicas em ácidos fenólicos, estilbenos, lignanas e flavonoides (Spencer *et al.*, 2008). Dentre os polifenóis, os estilbenos se destacam por sua ampla bioatividade e estabilidade química, sendo explorados como potenciais agentes terapêuticos em diversas patologias, incluindo as doenças neurodegenerativas (Giacomini *et al.*, 2016a; Navarro-Orcajada *et al.*, 2023; Socała *et al.*, 2024).

Os estilbenos são fitoalexinas que apresentam em sua estrutura química um esqueleto de carbono de 1,2-difeniletileno (C6-C2-C6), com uma ligação de etileno unindo dois anéis de benzeno (Keylor; Matsuura; Stephenson, 2015). Os mais de 400 derivados de estilbenos podem apresentar duas formas isoméricas: *trans*-estilbeno ((E)-estilbeno), sendo essa conformação mais estável; e *cis*-estilbeno ((Z)-estilbeno), que seria menos estável. Alguns dos *trans*-estilbenos biologicamente ativos incluem o oxiresveratrol, pterostilbeno, piceatanol e o RV (Shen; Wang; Lou, 2009). Esses

compostos apresentam ainda grande versatilidade, sendo passíveis a diferentes modificações estruturais uma vez que possuem estabilidade térmica e estrutural e importante capacidade de substituição das ligações de hidrogênio (Giacomini *et al.*, 2016b). Além disso, naturalmente, os estilbenos podem ser encontrados sob diferentes formas, em sua forma livre (aglicona), ligadas a outros grupamentos ou compostos, como açúcares (glicosídeos), ou ainda ligados a uma matriz extracelular (Flamini *et al.*, 2013).

Um dos estilbenos mais estudados é o RV, que pode ser encontrado em diversas plantas, incluindo uvas, amendoins e frutas vermelhas. Na defesa vegetal, o RV atua principalmente no combate de infecções fúngicas e danos causados por radiação ultravioleta ou variações ambientais (Bhat; Kosmeder; Pezzuto, 2001). Além de seu papel nas plantas, o RV tem despertado grande interesse no descobrimento de potenciais novos fármacos devido às suas diferentes funções fisiológicas e farmacológicas, principalmente suas propriedades antioxidantes (He; Yan, 2013), anti-inflamatória (Meng *et al.*, 2021) e antimicrobiana (Vestergaard; Ingmer, 2019).

2.3. Resveratrol

O RV (3,4',5-*trans*-trihidroxiestilbeno) é um polifenol, pertencente a classe dos estilbenos, sendo produzido por uma variedade de espécies vegetais em resposta a patógenos ou sob condições de estresse (Frémont, 2000) (Figura 3). O RV pode ser encontrado em amoras, amendoins, uvas e seus derivados, como vinhos e chás (Sparvoli *et al.*, 1994). Pesquisas têm relacionado a molécula com diferentes atividades biológicas e farmacológicas, incluindo atividade antioxidante (Bhat; Kosmeder; Pezzuto, 2001; Constantinescu; Mihis, 2023; Gülçin, 2010; Queiroz *et al.*, 2009) funções anti-inflamatória (Elmali *et al.*, 2007; Meng *et al.*, 2021; Sánchez-Fidalgo *et al.*, 2010), anticâncer (Jang *et al.*, 1997; Saiko *et al.*, 2008; Zykova *et al.*, 2008), antidiabética (Bhatt; Thomas; Nanjan, 2012; Brasnyó *et al.*, 2011; Milne *et al.*, 2007), cardioprotetora (Gal *et al.*, 2021; Hung, 2000; Magyar *et al.*, 2012), além de desempenhar papel importante na neuroproteção (Arbo *et al.*, 2020; dos Santos *et al.*, 2022; Moussa *et al.*, 2017; Turner *et al.*, 2015), agindo na prevenção de doenças neurodegenerativas como a DP.

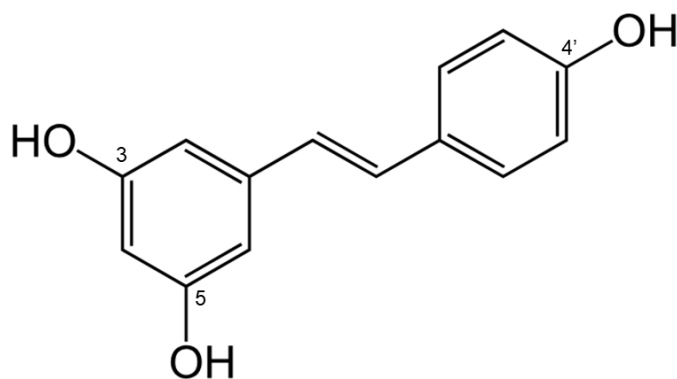


Figura 3. Estrutura química do resveratrol (3,4',5-*trans*-trihidroxiestilbeno).

Nos anos de 1940, as práticas das medicinas tradicionais japonesa e chinesa já destacam o RV como componente ativo com ação anti-inflamatória e antiplaquetária nas preparações medicinais utilizadas na época, principalmente no tratamento de distúrbios do fígado, pele, coração e metabolismo lipídico (Nonomura; Kanagawa; Makimoto, 1963; Song *et al.*, 2021). Na década de 1990, acreditava-se que o consumo moderado de álcool reduzia significativamente o risco de doença arterial coronariana. Porém, ainda se discutia se esse efeito protetor se aplicava a todas as bebidas alcoólicas ou se estava associado a presença de compostos específicos em algumas bebidas (Rimm *et al.*, 1996; Soleas; Diamandis; Goldberg, 1997). Em 1992, pesquisadores americanos relataram que o RV estava presente em concentrações significativas no vinho tinto (Siemann; Creasy, 1992). Tal estudo foi particularmente relevante devido à observação intrigante feita na época de que certas populações, especialmente os franceses, apresentavam baixa incidência de mortalidade causada pela doença arterial coronariana, mesmo fazendo o consumo de quantidades significativas de gorduras saturadas e apresentando outros fatores de risco semelhantes a outras populações, como tabagismo, sedentarismo e estresse. A propriedade cardioprotetora do RV associada a outros polifenóis presentes no vinho, forneceram uma possível explicação para o que na época foi chamado de “Paradoxo Francês”, reforçando a teoria de que componentes da dieta podem ter benefícios significativos para a saúde (Criqui; Ringel, 1994; Renaud; de Lorgeril, 1992). Ainda que a validade do Paradoxo Francês continue sendo questionada, a descoberta do RV no vinho impulsionou diferentes pesquisas sobre suas possíveis aplicações terapêuticas.

Embora o interesse inicial pelo RV tenha sido focado principalmente em suas propriedades antioxidantes, estudos relatam que o mesmo pode atuar sobre diversas vias

de transdução de sinal. O RV é um ativador das vias Sirtuína 1 (SIRT1) e fator nuclear eritroide 2 (Nrf2), responsáveis pela regulação da homeostase celular e expressão de genes de resposta antioxidante, respectivamente, promovendo efeitos antioxidantes e anti-inflamatórios (Borra; Smith; Denu, 2005; Farkhondeh *et al.*, 2020; Yang *et al.*, 2019). A inibição da ativação do fator nuclear kappa B (NF- κ B) também é uma ação anti-inflamatória induzida pelo resveratrol, reduzindo a produção de citocinas pró-inflamatórias (Xu *et al.*, 2018; Zhou *et al.*, 2017). O resveratrol é capaz ainda de modular as vias de sinalização da proteína quinase ativada por mitógenos (MAPK) (Kan *et al.*, 2023; Santos *et al.*, 2021), ou por adenosina monofosfato (AMPK) (Dasgupta; Milbrandt, 2007; Lan *et al.*, 2017; Pineda-Ramírez *et al.*, 2020) e PI3K/AKT/mTOR (Utpal *et al.*, 2024a; Wang *et al.*, 2022), exercendo efeitos de regulação da resposta ao estresse oxidativo, promoção da autofagia e modulação do metabolismo energético, resultando na redução da resposta inflamatória, proteção contra apoptose e melhora da homeostase celular.

Similar a outros antioxidantes, o RV pode atuar diretamente na neutralização das ERO e ERN, prevenindo a ação desses oxidantes sobre o organismo. Sua estrutura química apresenta três grupamentos hidroxila capazes de doar elétrons ou hidrogênios para radicais livres altamente reativos (ânion superóxido ou radical hidroxila), ou ainda neutralizá-los através da formação de compostos menos reativos (Stojanović; Sprinz; Brede, 2001). Assim, a maioria das atividades biológicas do RV é atribuída à sua capacidade intrínseca de eliminação de radicais livres (Pangeni *et al.*, 2014). Por outro lado, como mencionado acima, o RV pode atuar na modulação de vias antioxidantes endógenas, promovendo a expressão de importantes mediadores antioxidantes (Constantinescu; Mihis, 2023).

Além de modular a liberação de mediadores inflamatórios, o RV exerce sua ação anti-inflamatória regulando diferentes vias de sinalização intracelulares envolvidas na resposta inflamatória. Conforme mencionada anteriormente, o composto inibe a ativação do fator de transcrição NF- κ B, um dos principais regulares da expressão de genes pró-inflamatórios, além de reduzir a produção de óxido nítrico (NO) e de citocinas pró-inflamatórias, como o TNF- α e IL-6, atenuando a ativação da microglia (Feng *et al.*, 2002; Tsai; Lin-Shiau; Lin, 1999; Wadsworth; Koop, 1999). Além disso, o RV pode influenciar a polarização das células da microglia, promovendo um fenótipo anti-inflamatório (M2) em relação ao fenótipo pró-inflamatório (M1), o que contribui para a contenção da

1 inflamação. O composto também interage com diversos alvos funcionais no interior das
2 células, incluindo receptores de membrana e intracelulares, moléculas sinalizadoras,
3 fatores de transcrição, enzimas e mecanismos de reparo de DNA (Gatz; Wiesmuller, 2008;
4 Robb *et al.*, 2008). Sua capacidade de regular o equilíbrio entre processos inflamatórios
5 e mecanismos de reparo celular destaca seu potencial terapêutico em condições
6 associadas à inflamação crônica e disfunção metabólica.

7 Por apresentar atividades biológicas relevantes e a capacidade de modular
8 diferentes vias celulares, o RV tem sido amplamente investigado por suas propriedades
9 neuroprotetoras, especialmente no contexto da DP (Bhusal *et al.*, 2023b; Phukan *et al.*,
10 2023). O composto pode atuar na atenuação da neurodegeneração por meio da redução
11 do estresse oxidativo e da neuroinflamação, dois dos principais processos envolvidos na
12 degeneração dos neurônios dopaminérgicos (Bartra *et al.*, 2024; Bonsack; Alleyne;
13 Sukumari-Ramesh, 2017; dos Santos *et al.*, 2022). Sua capacidade de modular a resposta
14 inflamatória e antioxidante contribui para a preservação da integridade celular e a
15 manutenção da homeostase no sistema nervoso central (SNC) (Utpal *et al.*, 2024; Zhu *et al.*,
16 2025). Além disso, o RV favorece mecanismos como a autofagia, auxiliando na
17 eliminação de proteínas mal dobradas e na proteção contra danos celulares (Guo *et al.*,
18 2016; Hu *et al.*, 2017). Diferentes estudos experimentais têm demonstrado a melhora do
19 déficit locomotor (Palle; Neerati, 2018; Zhang *et al.*, 2018), aumento dos níveis de
20 dopamina e seus metabólitos (Anandhan *et al.*, 2010; Gaballah *et al.*, 2016; Srivastava *et al.*,
21 2012), além do aumento de enzimas relacionadas à síntese de dopamina (da Rocha
22 Lindner *et al.*, 2015; Okawara *et al.*, 2007), redução da expressão da α -sinucleína (Guo
23 *et al.*, 2016), melhora do status antioxidante (Gaballah *et al.*, 2016), bem como a
24 regulação da neuroinflamação (Jin *et al.*, 2008; Lofrumento *et al.*, 2014) e disfunção
25 mitocondrial (Gueguen *et al.*, 2015; Peng *et al.*, 2016; Satpute *et al.*, 2017). Essas
26 propriedades fazem do RV um candidato promissor para estratégias terapêuticas voltadas
27 à neuroproteção e à intervenção na progressão da doença.

28 Apesar de possuir diferentes propriedades farmacológicas interessantes à clínica,
29 a eficácia terapêutica do RV não é totalmente alcançada, uma vez que a molécula passa
30 por extenso metabolismo, sendo rapidamente excretada, além de possuir baixa
31 solubilidade em água (<0,05 mg/mL), o que impacta diretamente sua biodisponibilidade
32 (Hao *et al.*, 2015; Walle, 2011). Mesmo sendo altamente absorvido, através de difusão
33 passiva ou transportadores de membrana, o RV é metabolizado por enzimas da família

das UDP- glicuronosiltransferases e sulfotransferases, resultando na formação de conjugados como resveratrol-3-O-glucuronídeo, resveratrol-4'-O-glucuronídeo, resveratrol-3-O-sulfato (Böhmdorfer *et al.*, 2017; Walle *et al.*, 2004). Quando administrado em humanos, baixas doses do RV são biotransformadas majoritariamente em conjugados glucuronídeos, enquanto a ingestão de altas doses do RV leva a geração principalmente de conjugados sulfatados (Cottart; Nivet-Antoine; Beaudeau, 2014). Esses metabólitos formados, embora possam manter alguma atividade biológica (Baur; Sinclair, 2006; Calamini *et al.*, 2010; Miksits *et al.*, 2009), geralmente apresentam menor afinidade por alvos moleculares e dificuldades em atravessar as barreiras biológicas, limitando seus efeitos. Desse modo, faz-se necessário o desenvolvimento de uma estratégia plausível para melhorar sua solubilidade em água e aumentar sua biodisponibilidade.

2.4. Otimização estrutural: glicosilação do resveratrol

Naturalmente, na etapa final da síntese biológica, os compostos fenólicos sofrem modificações para alcançarem seu estado funcional através de reações como glicosilação, sulfatação, metilação ou acilação. Sinteticamente, essas reações de otimização estrutural podem ser induzidas através de métodos químicos ou enzimáticos. Além disso, tais alterações são responsáveis também pela modificação de propriedades biológicas como solubilidade, estabilidade, biodisponibilidade e bioatividade (De Winter *et al.*, 2015; Regev-Shoshani *et al.*, 2003; Salas; Méndez, 2007; Williams; Zhang; Thorson, 2007).

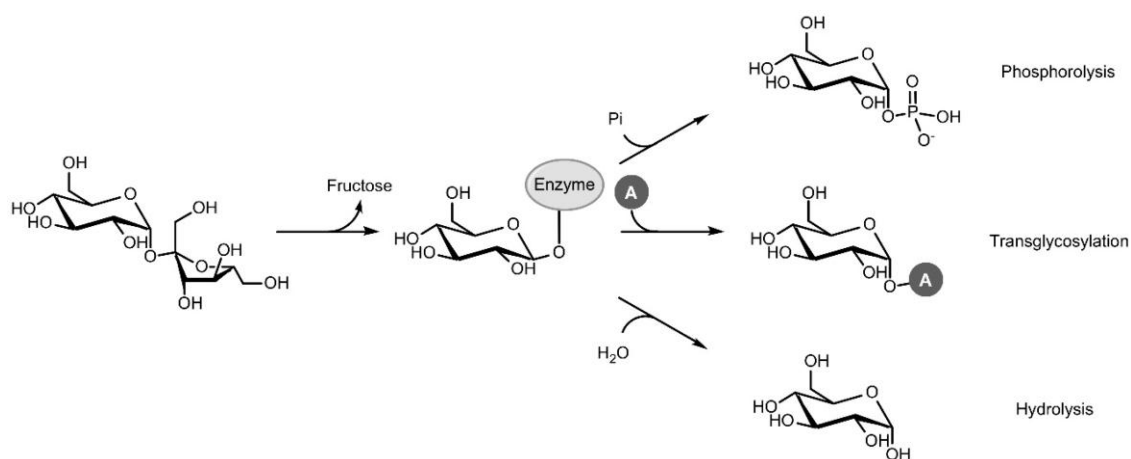
Grande parte dos produtos de origem natural biologicamente ativos tiveram adicionados às suas estruturas diferentes tipos de açúcares, com o intuito de melhorar suas propriedades físicas, químicas e biológicas (Abu-Salah, 1996; Davis; Robinson, 2002; Krawczyk *et al.*, 2020; Mizuma *et al.*, 1993; Schatz *et al.*, 2005; Tan *et al.*, 1967). A porção glicosídica adicionada pode ter influência sobre processos como absorção, distribuição, metabolismo e excreção da aglicona, servindo como estratégia no aumento da solubilidade de compostos naturais, melhorando sua biodisponibilidade e atividades biológicas (Biasutto *et al.*, 2009; Klingel *et al.*, 2019; Murota *et al.*, 2010; Slámová; Kapešová; Valentová, 2018; Thorson *et al.*, 2004). Estudos têm demonstrado atividade antioxidante (Wang *et al.*, 2015), anti-inflamatória (Larrosa *et al.*, 2010) e neuroprotetora (Peñalver *et al.*, 2018; Sato *et al.*, 2014) de diferentes moléculas glicosiladas derivadas do resveratrol, muitas superiores às apresentadas pela sua forma aglicona. Tal fato poderia

1 estar relacionado à capacidade destas formas agirem como pró-fármacos, entregando
2 maiores concentrações de resveratrol às células e de forma mais lenta, além de
3 diminuir sua velocidade de metabolização, aumentando assim seu efeito biológico
4 (Biasutto *et al.*, 2017; Falomir *et al.*, 2016; Larrosa *et al.*, 2010).

5 Atualmente, os compostos glicosilados podem ser obtidos por meio de três
6 principais abordagens: extração das fontes naturais, síntese química e síntese enzimática
7 (Elshahawi *et al.*, 2015; Kiessling; Splain, 2010a; Yu; Sun; Yang, 2012). A obtenção de
8 polifenóis glicosilados diretamente a partir de plantas é complexa e de baixo rendimento,
9 além da concentração estar sujeita a variações na composição, tornando a padronização
10 difícil e aumentando a necessidade de testes frequentes para garantir qualidade e
11 consistência do produto final (Alara; Abdurahman; Ukaegbu, 2021; Hu *et al.*, 2024).
12 Outra opção é a indução dessas reações de otimização estrutural através de procedimentos
13 sintéticos, como reações de proteção e desproteção de grupos funcionais (Kiessling;
14 Splain, 2010). Em relação às vias de acesso por síntese química, a funcionalização
15 controlada dessas unidades fenólicas também apresenta múltiplos obstáculos, como a
16 dificuldade em garantir regio e estereosseletividade e a necessidade do uso de solventes
17 e catalisadores tóxicos (Grogan *et al.*, 2002; Hanson *et al.*, 2004). Já as vias enzimáticas
18 de origem biológica revelam-se alternativas bastante promissoras devido à alta
19 especificidade das enzimas e ao seu caráter sustentável. Entretanto, as enzimas capazes
20 de funcionalizar seletivamente os polifenóis são raras, enquanto as necessidades de
21 antioxidantes funcionalizados tanto na pesquisa quanto na indústria são consideráveis
22 (Filice; Marciello, 2013; Muthana; Cao; Chen, 2009).

23 O uso das técnicas enzimáticas permite tanto a produção de polifenóis naturais
24 como sintéticos com rendimentos significativos. Dentre as principais enzimas utilizadas
25 estão as enzimas das famílias glicosiltransferases e glicosidases, capazes de catalisar a
26 reação de glicosilação de forma estéreo/regiosseletiva através de uma reação simples e de
27 forma sustentável. As reações envolvem basicamente o uso de uma molécula de açúcar,
28 que atuará como doador, e a molécula aceptora, ou seja, o polifenol de interesse (Křen;
29 Thiem, 1997; Xiao; Muzashvili; Georgiev, 2014). As glicosiltransferases catalisam a
30 transferência direta de açúcares para os polifenóis, garantindo alta especificidade e
31 regioseletividade, porém requer o uso de açúcares ativados de alto custo como um fosfo-
32 açúcar de nucleotídeo para atuarem como doadores (Křen; Thiem, 1997; Muthana; Cao;
33 Chen, 2009; Xiao; Muzashvili; Georgiev, 2014). Por outro lado, as glicosidases são

1 enzimas hidrolíticas que, normalmente, atuam clivando glicosídeos. No entanto, em
 2 condições específicas, podem atuar de forma reversa, promovendo a transglicosilação,
 3 um processo no qual quebram ligações glicosídicas e transferem moléculas de açúcares
 4 para polifenóis (Figura 4). As glicosidases apresentam duas grandes vantagens, a primeira
 5 é a capacidade de aceitar uma ampla gama de aceptores, o que permite que o conjugado
 6 intermediário de enzima e açúcar seja redirecionado para diferentes compostos que não
 7 apenas fosfato. A segunda grande vantagem é o açúcar utilizado como doador, a sacarose,
 8 sendo um substrato de baixo custo, tornando-se uma alternativa promissora para a
 9 produção de polifenóis glicosilados em escala industrial (Ara *et al.*, 2013; Hofer, 2016;
 10 Shaikh; Withers, 2008).



11
 12 **Figura 4.** Reações de fosforólise, transglicosilação e hidrólise catalisadas pela glicosidase sacarose
 13 fosforilase. Pi: fosfato inorgânico; A: aceitador (Fonte: Franceus; Desmet, 2020).

14 Entre as glicosidases utilizadas para a glicosilação de polifenóis, as enzimas
 15 sacarose fosforilases destacam-se como alternativas eficientes e econômicas devido à sua
 16 capacidade de transferir seletivamente o resíduo glicosilado da sacarose para aceptores
 17 fenólicos, possibilitando a síntese de derivados glicosilados do resveratrol com alta
 18 eficiência. Essas enzimas podem ser encontradas em diferentes fontes microbianas,
 19 incluindo a bactéria *Bifidobacterium adolescentis*, presente no trato gastrointestinal
 20 humano (Franceus; Desmet, 2020b; Kruschitz *et al.*, 2021; Xia *et al.*, 2021). As sacarose
 21 fosforilases, contudo, apresentam baixa eficiência na glicosilação do resveratrol e de
 22 outros polifenóis, devido a restrições espaciais em seu sítio ativo que limita o acesso de
 23 moléculas aromáticas maiores (Dirks-Hofmeister *et al.*, 2015; Kraus; Grimm; Seibel,
 24 2016). Recentemente, estratégias de engenharia enzimática vêm sendo desenvolvidas para
 25 melhorar a atividade catalítica dessas enzimas, como é o caso da mutação Q345F
 26 desenvolvida por Kraus e colaboradores (2017), responsável pela expansão da entrada do

sítio catalítico de 272 para 557 Å³, permitindo também a formação de interações hidrofóbicas e aromáticas entre o ligante (RV) e a proteína, importantes para uma melhor estabilização do complexo e eficiência da reação. Através dessa estratégia, o grupo de Kraus e colaboradores, sintetizou a forma glicosilada resveratrol-3- α -glicosídeo (resveratrol-3-*O*- α -D-glicosídeo) a partir do resveratrol com um rendimento de 97% (Figura 5B).

Como mencionado anteriormente, algumas formas glicosiladas do resveratrol podem ser encontradas naturalmente, como é o caso da polidatina (resveratrol-3-*O*- β -D-glicosídeo), molécula glicosilada precursora natural do RV (Figura 5A). Assim como o RV, a polidatina pode ser encontrada em uvas, vinho tinto, amendoim, além do cacau e seus derivados (Du; Peng; Zhang, 2013). A polidatina compartilha grande parte das atividades biológicas apresentadas pelo RV, incluindo a regulação da função inflamatória através da alta atividade antioxidante (Hosoda *et al.*, 2013; Kerem *et al.*, 2006), levando a inibição da produção de mediadores inflamatórios (Xu *et al.*, 2010). Como resultado dessas atividades, a polidatina exerce também efeitos neuroprotetores sobre diferentes patologias do SNC (Schimith *et al.*, 2022), como a doença de Parkinson (Ahmed *et al.*, 2018; Chen *et al.*, 2015; Zhang *et al.*, 2020), derrame cerebral (Gao *et al.*, 2016), isquemia cerebral (Ji *et al.*, 2012; Li *et al.*, 2012), lesões cerebrais traumáticas (Li *et al.*, 2019), lesões na medula espinhal (Kataria *et al.*, 2016; Zhan *et al.*, 2020), além de alterações decorrentes do processo de envelhecimento (Xu *et al.*, 2016).

A polidatina e o resveratrol-3- α -glicosídeo são derivados glicosilados do resveratrol, nos quais o glicosídeo se liga a posição C-3 do anel aromático A, substituindo um dos grupamentos hidroxila das moléculas. Apesar das semelhanças, as moléculas apresentam diferenças estruturais importantes. A polidatina é um glicosídeo do resveratrol com a ligação do açúcar em posição β , enquanto o resveratrol-3- α -glicosídeo apresenta uma ligação do açúcar em posição α (Figura 5). Acredita-se que variações do tipo de ligação glicosídica podem influenciar suas propriedades físico-químicas, como solubilidade, estabilidade e biodisponibilidade (Harris; Edwards; Morrison, 2017; Moradi *et al.*, 2016; Solá; Griebenow, 2009).

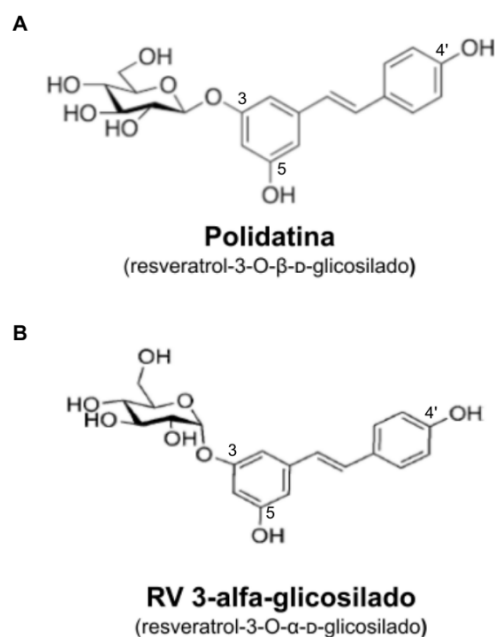


Figura 5. Estrutura química (A) polidatina e (B) resveratrol-3- α -glicosilado.

Embora haja um crescente interesse nos derivados glicosilados do resveratrol, as atividades biológicas do resveratrol-3- α -glicosídeo ainda foram pouco exploradas. Akash e colaboradores (2024) avaliaram recentemente o potencial dos derivados do resveratrol, incluindo a polidatina e o resveratrol-3- α -glicosídeo contra o câncer colorretal através de abordagens *in silico*. Enquanto outros glicosídeos do resveratrol, como a polidatina, tem servido como base para estudos que visam compreender os efeitos estruturais da glicosilação na bioatividade do resveratrol, há uma lacuna no conhecimento sobre os efeitos da modificação estrutural específica do resveratrol-3- α -glicosídeo, se essa poderia influenciar sua farmacocinética e bioatividade. A caracterização de suas propriedades biológicas é essencial para determinar seu potencial terapêutico, especialmente em relação à sua estabilidade, capacidade de atravessar barreiras biológicas e interação com alvos celulares. Estudos são necessários para avaliar se essa forma glicosilada pode apresentar vantagens em comparação ao resveratrol aglicona e a outros derivados, como a polidatina, contribuindo para o desenvolvimento de estratégias mais eficazes no tratamento de doenças neurodegenerativas.

2.5. Testes pré-clínicos

Apesar do potencial da glicosilação para otimizar a farmacocinética do resveratrol, sua eficácia terapêutica precisa ser validada por meio de testes pré-clínicos. Esses estudos são fundamentais para determinar sua segurança, biodisponibilidade e efeitos biológicos em modelos experimentais, auxiliando no desenvolvimento de

1 estratégias terapêuticas mais eficazes. A compreensão das diferenças geradas pela
2 glicosilação é essencial para o desenvolvimento de derivados otimizados com
3 características físico-químicas e farmacológicas potencialmente aprimoradas. Para
4 garantir que esses compostos atinjam seu potencial terapêutico sem causar efeitos
5 adversos, são necessários testes pré-clínicos detalhados (Shegokar, 2020). Esses testes
6 são fundamentais na avaliação da segurança e eficácia de novos compostos com potencial
7 para novos fármacos, antes da sua aplicação em humanos, contribuindo para evitar falhas
8 nos ensaios clínicos, além de otimizar tempo e recursos (Nuermberger, 2017; Singh,
9 2006).

10 A pesquisa pré-clínica baseia-se na condução de estudos *in silico*, *in vitro* e *in*
11 *vivo*. Usualmente, são empregas técnicas de cultura celular e testes em animais para
12 examinar a eficácia e segurança de novas moléculas, aliada ao uso inicial de técnicas de
13 triagens virtual e de alto rendimento (do inglês *high-throughput screening*) (Issa *et al.*,
14 2017; Menshykau, 2017). Entre as possíveis razões para falha de mais de 90% dos
15 candidatos a novos fármacos estão a falta de eficácia clínica, alta toxicidade e
16 propriedades farmacocinéticas incompatíveis (Sun *et al.*, 2022). Diante desse contexto, o
17 uso de inteligência artificial e aprendizado de máquina através de ferramentas *in silico*
18 têm se mostrado indispensáveis, permitindo a previsão da farmacocinética, interações
19 moleculares e toxicidade de substâncias. Essas ferramentas permitem realizar simulações
20 de como as moléculas se comportam em diferentes condições biológicas, antecipando
21 possíveis problemas ou identificando oportunidades de otimização antes dos testes
22 experimentais (Chou; Lin, 2023; Issa *et al.*, 2017; Lin; Chou, 2022; Tran *et al.*, 2023). No
23 entanto, para uma compreensão mais completa dos efeitos dos compostos, os estudos *in*
24 *vitro* e *in vivo* são igualmente cruciais (Hughes *et al.*, 2011). Os ensaios *in vitro* permitem
25 uma avaliação detalhada da atividade biológica e validação de alvos em ambientes
26 controlados, enquanto os modelos *in vivo*, oferecem uma abordagem mais próxima das
27 condições fisiológicas humanas, permitindo a observação de efeitos biológicos em
28 sistemas complexos e, conseqüentemente, uma validação mais robusta dos resultados e
29 otimização da eficácia (Biala *et al.*, 2023; Chi; Burrows; Anderson, 2022; Robuck;
30 Wurzelmann, 2005).

31 Diferentes modelos animais têm sido estabelecidos com o objetivo de mimetizar
32 condições patológicas humanas, sendo usados no processo de desenvolvimento e
33 avaliação farmacológica de candidatos a novos fármacos para prevenção e tratamento,

como é o caso da DP (Cassar *et al.*, 2020; Habjan *et al.*, 2024; Patton; Zon; Langenau, 2021; Vaz; Outeiro; Ferreira, 2018; Wang *et al.*, 2021). Por apresentar toxicidade seletiva a neurônios dopaminérgicos, o modelo induzido pelo MPTP causa parkinsonismo e tem sido amplamente utilizado para o estudo da patogênese da DP (Gao; Hong, 2008). O MPTP é um composto lipofílico que facilmente permeia a barreira hematoencefálica, mas que por si só não apresenta toxicidade. Uma vez no cérebro, o MPTP é rapidamente convertido em seu metabólito tóxico, o MPP⁺ (1-metil-4-fenilpiridínio), pela enzima monoamina oxidase B (Langston *et al.*, 1983). Quando absorvido pelos neurônios dopaminérgicos da substância negra, o MPP⁺ concentra-se nas mitocôndrias, onde, sob níveis tóxicos, inibe o complexo I da cadeia respiratória mitocondrial (Richardson *et al.*, 2007; Risiglione *et al.*, 2020; Singer; Ramsay, 1990). Em humanos, tanto a exposição ocupacional como acidental ao MPTP desencadeou características clínicas e patológicas da DP (Langston, 2017; Stern *et al.*, 1990). O MPTP é uma neurotoxina bem caracterizada, amplamente utilizada para induzir parkinsonismo em diversas espécies, devido à sua capacidade de atingir seletivamente e degenerar os neurônios dopaminérgicos da substância negra, mimetizando características patológicas-chave da DP (Lal *et al.*, 2024). A neurotoxina também se mostrou eficaz em danificar o sistema dopaminérgico central de camundongos (Heikkila; Sonsalla, 1987). Do mesmo modo, estudos demonstraram a existência de uma síndrome semelhante ao parkinsonismo induzida por MPTP em *goldfish* (Poli *et al.*, 1990; Pollard *et al.*, 1992). Após exposição ao MPTP, o peixe-zebra também se mostrou suscetível aos efeitos neurotóxicos da toxina, apresentando neurodegeneração, diminuição da atividade locomotora e declínio cognitivo, além da diminuição do número de neurônios dopaminérgicos no diencéfalo (Bashirzade *et al.*, 2022; Lu *et al.*, 2015; Omar; Kumar; Teoh, 2023; Razali *et al.*, 2022; Christensen *et al.*, 2020; Yao *et al.*, 2017; Zhao *et al.*, 2020).

O peixe-zebra (*Danio rerio*) tem se destacado como organismo modelo em ensaios pré-clínicos de avaliação da potencial toxicidade de candidatos a novos fármacos e também em estudos do sistema nervoso em função do seu curto tempo de geração, grande capacidade de reprodução, embriões transparentes, rápido desenvolvimento e fácil manipulação (Fontana *et al.*, 2018; Soussi-Yanicostas, 2022).

Por se tratar de um processo altamente coordenado e conservado, o peixe-zebra apresenta ainda grande similaridade com outros vertebrados no desenvolvimento do sistema nervoso, incluindo humanos, principalmente em processos como a proliferação,

diferenciação e migração de neurônios, bem como a formação de circuitos neuronais funcionais (Rupp *et al.*, 1996). No modelo do peixe-zebra, o SNC começa a se formar nas primeiras horas pós-fertilização (hpf), com a formação da placa neural e os primeiros grupamentos neuronais ocorrendo por volta das 10 hpf (Figura 6A). Seguido pelo desenvolvimento do tubo neural, as primeiras 24 horas de vida do embrião são caracterizadas pela formação das principais estruturas cerebrais, o prosencéfalo (telencéfalo e diencéfalo), mesencéfalo e rombencéfalo. Além disso, a maioria dos tipos de células neuronais, núcleos e circuitos neurais encontrados no organismo adulto, já estão formados e funcionais por volta dos 3 dias pós-fertilização (dpf), momento da eclosão do embrião, e a maturação dessas estruturas e redes continua nos dias subsequentes, permitindo a análise funcional do sistema nervoso ainda nos estágios larvais (Grinblat *et al.*, 1998, Hauptmann *et al.*, 2002; Kaslin; Ganz, 2020).

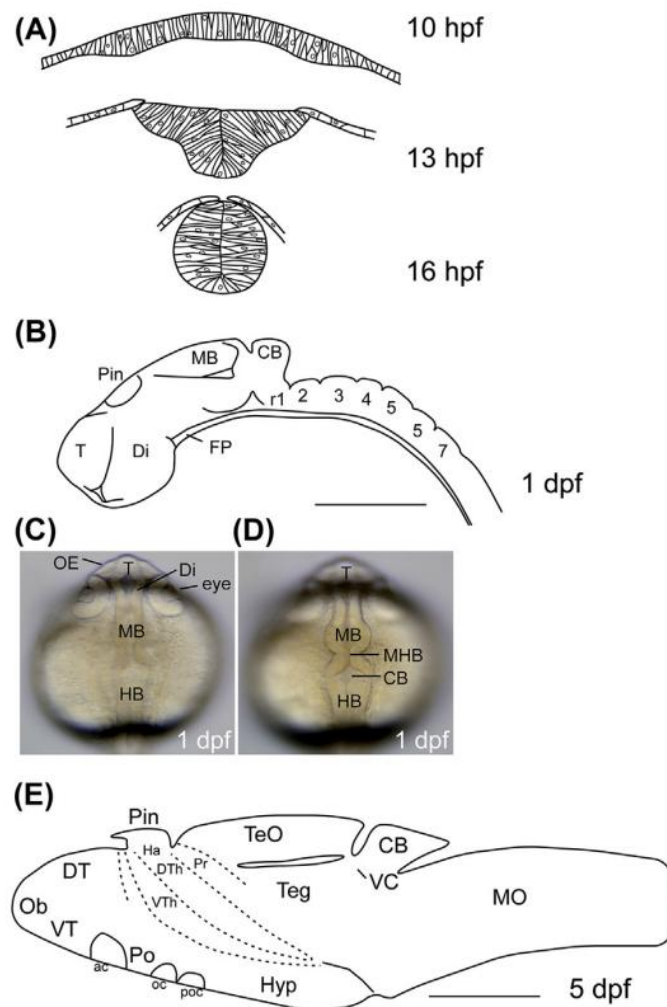
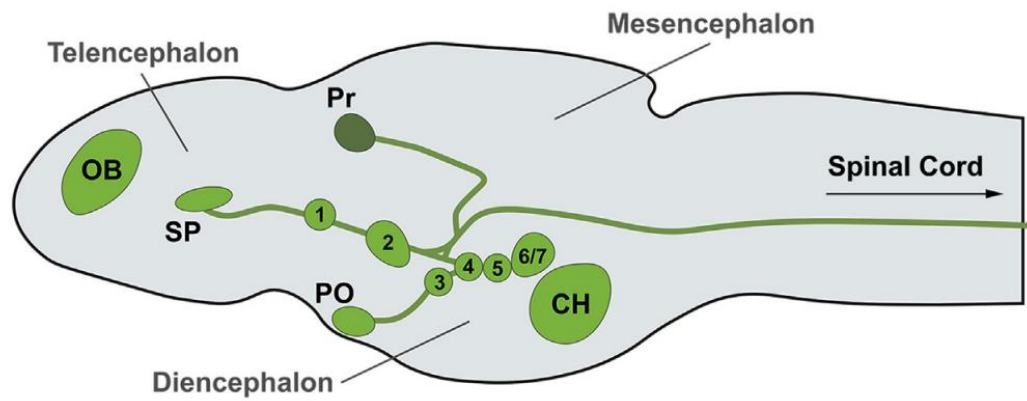


Figura 6. Desenvolvimento inicial do tubo neural e das principais das principais regiões cerebrais do peixe-zebra em 1 e 5 dias pós-fertilização. (A) Formação da placa neural; (B) formação das principais estruturas cerebrais; (C, D) vista dorsal do embrião de peixe-zebra com 1 dpf; (E) cérebro da larva de peixe-zebra com 5 dpf com as principais regiões do encéfalo já subdivididas. Hpf, horas pós-fertilização; dpf, dias pós

1 fertilização; CB, cerebelo; Di, diencéfalo; DT, telencéfalo dorsal; DTh, tálamo dorsal; Pin, pineal; HB,
2 rombencéfalo; Hyp, hipotálamo; MB, mesencéfalo; Ob, bulbo olfatório; OE, epitélio olfativo; Teg,
3 tegmento; T, telencéfalo; VT, telencéfalo ventral; VTh, tálamo ventral. (Fonte: Kaslin; Ganz, 2020)

4 O peixe-zebra possui todas as principais estruturas básicas do SNC encontradas
5 no cérebro de mamíferos, incluindo o sistema catecolaminérgico (Schmidt; Strähle;
6 Scholpp, 2013). Dentre os sistemas de neurotransmissores que emergem durante o
7 desenvolvimento, o sistema dopaminérgico possui particular interesse devido ao seu
8 papel central na modulação do comportamento motor e da cognição (Klein et al., 2018;
9 Chen et al., 2024). Diferentemente dos mamíferos, os neurônios dopaminérgicos do
10 peixe-zebra não se desenvolvem na região do mesencéfalo (Du *et al.*, 2016). A
11 diferenciação dos primeiros neurônios dopaminérgicos ocorre por volta de 18 hpf, na
12 região do diencéfalo, expandindo-se progressivamente até formar circuitos funcionais
13 completos entre 4 e 5 dpf com projeções para a região do subpallium (Schweitzer;
14 Driever, 2009) (Figura 7). Embora o peixe-zebra não possua uma região caracterizada
15 como substância negra compacta equivalente à dos mamíferos, essas projeções
16 dopaminérgicas localizadas na porção ventral do telencéfalo formam uma estrutura
17 anatômica homóloga ao sistema nigroestriatal humano, tornando-o um modelo valioso
18 para o estudo da neurobiologia dopaminérgica (Rink; Wullimann, 2001, 2002;
19 Wullimann; Rink, 2002) (Figura 7). Além disso, durante esse período inicial do
20 desenvolvimento, as larvas de peixe-zebra já apresentam as proteínas-chave envolvidas
21 na síntese e degradação da dopamina funcionalmente ativas, tais como a enzima tirosina
22 hidroxilase e os transportadores e receptores de dopamina, permitindo a realização de
23 estudos neuroquímicos e comportamentais (Schweitzer; Driever, 2009). O uso do peixe-
24 zebra no seu estágio larval permite ainda a realização de triagens de alto rendimento,
25 através da avaliação automatizada e simultânea da função motora de múltiplos animais
26 em microplacas (Bartel et al., 2020). Essas características, aliadas a transparência que o
27 peixe-zebra apresenta em seus estágios iniciais do desenvolvimento, viabilizam
28 investigações detalhadas sobre a formação e degeneração do sistema dopaminérgico,
29 tornando-o um modelo promissor para a compreensão dos mecanismos envolvidos na DP,
30 bem como para a triagem de novos compostos com potencial neuroprotetor (Briñez-
31 Gallego *et al.*, 2023).



1

2 **Figura 7.** Sistema dopaminérgico peixe-zebra. Representação apresentando a distribuição dos principais
 3 grupos de neurônios dopaminérgicos, numerados de 1-7, no cérebro da larva de peixe-zebra com 5 dias
 4 pós-fertilização. CH, hipotálamo caudal; OB, bulbo olfatório; PO, área pré-óptica; Pr, pretectum; SP,
 5 subpallium. (Fonte: Bartel *et al.*, 2020).

3. JUSTIFICATIVA

Tendo em vista a carência de estratégias efetivas na prevenção e tratamento da DP, faz-se necessário o desenvolvimento de potenciais novos fármacos. Diferentes estudos têm demonstrado nos últimos anos o potencial terapêutico do RV sobre as doenças neurodegenerativas, uma vez que a molécula possui, dentre outras, atividade antioxidante e anti-inflamatória. Apesar de possuir diferentes atividades farmacológicas interessantes à clínica, a eficácia terapêutica do RV pode ser limitada, uma vez que a molécula passa por extenso metabolismo, sendo rapidamente excretada, possui baixa solubilidade em água, o que impacta diretamente em sua biodisponibilidade. Desse modo, estratégias de modificação estrutural podem vir a aprimorar as propriedades farmacológicas desta molécula, principalmente através da produção de formas glicosiladas. A avaliação toxicológica e dos efeitos farmacológicos são etapas cruciais no desenvolvimento de candidatos a novos fármacos. No caso do RV e seus derivados, a glicosilação pode influenciar propriedades como solubilidade, biodisponibilidade e atividade biológica. No entanto, os mecanismos pelos quais as formas glicosiladas do RV exercem seus efeitos neuroprotetores ainda não são completamente compreendidos, especialmente em modelos *in vivo*. Além disso, a investigação sobre o impacto do tipo de ligação glicosídica, alfa ou beta, é importante para elucidar como essa modificação estrutural pode influenciar as propriedades farmacocinéticas e a atividade neuroprotetora desses compostos. Dessa forma, investigar essas moléculas em um modelo de parkinsonismo pode fornecer perspectivas valiosas sobre seu potencial terapêutico e suas vantagens em relação ao RV não modificado.

4. OBJETIVOS

4.1. Objetivo geral

Avaliar a toxicidade e os efeitos farmacológicos do resveratrol e suas formas glicosiladas no contexto da Doença de Parkinson, por meio de abordagens *in silico* e *in vivo* em larvas de peixe-zebra (*Danio rerio*).

4.2. Objetivos específicos

- Investigar o impacto do tipo de ligação (alfa ou beta) entre a molécula de glicose e o resveratrol nas propriedades farmacológicas;
- Predizer as interações moleculares e propriedades farmacocinéticas e farmacodinâmicas do resveratrol, da polidatina e do resveratrol-3- α -glicosídeo por meio de análises de farmacologia de rede e docking molecular;
- Avaliar a toxicidade aguda da polidatina em larvas de peixe-zebra, identificando concentrações seguras para ensaios no modelo de Doença de Parkinson;
- Investigar os efeitos da polidatina e do resveratrol-3- α -glicosídeo na modulação da atividade locomotora em larvas de peixe-zebra expostas à neurotoxina MPTP.

BIBLIOGRAFIA

- ABU-SALAH, K M. Amphotericin B: an update. **British journal of biomedical science**, [s. l.], v. 53, n. 2, p. 122–33, 1996. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/8757689>.
- ADEBOOYE, Odunayo C.; ALASHI, Adeola M.; ALUKO, Rotimi E. A brief review on emerging trends in global polyphenol research. **Journal of Food Biochemistry**, [s. l.], v. 42, n. 4, p. e12519, 2018.
- AHMED, Muhammad Rashid *et al.* Neuroprotective potential of polydatin against motor abnormalities and dopaminergic neuronal loss in rotenone induced parkinson model. **International Journal of Morphology**, [s. l.], v. 36, n. 2, p. 584–591, 2018.
- AKASH, Shopnil *et al.* In silico evaluation of anti-colorectal cancer inhibitors by Resveratrol derivatives targeting Armadillo repeats domain of APC: molecular docking and molecular dynamics simulation. **Frontiers in Oncology**, [s. l.], v. 14, 2024.
- ALARA, Oluwaseun Ruth; ABDURAHMAN, Nour Hamid; UKAEGBU, Chinonso Ishamel. Extraction of phenolic compounds: A review. **Current Research in Food Science**, [s. l.], v. 4, p. 200–214, 2021.
- ALDAKHEEL, Amaal; KALIA, Lorraine V.; LANG, Anthony E. Pathogenesis-Targeted, Disease-Modifying Therapies in Parkinson Disease. **Neurotherapeutics**, [s. l.], v. 11, n. 1, p. 6–23, 2014.
- ALIZADEH, Mohammad; KHEIROURI, Sorayya; KERAMATI, Majid. What Dietary Vitamins and Minerals Might Be Protective against Parkinson’s Disease? **Brain Sciences**, [s. l.], v. 13, n. 7, p. 1119, 2023.
- AMRI, A. *et al.* Administration of resveratrol: What formulation solutions to bioavailability limitations?. **Journal of Controlled Release**, [s. l.], v. 158, n. 2, p. 182–193, 2012.

- ANANDHAN, Anandurai *et al.* Resveratrol attenuates oxidative stress and improves behaviour in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) challenged parkinsonic mice. **Annals of neurosciences**, [s. l.], v. 17, n. 3, 2010.
- ARA, Kazi Zubaida Gulshan *et al.* Glycoside Hydrolases for Extraction and Modification of Polyphenolic Antioxidants. *In: ADVANCES IN ENZYME BIOTECHNOLOGY*. New Delhi: Springer India, 2013. p. 9–21.
- ARBO, Bruno Dutra *et al.* Resveratrol derivatives as potential treatments for Alzheimer's and Parkinson's disease. **Frontiers in Aging Neuroscience**, [s. l.], v. 12, n. 103, 2020. Disponível em: <https://www.frontiersin.org/article/10.3389/fnagi.2020.00103/full>.
- BARBEAU, André *et al.* Ecogenetics of Parkinson's Disease: Prevalence and Environmental Aspects in Rural Areas. **Canadian Journal of Neurological Sciences / Journal Canadien des Sciences Neurologiques**, [s. l.], v. 14, n. 1, p. 36–41, 1987.
- BARTRA, Clara *et al.* Resveratrol Activates Antioxidant Protective Mechanisms in Cellular Models of Alzheimer's Disease Inflammation. **Antioxidants**, [s. l.], v. 13, n. 2, p. 177, 2024.
- BARTEL, W. Philip, VAN LAAR, V.S., BURTON, E.A., Parkinson's disease, in: R.T. Gerlai (Ed.), **Behavioral and Neural Genetics of Zebrafish**, Elsevier, Academic Press, p. 377–412, 2020.
- BASHIRZADE, Alim A. O. *et al.* MPTP-Treated Zebrafish Recapitulate 'Late-Stage' Parkinson's-like Cognitive Decline. **Toxics**, [s. l.], v. 10, n. 2, p. 69, 2022.
- BAUR, Joseph A; SINCLAIR, David A. Therapeutic potential of resveratrol: the in vivo evidence. **Nature reviews. Drug discovery**, [s. l.], v. 5, n. 6, p. 493–506, 2006.
- BHAT, Krishna P.L.; KOSMEDER, Jerome W.; PEZZUTO, John M. Biological Effects of Resveratrol. **Antioxidants & Redox Signaling**, [s. l.], v. 3, n. 6, p. 1041–1064, 2001.

- BHATT, Jayesh Kumar; THOMAS, Sabin; NANJAN, Moola Joghee. Resveratrol supplementation improves glycemic control in type 2 diabetes mellitus. **Nutrition Research**, [s. l.], v. 32, n. 7, p. 537–541, 2012.
- BHUSAL, Chandra Kanta *et al.* Unveiling Nature's potential: Promising natural compounds in Parkinson's disease management. **Parkinsonism & Related Disorders**, [s. l.], v. 115, p. 105799, 2023a.
- BHUSAL, Chandra Kanta *et al.* Unveiling Nature's potential: Promising natural compounds in Parkinson's disease management. **Parkinsonism & Related Disorders**, [s. l.], v. 115, p. 105799, 2023b.
- BIALA, Grazyna *et al.* Research in the Field of Drug Design and Development. **Pharmaceuticals**, [s. l.], v. 16, n. 9, p. 1283, 2023.
- BIASUTTO, Lucia *et al.* Resveratrol derivatives as a pharmacological tool. **Annals of the New York Academy of Sciences**, [s. l.], v. 1403, n. 1, p. 27–37, 2017.
- BIASUTTO, Lucia *et al.* Soluble polyphenols: Synthesis and bioavailability of 3,4',5-tri(α -d-glucose-3-O-succinyl) resveratrol. **Bioorganic & Medicinal Chemistry Letters**, [s. l.], v. 19, n. 23, p. 6721–6724, 2009. Disponível em: <https://linkinghub.elsevier.com/retrieve/pii/S0960894X09013821>.
- BÖHMDORFER, Michaela *et al.* Involvement of UDP-Glucuronosyltransferases and Sulfotransferases in the Excretion and Tissue Distribution of Resveratrol in Mice. **Nutrients**, [s. l.], v. 9, n. 12, p. 1347, 2017.
- BONIFATI, Vincenzo *et al.* Mutations in the DJ-1 Gene Associated with Autosomal Recessive Early-Onset Parkinsonism. **Science**, [s. l.], v. 299, n. 5604, p. 256–259, 2003.
- BONSACK, Frederick; ALLEYNE, Cargill H.; SUKUMARI-RAMESH, Sangeetha. Resveratrol Attenuates Neurodegeneration and Improves Neurological Outcomes after Intracerebral Hemorrhage in Mice. **Frontiers in Cellular Neuroscience**, [s. l.], v. 11, 2017.

- 1
2 BORODZIUK, Barbara *et al.* Preventing Progression: A Comprehensive Approach to
3 Managing Parkinson's Disease. **Quality in Sport**, [s. l.], v. 15, p. 52254, 2024.
4
- 5 BORRA, Margie T.; SMITH, Brian C.; DENU, John M. Mechanism of Human SIRT1
6 Activation by Resveratrol. **Journal of Biological Chemistry**, [s. l.], v. 280, n. 17, p.
7 17187–17195, 2005.
8
- 9 BOSE, Anindita; BEAL, M. Flint. Mitochondrial dysfunction in Parkinson's disease.
10 **Journal of Neurochemistry**, [s. l.], v. 139, n. S1, p. 216–231, 2016.
11
- 12 BRASIL, Ministério da Saúde. Protocolo clínico e diretrizes terapêuticas Doença de
13 Parkinson. Conselho Nacional de Incorporação de Tecnologias no SUS. 2017.
14 [https://www.gov.br/conitec/pt-](https://www.gov.br/conitec/pt-br/midias/protocolos/relatorio_pcdt_doenca_de_parkinson_final_291_2017.pdf)
15 [br/midias/protocolos/relatorio_pcdt_doenca_de_parkinson_final_291_2017.pdf](https://www.gov.br/conitec/pt-br/midias/protocolos/relatorio_pcdt_doenca_de_parkinson_final_291_2017.pdf)
16
- 17 BRASNYÓ, Pál *et al.* Resveratrol improves insulin sensitivity, reduces oxidative stress
18 and activates the Akt pathway in type 2 diabetic patients. **British Journal of Nutrition**,
19 [s. l.], v. 106, n. 3, p. 383–389, 2011.
20
- 21 BRECKENRIDGE, Charles B. *et al.* Association between Parkinson's Disease and
22 Cigarette Smoking, Rural Living, Well-Water Consumption, Farming and Pesticide Use:
23 Systematic Review and Meta-Analysis. **PLOS ONE**, [s. l.], v. 11, n. 4, p. e0151841,
24 2016.
25
- 26 BRÍÑEZ-GALLEGO, Paola *et al.* Experimental models of chemically induced
27 Parkinson's disease in zebrafish at the embryonic larval stage: a systematic review.
28 **Journal of Toxicology and Environmental Health, Part B**, [s. l.], v. 26, n. 4, p. 201–
29 237, 2023.
30
- 31 BURRÉ, Jacqueline *et al.* α -Synuclein Promotes SNARE-Complex Assembly in Vivo
32 and in Vitro. **Science**, [s. l.], v. 329, n. 5999, p. 1663–1667, 2010.
33

- CALAMINI, Barbara *et al.* Pleiotropic mechanisms facilitated by resveratrol and its metabolites. **Biochemical Journal**, [s. l.], v. 429, n. 2, p. 273–282, 2010.
- CASSAR, Steven *et al.* Use of Zebrafish in Drug Discovery Toxicology. **Chemical Research in Toxicology**, [s. l.], v. 33, n. 1, p. 95–118, 2020.
- CENCI, M. Angela. Presynaptic Mechanisms of l-DOPA-Induced Dyskinesia: The Findings, the Debate, and the Therapeutic Implications. **Frontiers in Neurology**, [s. l.], v. 5, n. NOV, p. 1–15, 2014. Disponível em: <http://journal.frontiersin.org/article/10.3389/fneur.2014.00242/abstract>.
- CHAUDHURI, K. Ray *et al.* Economic Burden of Parkinson's Disease: A Multinational, Real-World, Cost-of-Illness Study. **Drugs - Real World Outcomes**, [s. l.], v. 11, n. 1, p. 1–11, 2024.
- CHAUDHURI, K Ray; SCHAPIRA, Anthony HV. Non-motor symptoms of Parkinson's disease: dopaminergic pathophysiology and treatment. **The Lancet Neurology**, [s. l.], v. 8, n. 5, p. 464–474, 2009. Disponível em: <https://linkinghub.elsevier.com/retrieve/pii/S1474442209700687>.
- CHEN, Y. *et al.* Anti-oxidant polydatin (piceid) protects against substantia nigral motor degeneration in multiple rodent models of Parkinson's disease. **Molecular Neurodegeneration**, [s. l.], v. 10, n. 1, p. 1–14, 2015.
- CHEN, H. *et al.* Dopaminergic system and neurons: Role in multiple neurological diseases. **Neuropharmacology**, Dec 1;260:110133, 2024.
- CHI, Lap Hing; BURROWS, Allan D.; ANDERSON, Robin L. Can preclinical drug development help to predict adverse events in clinical trials?. **Drug Discovery Today**, [s. l.], v. 27, n. 1, p. 257–268, 2022.
- CHOU, Wei-Chun; LIN, Zhoumeng. Machine learning and artificial intelligence in physiologically based pharmacokinetic modeling. **Toxicological Sciences**, [s. l.], v. 191, n. 1, p. 1–14, 2023.

CHRISTENSEN, Christian *et al.* Multi-parameter Behavioral Phenotyping of the MPP+ Model of Parkinson's Disease in Zebrafish. **Frontiers in Behavioral Neuroscience**, [s. l.], v. 14, 2020.

CIULLA, Michele *et al.* Role of dietary supplements in the management of parkinson's disease. **Biomolecules**, [s. l.], v. 9, n. 7, 2019.

CONNOLLY, Barbara S.; LANG, Anthony E. Pharmacological treatment of Parkinson disease: A review. **JAMA - Journal of the American Medical Association**, [s. l.], v. 311, n. 16, p. 1670–1683, 2014.

CONSTANTINESCU, Teodora; MIHIS, Alin Grig. Resveratrol as a privileged molecule with antioxidant activity. **Food Chemistry Advances**, [s. l.], v. 3, p. 100539, 2023.

COSTA, Helena Nunes *et al.* Parkinson's Disease: A Multisystem Disorder. **Neuroscience Bulletin**, [s. l.], v. 39, n. 1, p. 113–124, 2023.

COTTART, Charles-Henry; NIVET-ANTOINE, Valérie; BEAUDEUX, Jean-Louis. Review of recent data on the metabolism, biological effects, and toxicity of resveratrol in humans. **Molecular Nutrition & Food Research**, [s. l.], v. 58, n. 1, p. 7–21, 2014.

CRIQUI, M H; RINGEL, B L. Does diet or alcohol explain the French paradox?. **The Lancet**, [s. l.], v. 344, n. 8939–8940, p. 1719–1723, 1994.

DA ROCHA LINDNER, Gabriela *et al.* Improved Neuroprotective Effects of Resveratrol-Loaded Polysorbate 80-Coated Poly(Lactide) Nanoparticles in MPTP-Induced Parkinsonism. **Nanomedicine**, [s. l.], v. 10, n. 7, p. 1127–1138, 2015.

DAI, Jin; MUMPER, Russell J. Plant Phenolics: Extraction, Analysis and Their Antioxidant and Anticancer Properties. **Molecules**, [s. l.], v. 15, n. 10, p. 7313–7352, 2010.

- DAR, Refaz Ahmad *et al.* Natural product medicines: A literature update. **The Journal of Phytopharmacology**, [s. l.], v. 6, n. 6, p. 340–342, 2017.
- DASGUPTA, Biplab; MILBRANDT, Jeffrey. Resveratrol stimulates AMP kinase activity in neurons. **Proceedings of the National Academy of Sciences**, [s. l.], v. 104, n. 17, p. 7217–7222, 2007.
- DAVIS, Benjamin G; ROBINSON, Mark A. Drug delivery systems based on sugar-macromolecule conjugates. **Current opinion in drug discovery & development**, [s. l.], v. 5, n. 2, p. 279–88, 2002.
- DE LAU, Lonneke M L; BRETHER, Monique M B. The Epidemiology of Parkinson's Disease. **Lancet Neurol**, [s. l.], v. 5, n. 6, p. 535–35, 2006. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S1016319010600444>.
- DE WINTER, Karel *et al.* Enzymatic Glycosylation of Phenolic Antioxidants: Phosphorylase-Mediated Synthesis and Characterization. **Journal of Agricultural and Food Chemistry**, [s. l.], v. 63, n. 46, p. 10131–10139, 2015. Disponível em: <https://pubs.acs.org/doi/10.1021/acs.jafc.5b04380>.
- DICKSON, Dennis W *et al.* Neuropathological assessment of Parkinson's disease: refining the diagnostic criteria. **The Lancet Neurology**, [s. l.], v. 8, n. 12, p. 1150–1157, 2009.
- DICKSON, Dennis W. Neuropathology of Parkinson disease. **Parkinsonism & Related Disorders**, [s. l.], v. 46, p. S30–S33, 2018.
- DIRKS-HOFMEISTER, Mareike E. *et al.* Creating Space for Large Acceptors: Rational Biocatalyst Design for Resveratrol Glycosylation in an Aqueous System. **Angewandte Chemie - International Edition**, [s. l.], v. 54, n. 32, p. 9289–9292, 2015.
- DORSEY, E. R. *et al.* Projected number of people with Parkinson disease in the most populous nations, 2005 through 2030. **Neurology**, [s. l.], v. 68, n. 5, p. 384–386, 2007.

DOS SANTOS, Michele Goulart *et al.* Neuroprotective Effects of Resveratrol in In vivo and In vitro Experimental Models of Parkinson's Disease: a Systematic Review. **Neurotoxicity Research**, [s. l.], v. 40, n. 1, p. 319–345, 2022.

DU, Q. H.; PENG, C.; ZHANG, H. Polydatin: A review of pharmacology and pharmacokinetics. **Pharmaceutical Biology**, [s. l.], v. 51, n. 11, p. 1347–1354, 2013. Disponível em: <http://www.tandfonline.com/doi/full/10.3109/13880209.2013.792849>.

DU, Y., *et al.* Spatial and Temporal Distribution of Dopaminergic Neurons during Development in Zebrafish. **Front Neuroanat**, v. 10, n 115, 2016.

ELMALI, N. *et al.* Effects of Resveratrol in Inflammatory Arthritis. **Inflammation**, [s. l.], v. 30, n. 1–2, p. 1–6, 2007. Disponível em: <http://link.springer.com/10.1007/s10753-006-9012-0>.

ELSHAHAWI, Sherif I. *et al.* A comprehensive review of glycosylated bacterial natural products. **Chemical Society Reviews**, [s. l.], v. 44, n. 21, p. 7591–7697, 2015.

FALOMIR, Eva *et al.* Cytotoxic, Antiangiogenic and Antitelomerase Activity of Glucosyl- and Acyl- Resveratrol Prodrugs and Resveratrol Sulfate Metabolites. **ChemBioChem**, [s. l.], v. 17, n. 14, p. 1343–1348, 2016. Disponível em: <http://doi.wiley.com/10.1002/cbic.201600084>.

FARKHONDEH, Tahereh *et al.* The therapeutic effect of resveratrol: Focusing on the Nrf2 signaling pathway. **Biomedicine & Pharmacotherapy**, [s. l.], v. 127, p. 110234, 2020.

FEIGIN, Valery L *et al.* Global, regional, and national burden of neurological disorders, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. **The Lancet Neurology**, [s. l.], v. 18, n. 5, p. 459–480, 2019.

FENG, Yong-Hong *et al.* Low dose of resveratrol enhanced immune response of mice. **Acta pharmacologica Sinica**, [s. l.], v. 23, n. 10, p. 893–7, 2002.

- FILICE, Marco; MARCIELLO, Marzia. Enzymatic Synthesis of Oligosaccharides: A Powerful Tool for a Sweet Challenge. **Current Organic Chemistry**, [s. l.], v. 17, n. 7, p. 701–718, 2013.
- FINK, Anthony L. The Aggregation and Fibrillation of α -Synuclein. **Accounts of Chemical Research**, [s. l.], v. 39, n. 9, p. 628–634, 2006.
- FLAMINI, Riccardo *et al.* Advanced Knowledge of Three Important Classes of Grape Phenolics: Anthocyanins, Stilbenes and Flavonols. **International Journal of Molecular Sciences**, [s. l.], v. 14, n. 10, p. 19651–19669, 2013.
- FONTANA, Barbara D. *et al.* The developing utility of zebrafish models of neurological and neuropsychiatric disorders: A critical review. **Experimental Neurology**, [s. l.], v. 299, p. 157–171, 2018.
- FRANCEUS, Jorick; DESMET, Tom. Sucrose Phosphorylase and Related Enzymes in Glycoside Hydrolase Family 13: Discovery, Application and Engineering. **International Journal of Molecular Sciences**, [s. l.], v. 21, n. 7, p. 2526, 2020a.
- FRANCEUS, Jorick; DESMET, Tom. Sucrose Phosphorylase and Related Enzymes in Glycoside Hydrolase Family 13: Discovery, Application and Engineering. **International Journal of Molecular Sciences**, [s. l.], v. 21, n. 7, p. 2526, 2020b.
- FRANCO-IBORRA, Sandra; VILA, Miquel; PERIER, Celine. The Parkinson Disease Mitochondrial Hypothesis. **The Neuroscientist**, [s. l.], v. 22, n. 3, p. 266–277, 2016.
- FRÉMONT, Lucie. Biological effects of resveratrol. **Life Sciences**, [s. l.], v. 66, n. 8, p. 663–673, 2000. Disponível em:
<https://linkinghub.elsevier.com/retrieve/pii/S0024320599004105>.
- GABALLAH, Hanaa Hibishy *et al.* Modulatory effects of resveratrol on endoplasmic reticulum stress-associated apoptosis and oxido-inflammatory markers in a rat model of

rotenone-induced Parkinson's disease. **Chemico-Biological Interactions**, [s. l.], v. 251, p. 10–16, 2016.

GAL, Roland *et al.* The Effect of Resveratrol on the Cardiovascular System from Molecular Mechanisms to Clinical Results. **International Journal of Molecular Sciences**, [s. l.], v. 22, n. 18, p. 10152, 2021.

GAO, Youguang *et al.* Neuroprotective effects of polydatin against mitochondrial-dependent apoptosis in the rat cerebral cortex following ischemia/reperfusion injury. **Molecular Medicine Reports**, [s. l.], v. 14, n. 6, p. 5481–5488, 2016.

GAO, Hui-Ming; HONG, Jau-Shyong. Why neurodegenerative diseases are progressive: uncontrolled inflammation drives disease progression. **Trends in Immunology**, [s. l.], v. 29, n. 8, p. 357–365, 2008. Disponível em: <https://linkinghub.elsevier.com/retrieve/pii/S1471490608001567>.

GATZ, S. A.; WIESMULLER, L. Take a break--resveratrol in action on DNA. **Carcinogenesis**, [s. l.], v. 29, n. 2, p. 321–332, 2008.

GIACOMINI, Elisa *et al.* The Use of Stilbene Scaffold in Medicinal Chemistry and Multi- Target Drug Design. **Current Medicinal Chemistry**, [s. l.], v. 23, n. 23, p. 2439–2489, 2016a.

GIACOMINI, Elisa *et al.* The Use of Stilbene Scaffold in Medicinal Chemistry and Multi- Target Drug Design. **Current Medicinal Chemistry**, [s. l.], v. 23, n. 23, p. 2439–2489, 2016b.

GOLAN, D.E. *et al.* **Principles of Pharmacology: The Pathophysiologic Basis of Drug Therapy**. 3rd. ed. [S. l.: s. n.], 2012.

GOLDENBER, M.M. MEDICAL MANAGEMENT OF PARKINSON'S DISEASE. **P&T**, [s. l.], v. 33, n. 10, p. 590–606, 2008. Disponível em: https://jnnp.bmj.com/lookup/doi/10.1136/jnnp.72.suppl_1.i22.

- 1 GOSAVI, Nirmal *et al.* Golgi Fragmentation Occurs in the Cells with Prefibrillar α -
2 Synuclein Aggregates and Precedes the Formation of Fibrillar Inclusion. **Journal of**
3 **Biological Chemistry**, [s. l.], v. 277, n. 50, p. 48984–48992, 2002.
- 4 GRINBLAT, Y., GAMSE, J., PATEL, M., & SIVe, H. Determination of the zebrafish
5 forebrain: Induction and patterning. **Development**, 125(22), 4403e4416, 1998.
- 6
- 7 GROGAN, Michael J. *et al.* Homogeneous Glycopeptides and Glycoproteins for
8 Biological Investigation. **Annual Review of Biochemistry**, [s. l.], v. 71, n. 1, p. 593–
9 634, 2002.
- 10
- 11 GUEGUEN, Naïg *et al.* Resveratrol Directly Binds to Mitochondrial Complex I and
12 Increases Oxidative Stress in Brain Mitochondria of Aged Mice. **PLOS ONE**, [s. l.], v.
13 10, n. 12, p. e0144290, 2015.
- 14
- 15 GÜLÇİN, İlhami. Antioxidant properties of resveratrol: A structure–activity insight.
16 **Innovative Food Science & Emerging Technologies**, [s. l.], v. 11, n. 1, p. 210–218,
17 2010.
- 18
- 19 GUO, Yan-Jie *et al.* Resveratrol alleviates MPTP-induced motor impairments and
20 pathological changes by autophagic degradation of α -synuclein via SIRT1-deacetylated
21 LC3. **Molecular Nutrition & Food Research**, [s. l.], v. 60, n. 10, p. 2161–2175, 2016.
22 Disponível em: <https://onlinelibrary.wiley.com/doi/10.1002/mnfr.201600111>.
- 23
- 24 HABJAN, Eva *et al.* Diving into drug-screening: zebrafish embryos as an in vivo
25 platform for antimicrobial drug discovery and assessment. **FEMS Microbiology**
26 **Reviews**, [s. l.], v. 48, n. 3, 2024.
- 27
- 28 HALLIDAY, Glenda M. *et al.* Neuropathology underlying clinical variability in patients
29 with synucleinopathies. **Acta Neuropathologica**, [s. l.], v. 122, n. 2, p. 187–204, 2011.
30 Disponível em: <http://link.springer.com/10.1007/s00401-011-0852-9>.
- 31
- 32 HANSON, S *et al.* Chemoenzymatic synthesis of oligosaccharides and glycoproteins.
33 **Trends in Biochemical Sciences**, [s. l.], v. 29, n. 12, p. 656–663, 2004.
- 34

- 1 HANUKA KATZ, Inbal *et al.* The Link between Polyphenol Structure, Antioxidant
2 Capacity and Shelf-Life Stability in the Presence of Fructose and Ascorbic Acid.
3 **Molecules**, [s. l.], v. 25, n. 1, p. 225, 2020.
4
- 5 HAO, Jifu *et al.* Preparation and Optimization of Resveratrol Nanosuspensions by
6 Antisolvent Precipitation Using Box-Behnken Design. **AAPS PharmSciTech**, [s. l.], v.
7 16, n. 1, p. 118–128, 2015. Disponível em: [http://link.springer.com/10.1208/s12249-](http://link.springer.com/10.1208/s12249-014-0211-y)
8 014-0211-y.
9
- 10 HARRIS, Hannah; EDWARDS, Christine; MORRISON, Douglas. Impact of Glycosidic
11 Bond Configuration on Short Chain Fatty Acid Production from Model Fermentable
12 Carbohydrates by the Human Gut Microbiota. **Nutrients**, [s. l.], v. 9, n. 1, p. 26, 2017.
13
- 14 HAUPTMANN, G., SOLL, I., & GERSTER, T. The early embryonic zebrafish forebrain
15 is subdivided into molecularly distinct transverse and longitudinal domains. **Brain**
16 **Research Bulletin**, 57(3e4), 371e375, 2002.
17
- 18 HE, Shan; YAN, Xiaojun. From Resveratrol to Its Derivatives: New Sources of Natural
19 Antioxidant. **Current Medicinal Chemistry**, [s. l.], v. 20, n. 8, p. 1005–1017, 2013.
20
- 21 HEIKKILA, Richard E.; SONSALLA, Patricia K. The Use of the MPTP-Treated Mouse
22 as an Animal Model of Parkinsonism. **Canadian Journal of Neurological Sciences /**
23 **Journal Canadien des Sciences Neurologiques**, [s. l.], v. 14, n. S3, p. 436–440, 1987.
24
- 25 HENCHCLIFFE, Claire; BEAL, M Flint. Mitochondrial biology and oxidative stress in
26 Parkinson disease pathogenesis. **Nature Clinical Practice Neurology**, [s. l.], v. 4, n. 11,
27 p. 600–609, 2008.
28
- 29 HENRICH, Martin T. *et al.* Mitochondrial dysfunction in Parkinson's disease – a key
30 disease hallmark with therapeutic potential. **Molecular Neurodegeneration**, [s. l.], v.
31 18, n. 1, p. 83, 2023.
32

HERMANOWICZ, Neal; JONES, Sarah A; HAUSER, Robert A. Impact of non-motor symptoms in Parkinson's disease: a PMDAAlliance survey. **Neuropsychiatric Disease and Treatment**, [s. l.], v. Volume 15, p. 2205–2212, 2019.

HOFER, Bernd. Recent developments in the enzymatic O-glycosylation of flavonoids. **Applied Microbiology and Biotechnology**, [s. l.], v. 100, n. 10, p. 4269–4281, 2016.

HOOZEMANS, J.J.M. *et al.* Activation of the unfolded protein response in Parkinson's disease. **Biochemical and Biophysical Research Communications**, [s. l.], v. 354, n. 3, p. 707–711, 2007.

HOSODA, Ryusuke *et al.* Differential Cell-Protective Function of Two Resveratrol (Trans -3,5,4'-trihydroxystilbene) Glucosides against Oxidative Stress. **Journal of Pharmacology and Experimental Therapeutics**, [s. l.], v. 344, n. 1, p. 124–132, 2013. Disponível em: <http://jpet.aspetjournals.org/lookup/doi/10.1124/jpet.112.198937>.

HU, Shunyang *et al.* Advances and Challenges in Biomanufacturing of Glycosylation of Natural Products. **Fermentation**, [s. l.], v. 10, n. 7, p. 349, 2024.

HU, Jinquan *et al.* Resveratrol improves neuron protection and functional recovery through enhancement of autophagy after spinal cord injury in mice. **American journal of translational research**, [s. l.], v. 9, n. 10, p. 4607–4616, 2017.

HUGHES, JP *et al.* Principles of early drug discovery. **British Journal of Pharmacology**, [s. l.], v. 162, n. 6, p. 1239–1249, 2011.

HUNG, L. Cardioprotective effect of resveratrol, a natural antioxidant derived from grapes. **Cardiovascular Research**, [s. l.], v. 47, n. 3, p. 549–555, 2000.

IRWIN, David J.; LEE, Virginia M.-Y.; TROJANOWSKI, John Q. Parkinson's disease dementia: convergence of α -synuclein, tau and amyloid- β pathologies. **Nature Reviews Neuroscience**, [s. l.], v. 14, n. 9, p. 626–636, 2013.

- 1 ISIK, Sevim *et al.* Microglia Mediated Neuroinflammation in Parkinson's Disease.
- 2 **Cells**, [s. l.], v. 12, n. 7, p. 1012, 2023.
- 3
- 4 ISSA, Naiem T. *et al.* Drug Metabolism in Preclinical Drug Development: A Survey of
- 5 the Discovery Process, Toxicology, and Computational Tools. **Current Drug**
- 6 **Metabolism**, [s. l.], v. 18, n. 6, 2017.
- 7
- 8 JANG, Meishiang *et al.* Cancer Chemopreventive Activity of Resveratrol, a Natural
- 9 Product Derived from Grapes. **Science**, [s. l.], v. 275, n. 5297, p. 218–220, 1997.
- 10
- 11 JELLINGER, Kurt A. Basic mechanisms of neurodegeneration: a critical update.
- 12 **Journal of Cellular and Molecular Medicine**, [s. l.], v. 14, n. 3, p. 457–487, 2010.
- 13
- 14 JELLINGER, Kurt A. Recent advances in our understanding of neurodegeneration.
- 15 **Journal of Neural Transmission**, [s. l.], v. 116, n. 9, p. 1111–1162, 2009.
- 16
- 17 JENNER, P.; OLANOW, C. W. Oxidative stress and the pathogenesis of Parkinson's
- 18 disease. **Neurology**, [s. l.], v. 47, n. Issue 6, Supplement 3, p. 161S-170S, 1996.
- 19 Disponível em: http://www.neurology.org/cgi/doi/10.1212/WNL.47.6_Suppl_3.161S.
- 20
- 21 JI, Hui *et al.* Polydatin modulates inflammation by decreasing NF-κB activation and
- 22 oxidative stress by increasing Gli1, Ptch1, SOD1 expression and ameliorates blood-
- 23 brain barrier permeability for its neuroprotective effect in pMCAO rat brain. **Brain**
- 24 **Research Bulletin**, [s. l.], v. 87, n. 1, p. 50–59, 2012. Disponível em:
- 25 <http://dx.doi.org/10.1016/j.brainresbull.2011.09.021>.
- 26
- 27 JIN, Feng *et al.* Neuroprotective effect of resveratrol on 6-OHDA-induced Parkinson's
- 28 disease in rats. **European Journal of Pharmacology**, [s. l.], v. 600, n. 1–3, p. 78–82,
- 29 2008. Disponível em: <https://linkinghub.elsevier.com/retrieve/pii/S0014299908010248>.
- 30
- 31 KALIA, Lorraine V.; LANG, Anthony E. Parkinson's disease. **The Lancet**, [s. l.], v.
- 32 386, n. 9996, p. 896–912, 2015. Disponível em: [http://dx.doi.org/10.1016/S0140-](http://dx.doi.org/10.1016/S0140-6736(14)61393-3)
- 33 [6736\(14\)61393-3](http://dx.doi.org/10.1016/S0140-6736(14)61393-3).
- 34

- KAM, Tae-In *et al.* Microglia and astrocyte dysfunction in parkinson's disease. **Neurobiology of Disease**, [s. l.], v. 144, p. 105028, 2020.
- KAN, Shunli *et al.* Resveratrol improves the prognosis of rats after spinal cord injury by inhibiting mitogen-activated protein kinases signaling pathway. **Scientific Reports**, [s. l.], v. 13, n. 1, p. 19723, 2023.
- KASLIN, J. and GANZ, J. Zebrafish Nervous Systems. In *The Zebrafish in Biomedical Research*, S.C. Cartner, J.S. Eisen, S.C. Farmer, K.J. Guillemin, M.L. Kent, and G.E. Sanders, eds. **Academic Press**, Elsevier, pp. 181–189, 2020.
- KATARIA, Hardeep *et al.* Small molecule agonists of cell adhesion molecule L1 mimic L1 functions in vivo. **Molecular Neurobiology**, [s. l.], v. 53, n. 7, p. 4461–4483, 2016.
- KEREM, Zohar *et al.* Antioxidant activity and inhibition of alpha-glucosidase by trans-resveratrol, piceid, and a novel trans-stilbene from the roots of Israeli Rumex bucephalophorus L. **Journal of agricultural and food chemistry**, [s. l.], v. 54, n. 4, p. 1243–7, 2006.
- KEYLOR, Mitchell H.; MATSUURA, Bryan S.; STEPHENSON, Corey R. J. Chemistry and Biology of Resveratrol-Derived Natural Products. **Chemical Reviews**, [s. l.], v. 115, n. 17, p. 8976–9027, 2015.
- KIESSLING, Laura L.; SPLAIN, Rebecca A. Chemical Approaches to Glycobiology. **Annual Review of Biochemistry**, [s. l.], v. 79, n. 1, p. 619–653, 2010a.
- KIESSLING, Laura L.; SPLAIN, Rebecca A. Chemical Approaches to Glycobiology. **Annual Review of Biochemistry**, [s. l.], v. 79, n. 1, p. 619–653, 2010b.
- KISPOTTA, Sneha; DAS, Debajyoti; PRUSTY, Shakti Ketan. A recent update on drugs and alternative approaches for parkinsonism. **Neuropeptides**, [s. l.], v. 104, p. 102415, 2024.

KITADA, Tohru *et al.* Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. **Nature**, [s. l.], v. 392, n. 6676, p. 605–608, 1998.

KLEIN MO, *et al.* Dopamine: Functions, Signaling, and Association with Neurological Diseases. **Cell Mol Neurobiol.** Jan;39(1):31-59. doi: 10.1007/s10571-018-0632-3. Epub 2018 Nov 16, 2019.

KLINGEL, Tizian *et al.* Glucosylation of flavonoids and flavonoid glycosides by mutant dextranucrase from *Lactobacillus reuteri* TMW 1.106. **Carbohydrate Research**, [s. l.], v. 483, p. 107741, 2019. Disponível em: <https://linkinghub.elsevier.com/retrieve/pii/S0008621519302782>.

KOULI, Antonina; TORSNEY, Kelli M.; KUAN, Wei-Li. Parkinson's Disease: Etiology, Neuropathology, and Pathogenesis. *In*: PARKINSON'S DISEASE: PATHOGENESIS AND CLINICAL ASPECTS. [S. l.]: Codon Publications, 2018. p. 3–26.

KRAUS, Michael; GRIMM, Clemens; SEIBEL, Jürgen. Redesign of the Active Site of Sucrose Phosphorylase through a Clash-Induced Cascade of Loop Shifts. **ChemBioChem**, [s. l.], v. 17, n. 1, p. 33–36, 2016.

KRAUS, Michael; GRIMM, Clemens; SEIBEL, Jürgen. Switching enzyme specificity from phosphate to resveratrol glucosylation. **Chemical Communications**, [s. l.], v. 53, n. 90, p. 12181–12184, 2017.

KRAWCZYK, Monika *et al.* 8-Hydroxyquinoline Glycoconjugates Containing Sulfur at the Sugar Anomeric Position—Synthesis and Preliminary Evaluation of Their Cytotoxicity. **Molecules**, [s. l.], v. 25, n. 18, p. 4174, 2020.

KŘEN, Vladimír; THIEM, Joachim. Glycosylation employing bio-systems: from enzymes to whole cells. **Chem. Soc. Rev.**, [s. l.], v. 26, n. 6, p. 463–473, 1997.

- 1 KRUSCHITZ, Andreas *et al.* Continuous process technology for glucoside production
2 from sucrose using a whole cell-derived solid catalyst of sucrose phosphorylase.
3 **Applied Microbiology and Biotechnology**, [s. l.], v. 105, n. 13, p. 5383–5394, 2021.
4
- 5 LAL, Roshan *et al.* Experimental models of Parkinson's disease: Challenges and
6 Opportunities. **European Journal of Pharmacology**, [s. l.], v. 980, p. 176819, 2024.
7
- 8 LAN, Fan *et al.* Resveratrol-Induced AMP-Activated Protein Kinase Activation Is Cell-
9 Type Dependent: Lessons from Basic Research for Clinical Application. **Nutrients**, [s.
10 l.], v. 9, n. 7, p. 751, 2017.
11
- 12 LANGSTON, J. William *et al.* Chronic Parkinsonism in Humans Due to a Product of
13 Meperidine-Analog Synthesis. **Science**, [s. l.], v. 219, n. 4587, p. 979–980, 1983.
14
- 15 LANGSTON, J. William. The MPTP Story. **Journal of Parkinson's Disease**, [s. l.], v.
16 7, n. s1, p. S11–S19, 2017.
17
- 18 LARROSA, Mar *et al.* Preventive Oral Treatment with Resveratrol Pro-prodrugs
19 Drastically Reduce Colon Inflammation in Rodents. **Journal of Medicinal Chemistry**,
20 [s. l.], v. 53, n. 20, p. 7365–7376, 2010. Disponível em:
21 <https://pubs.acs.org/doi/10.1021/jm1007006>.
22
- 23 LEE, He-Jin *et al.* Clearance of α -Synuclein Oligomeric Intermediates via the
24 Lysosomal Degradation Pathway. **The Journal of Neuroscience**, [s. l.], v. 24, n. 8, p.
25 1888–1896, 2004.
26
- 27 LEES, Andrew J.; HARDY, John; REVESZ, Tamas. Parkinson's disease. **The Lancet**,
28 [s. l.], v. 373, n. 9680, p. 2055–2066, 2009. Disponível em:
29 [http://dx.doi.org/10.1016/S0140-6736\(09\)60492-X](http://dx.doi.org/10.1016/S0140-6736(09)60492-X).
30
- 31 LI, Wen-Wei *et al.* Localization of α -synuclein to mitochondria within midbrain of
32 mice. **NeuroReport**, [s. l.], v. 18, n. 15, p. 1543–1546, 2007.
33

- 1 LI, Li *et al.* Polydatin prevents the induction of secondary brain injury after traumatic
2 brain injury by protecting neuronal mitochondria. **Neural Regeneration Research**, [s.
3 l.], v. 14, n. 9, p. 1573–1582, 2019.
- 4
- 5 LI, Run Ping *et al.* Polydatin protects learning and memory impairments in a rat model
6 of vascular dementia. **Phytomedicine**, [s. l.], v. 19, n. 8–9, p. 677–681, 2012.
7 Disponível em: <http://dx.doi.org/10.1016/j.phymed.2012.03.002>.
- 8
- 9 LI, Qingyun; BARRES, Ben A. Microglia and macrophages in brain homeostasis and
10 disease. **Nature Reviews Immunology**, [s. l.], v. 18, n. 4, p. 225–242, 2018.
- 11
- 12 LIM, Ee Wei *et al.* Amyloid- β and Parkinson's disease. **Journal of Neurology**, [s. l.], v.
13 266, n. 11, p. 2605–2619, 2019.
- 14
- 15 LIN, Zhoumeng; CHOU, Wei-Chun. Machine Learning and Artificial Intelligence in
16 Toxicological Sciences. **Toxicological Sciences**, [s. l.], v. 189, n. 1, p. 7–19, 2022.
- 17
- 18 LIU, Qianqian *et al.* Resveratrol synergizes with low doses of L-DOPA to improve
19 MPTP-induced Parkinson disease in mice. **Behavioural Brain Research**, [s. l.], v. 367,
20 p. 10–18, 2019.
- 21
- 22 LOFRUMENTO, Dario D *et al.* Neuroprotective effects of resveratrol in an MPTP
23 mouse model of Parkinson's-like disease: Possible role of SOCS-1 in reducing pro-
24 inflammatory responses. **Innate Immunity**, [s. l.], v. 20, n. 3, p. 249–260, 2014.
25 Disponível em: <http://journals.sagepub.com/doi/10.1177/1753425913488429>.
- 26
- 27 LU, Xi-Lin *et al.* Paeonolum protects against MPP⁺-induced neurotoxicity in zebrafish
28 and PC12 cells. **BMC Complementary and Alternative Medicine**, [s. l.], v. 15, n. 1, p.
29 137, 2015.
- 30
- 31 LUO, Yuanrong *et al.* Global, regional, national epidemiology and trends of Parkinson's
32 disease from 1990 to 2021: findings from the Global Burden of Disease Study 2021.
33 **Frontiers in aging neuroscience**, [s. l.], v. 16, p. 1498756, 2025.
- 34

- 1 MACCHI, Zachary A. *et al.* Patient and caregiver characteristics associated with
2 caregiver burden in Parkinson's disease: a palliative care approach. **Annals of**
3 **Palliative Medicine**, [s. l.], v. 9, n. S1, p. S24–S33, 2020.
- 4
- 5 MAGYAR, K. *et al.* Cardioprotection by resveratrol: A human clinical trial in patients
6 with stable coronary artery disease. **Clinical Hemorheology and Microcirculation**, [s.
7 l.], v. 50, n. 3, p. 179–187, 2012.
- 8
- 9 MAROTEAUX, L; CAMPANELLI, JT; SCHELLER, RH. Synuclein: a neuron-specific
10 protein localized to the nucleus and presynaptic nerve terminal. **The Journal of**
11 **Neuroscience**, [s. l.], v. 8, n. 8, p. 2804–2815, 1988.
- 12
- 13 MENG, Tiantian *et al.* Anti-Inflammatory Action and Mechanisms of Resveratrol.
14 **Molecules**, [s. l.], v. 26, n. 1, p. 229, 2021.
- 15
- 16 MENSHYKAU, Denis. Emerging technologies for prediction of drug candidate efficacy
17 in the preclinical pipeline. **Drug Discovery Today**, [s. l.], v. 22, n. 11, p. 1598–1603,
18 2017.
- 19
- 20 MIKSITS, Michaela *et al.* Antitumor Activity of Resveratrol and its Sulfated
21 Metabolites against Human Breast Cancer Cells. **Planta Medica**, [s. l.], v. 75, n. 11, p.
22 1227–1230, 2009.
- 23
- 24 MILNE, Jill C. *et al.* Small molecule activators of SIRT1 as therapeutics for the
25 treatment of type 2 diabetes. **Nature**, [s. l.], v. 450, n. 7170, p. 712–716, 2007.
- 26
- 27 MIZUMA, Takashi *et al.* Improvement of intestinal transport of poorly absorbed drugs
28 by sugar conjugation. **Drug Metabolism and Pharmacokinetics**, [s. l.], v. 8, n.
29 supplement, p. 739–742, 1993.
- 30
- 31 MOEHLE, M.S.; WEST, A.B. M1 and M2 immune activation in Parkinson's Disease:
32 Foe and ally? **Neuroscience**, [s. l.], v. 302, p. 59–73, 2015.
- 33

- MORADI, Shayli Varasteh *et al.* Glycosylation, an effective synthetic strategy to improve the bioavailability of therapeutic peptides. **Chemical Science**, [s. l.], v. 7, n. 4, p. 2492–2500, 2016.
- MOUSSA, Charbel *et al.* Resveratrol regulates neuro-inflammation and induces adaptive immunity in Alzheimer's disease. **Journal of Neuroinflammation**, [s. l.], v. 14, n. 1, p. 1, 2017.
- MURAKAMI, Hidetomo *et al.* Recent Advances in Drug Therapy for Parkinson's Disease. **Internal Medicine**, [s. l.], v. 62, n. 1, p. 8940–21, 2023.
- MUROTA, Kaeko *et al.* α -Oligoglucosylation of a sugar moiety enhances the bioavailability of quercetin glucosides in humans. **Archives of Biochemistry and Biophysics**, [s. l.], v. 501, n. 1, p. 91–97, 2010.
- MUTHANA, Saddam; CAO, Hongzhi; CHEN, Xi. Recent progress in chemical and chemoenzymatic synthesis of carbohydrates. **Current Opinion in Chemical Biology**, [s. l.], v. 13, n. 5–6, p. 573–581, 2009.
- NAVARRO-ORCAJADA, Silvia *et al.* Stilbenes: Characterization, bioactivity, encapsulation and structural modifications. A review of their current limitations and promising approaches. **Critical Reviews in Food Science and Nutrition**, [s. l.], v. 63, n. 25, p. 7269–7287, 2023.
- NONOMURA, Susumu; KANAGAWA, Hiroshi; MAKIMOTO, Arihiro. Chemical Constituents of Polygonaceous Plants. I. **YAKUGAKU ZASSHI**, [s. l.], v. 83, n. 10, p. 988–990, 1963.
- NUERMBERGER, Eric L. Preclinical Efficacy Testing of New Drug Candidates. **Microbiology Spectrum**, [s. l.], v. 5, n. 3, 2017.
- OKAWARA, Mitsugi *et al.* Resveratrol protects dopaminergic neurons in midbrain slice culture from multiple insults. **Biochemical Pharmacology**, [s. l.], v. 73, n. 4, p. 550–560, 2007.

- OLANOW, C. Warren; SCHAPIRA, Anthony H. V.; AGID, Yves. Neuroprotection for Parkinson's disease: Prospects and promises. **Annals of Neurology**, [s. l.], v. 53, n. S3, p. S1–S2, 2003. Disponível em: <https://onlinelibrary.wiley.com/doi/10.1002/ana.10566>.
- OMAR, Noor Azzizah; KUMAR, Jaya; TEOH, Seong Lin. Neuroprotective effects of Neurotrophin-3 in MPTP-induced zebrafish Parkinson's disease model. **Frontiers in Pharmacology**, [s. l.], v. 14, 2023.
- ORGANIZAÇÃO MUNDIAL DA SAÚDE (OMS). **Parkinson disease**. [S. l.], 2025.
- PAISÁN-RUIZ, Coro *et al.* Cloning of the Gene Containing Mutations that Cause PARK8-Linked Parkinson's Disease. **Neuron**, [s. l.], v. 44, n. 4, p. 595–600, 2004.
- PAJARES, Marta *et al.* Inflammation in Parkinson's Disease: Mechanisms and Therapeutic Implications. **Cells**, [s. l.], v. 9, n. 7, p. 1687, 2020. Disponível em: <https://www.mdpi.com/2073-4409/9/7/1687>.
- PALLE, Suresh; NEERATI, Prasad. Improved neuroprotective effect of resveratrol nanoparticles as evinced by abrogation of rotenone-induced behavioral deficits and oxidative and mitochondrial dysfunctions in rat model of Parkinson's disease. **Naunyn-Schmiedeberg's Archives of Pharmacology**, [s. l.], v. 391, n. 4, p. 445–453, 2018.
- PANGENI, Rudra *et al.* Resveratrol: review on therapeutic potential and recent advances in drug delivery. **Expert Opinion on Drug Delivery**, [s. l.], v. 11, n. 8, p. 1285–1298, 2014. Disponível em: <http://www.tandfonline.com/doi/full/10.1517/17425247.2014.919253>.
- PATTON, E. Elizabeth; ZON, Leonard I.; LANGENAU, David M. Zebrafish disease models in drug discovery: from preclinical modelling to clinical trials. **Nature Reviews Drug Discovery**, [s. l.], v. 20, n. 8, p. 611–628, 2021.
- PEÑALVER, Pablo *et al.* Alkylated resveratrol prodrugs and metabolites as potential therapeutics for neurodegenerative diseases. **European Journal of Medicinal**

- Chemistry**, [s. l.], v. 146, p. 123–138, 2018. Disponível em:
<https://linkinghub.elsevier.com/retrieve/pii/S0223523418300503>.
- PENG, Kaige *et al.* Resveratrol Regulates Mitochondrial Biogenesis and Fission/Fusion to Attenuate Rotenone-Induced Neurotoxicity. **Oxidative Medicine and Cellular Longevity**, [s. l.], v. 2016, n. 1, 2016.
- PERFEITO, Rita *et al.* Linking alpha-synuclein phosphorylation to reactive oxygen species formation and mitochondrial dysfunction in SH-SY5Y cells. **Molecular and Cellular Neuroscience**, [s. l.], v. 62, p. 51–59, 2014.
- PHANI, Sudarshan; LOIKE, John D.; PRZEDBORSKI, Serge. Neurodegeneration and Inflammation in Parkinson's disease. **Parkinsonism & Related Disorders**, [s. l.], v. 18, p. S207–S209, 2012. Disponível em:
<https://linkinghub.elsevier.com/retrieve/pii/S1353802011700645>.
- PHUKAN, Banashree Chetia *et al.* Therapeutic considerations of bioactive compounds in Alzheimer's disease and Parkinson's disease: Dissecting the molecular pathways. **Phytotherapy Research**, [s. l.], v. 37, n. 12, p. 5657–5699, 2023.
- PINEDA-RAMÍREZ, Narayana *et al.* Resveratrol Activates Neuronal Autophagy Through AMPK in the Ischemic Brain. **Molecular Neurobiology**, [s. l.], v. 57, n. 2, p. 1055–1069, 2020.
- POEWE, Werner *et al.* Parkinson disease. **Nature Reviews Disease Primers**, [s. l.], v. 3, n. 1, p. 17013, 2017.
- POHL, Franziska; KONG THOO LIN, Paul. The Potential Use of Plant Natural Products and Plant Extracts with Antioxidant Properties for the Prevention/Treatment of Neurodegenerative Diseases: In Vitro, In Vivo and Clinical Trials. **Molecules**, [s. l.], v. 23, n. 12, p. 3283, 2018.
- POLI, A. *et al.* Effect of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in goldfish brain. **Brain Research**, [s. l.], v. 534, n. 1–2, p. 45–50, 1990.

- POLLARD, H. B. *et al.* A parkinsonian syndrome induced in the goldfish by the neurotoxin MPTP. **The FASEB Journal**, [s. l.], v. 6, n. 12, p. 3108–3116, 1992.
- POLYMEROPOULOS, Mihael H. *et al.* Mutation in the α -Synuclein Gene Identified in Families with Parkinson's Disease. **Science**, [s. l.], v. 276, n. 5321, p. 2045–2047, 1997.
- PRINGSHEIM, Tamara *et al.* The prevalence of Parkinson's disease: A systematic review and meta-analysis. **Movement Disorders**, [s. l.], v. 29, n. 13, p. 1583–1590, 2014.
- QUEIROZ, Auriekson N. *et al.* A theoretical antioxidant pharmacophore for resveratrol. **European Journal of Medicinal Chemistry**, [s. l.], v. 44, n. 4, p. 1644–1649, 2009.
Disponível em: <https://linkinghub.elsevier.com/retrieve/pii/S0223523408004248>.
- RAHMAN, Md. Mominur *et al.* Multifunctional role of natural products for the treatment of Parkinson's disease: At a glance. **Frontiers in Pharmacology**, [s. l.], v. 13, n. 976385, 2022.
- RAZALI, Khairiah *et al.* Characterization of neurobehavioral pattern in a zebrafish 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced model: A 96-hour behavioral study. **PLOS ONE**, [s. l.], v. 17, n. 10, p. e0274844, 2022.
- REGEV-SHOSHANI, Gilly *et al.* Glycosylation of resveratrol protects it from enzymic oxidation. **Biochemical Journal**, [s. l.], v. 374, n. 1, p. 157–163, 2003.
- RENAUD, S.; DE LORGERIL, M. Wine, alcohol, platelets, and the French paradox for coronary heart disease. **The Lancet**, [s. l.], v. 339, n. 8808, p. 1523–1526, 1992.
- RICHARDSON, Jason R. *et al.* Obligatory Role for Complex I Inhibition in the Dopaminergic Neurotoxicity of 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). **Toxicological Sciences**, [s. l.], v. 95, n. 1, p. 196–204, 2007.

- 1 RIMM, E. B *et al.* Review of moderate alcohol consumption and reduced risk of
2 coronary heart disease: is the effect due to beer, wine, or spirits?. **BMJ**, [s. l.], v. 312, n.
3 7033, p. 731–736, 1996.
- 4
- 5 RISIGLIONE, Pierpaolo *et al.* High-Resolution Respirometry Reveals MPP+
6 Mitochondrial Toxicity Mechanism in a Cellular Model of Parkinson's Disease.
7 **International Journal of Molecular Sciences**, [s. l.], v. 21, n. 21, p. 7809, 2020.
- 8
- 9 ROBB, Ellen L. *et al.* Molecular mechanisms of oxidative stress resistance induced by
10 resveratrol: Specific and progressive induction of MnSOD. **Biochemical and**
11 **Biophysical Research Communications**, [s. l.], v. 367, n. 2, p. 406–412, 2008.
- 12
- 13 ROBUCK, Patricia R; WURZELMANN, John I. Understanding the Drug Development
14 Process. **Inflammatory Bowel Diseases**, [s. l.], v. 11, p. S13–S16, 2005.
- 15
- 16 RODRIGUEZ-OROZ, Maria C. *et al.* Initial clinical manifestations of Parkinson's
17 disease: features and pathophysiological mechanisms. **The Lancet Neurology**, [s. l.], v.
18 8, n. 12, p. 1128–1139, 2009. Disponível em: [http://dx.doi.org/10.1016/S1474-](http://dx.doi.org/10.1016/S1474-4422(09)70293-5)
19 [4422\(09\)70293-5](http://dx.doi.org/10.1016/S1474-4422(09)70293-5).
- 20
- 21 ROSS, Christopher A.; POIRIER, Michelle A. What is the role of protein aggregation in
22 neurodegeneration?. **Nature Reviews Molecular Cell Biology**, [s. l.], v. 6, n. 11, p.
23 891–898, 2005.
- 24
- 25 ROUAUD, T. *et al.* Pathophysiology of Parkinson's disease: Mitochondria, alpha-
26 synuclein and much more.... **Revue Neurologique**, [s. l.], v. 177, n. 3, p. 260–271,
27 2021. Disponível em: <https://linkinghub.elsevier.com/retrieve/pii/S0035378720306767>.
- 28
- 29 RUPP, B., WULLIMANN, M.F., REICHERT, H. The zebrafish brain: a
30 neuroanatomical comparison with the goldfish. **Anatomy and Embryology** 194,
31 187e203, 1996.
- 32
- 33 SAIKO, Philipp *et al.* Resveratrol and its analogs: Defense against cancer, coronary
34 disease and neurodegenerative maladies or just a fad?. **Mutation Research/Reviews in**

- 1 **Mutation Research**, [s. l.], v. 658, n. 1–2, p. 68–94, 2008. Disponível em:
2 <https://linkinghub.elsevier.com/retrieve/pii/S1383574207000324>.
3
- 4 SALAS, José A.; MÉNDEZ, Carmen. Engineering the glycosylation of natural products
5 in actinomycetes. **Trends in Microbiology**, [s. l.], v. 15, n. 5, p. 219–232, 2007.
6
- 7 SALLA, Mohamed *et al.* Enhancing the Bioavailability of Resveratrol: Combine It,
8 Derivatize It, or Encapsulate It?. **Pharmaceutics**, [s. l.], v. 16, n. 4, p. 569, 2024.
9
- 10 SAMUELSSON, Gunnar. **Drugs of Natural Origin: A Textbook of Pharmacognosy**
11 **(5th edition)**. 5th. ed. [S. l.: s. n.], 2004.
12
- 13 SÁNCHEZ-FIDALGO, Susana *et al.* Dietary supplementation of resveratrol attenuates
14 chronic colonic inflammation in mice. **European Journal of Pharmacology**, [s. l.], v.
15 633, n. 1–3, p. 78–84, 2010. Disponível em:
16 <http://dx.doi.org/10.1016/j.ejphar.2010.01.025>.
17
- 18 SANTOS, Milena Almeida *et al.* Antioxidant effect of Resveratrol: Change in MAPK
19 cell signaling pathway during the aging process. **Archives of Gerontology and**
20 **Geriatrics**, [s. l.], v. 92, p. 104266, 2021.
21
- 22 SATO, Daisuke *et al.* Synthesis of glycosides of resveratrol, pterostilbene, and
23 piceatannol, and their anti-oxidant, anti-allergic, and neuroprotective activities.
24 **Bioscience, Biotechnology, and Biochemistry**, [s. l.], v. 78, n. 7, p. 1123–1128, 2014.
25
- 26 SATPUTE, RM *et al.* Effect of resveratrol and tetracycline on the subacute paraquat
27 toxicity in mice. **Human & Experimental Toxicology**, [s. l.], v. 36, n. 12, p. 1303–
28 1314, 2017.
29
- 30 SCHAPIRA, Anthony H.V.; CHAUDHURI, K. Ray; JENNER, Peter. Non-motor
31 features of Parkinson disease. **Nature Reviews Neuroscience**, [s. l.], v. 18, n. 7, p. 435–
32 450, 2017.
33

SCHATZ, Albert *et al.* The Classic: Streptomycin, a Substance Exhibiting Antibiotic Activity against Gram-Positive and Gram-Negative Bacteria. **Clinical Orthopaedics and Related Research**, [s. l.], v. NA;, n. 437, p. 3–6, 2005. Disponível em: <http://journals.lww.com/00003086-200508000-00002>.

SCHEIFE, Richard T. *et al.* Impact of Parkinson's disease and its pharmacologic treatment on quality of life and economic outcomes. **American Journal of Health-System Pharmacy**, [s. l.], v. 57, n. 10, p. 953–962, 2000.

SCHIMITH, Lucia E. *et al.* Polydatin as a therapeutic alternative for central nervous system disorders: A systematic review of animal studies. **Phytotherapy Research**, [s. l.], v. 36, n. 7, p. 2852–2877, 2022. Disponível em: <https://onlinelibrary.wiley.com/doi/10.1002/ptr.7497>.

SCHMIDT, Rebecca; STRÄHLE, Uwe; SCHOLPP, Steffen. Neurogenesis in zebrafish – from embryo to adult. **Neural Development**, [s. l.], v. 8, n. 1, p. 3, 2013.

SCHNEIDER MEDEIROS, Márcio *et al.* Occupational pesticide exposure and the risk of death in patients with Parkinson's disease: an observational study in southern Brazil. **Environmental Health**, [s. l.], v. 19, n. 1, p. 68, 2020.

SCHWEITZER, J., DRIEVER, W. Development of the Dopamine Systems in Zebrafish. In: Pasterkamp, R.J., Smidt, M.P., Burbach, J.P.H. (eds) Development and Engineering of Dopamine Neurons. **Advances in Experimental Medicine and Biology**, vol 651, 2009.

SHAIKH, Fathima Aidha; WITHERS, Stephen G. Teaching old enzymes new tricks: engineering and evolution of glycosidases and glycosyl transferases for improved glycoside synthesis. **Biochemistry and Cell Biology**, [s. l.], v. 86, n. 2, p. 169–177, 2008.

SHEGOKAR, Ranjita. Preclinical testing—Understanding the basics first. In: DRUG DELIVERY ASPECTS. [S. l.]: Elsevier, 2020. p. 19–32.

- 1 SHEN, Tao; WANG, Xiao-Ning; LOU, Hong-Xiang. Natural stilbenes: an overview.
2 **Natural Product Reports**, [s. l.], v. 26, n. 7, p. 916, 2009.
- 3
- 4 SIDRANSKY, Ellen; LOPEZ, Grisel. The link between the GBA gene and
5 parkinsonism. **The Lancet Neurology**, [s. l.], v. 11, n. 11, p. 986–998, 2012.
- 6
- 7 SIEMANN, E. H.; CREASY, L. L. Concentration of the Phytoalexin Resveratrol in
8 Wine. **American Journal of Enology and Viticulture**, [s. l.], v. 43, n. 1, p. 49–52,
9 1992.
- 10
- 11 SINGER, T P; RAMSAY, R R. Mechanism of the neurotoxicity of MPTP. An update.
12 **FEBS letters**, [s. l.], v. 274, n. 1–2, p. 1–8, 1990.
- 13
- 14 SINGH, Sonu. Preclinical Pharmacokinetics: An Approach Towards Safer and
15 Efficacious Drugs. **Current Drug Metabolism**, [s. l.], v. 7, n. 2, p. 165–182, 2006.
- 16
- 17 SLÁMOVÁ, Kristýna; KAPEŠOVÁ, Jana; VALENTOVÁ, Kateřina. “Sweet
18 Flavonoids”: Glycosidase-Catalyzed Modifications. **International Journal of**
19 **Molecular Sciences**, [s. l.], v. 19, n. 7, p. 2126, 2018.
- 20
- 21 SOCAŁA, Katarzyna *et al.* Therapeutic potential of stilbenes in neuropsychiatric and
22 neurological disorders: A comprehensive review of preclinical and clinical evidence.
23 **Phytotherapy Research**, [s. l.], v. 38, n. 3, p. 1400–1461, 2024.
- 24
- 25 SOLÁ, Ricardo J.; GRIEBENOW, Kai. Effects of glycosylation on the stability of
26 protein pharmaceuticals. **Journal of Pharmaceutical Sciences**, [s. l.], v. 98, n. 4, p.
27 1223–1245, 2009.
- 28
- 29 SOLEAS, George J.; DIAMANDIS, Eleftherios P.; GOLDBERG, David M.
30 Resveratrol: A molecule whose time has come? And gone?. **Clinical Biochemistry**, [s.
31 l.], v. 30, n. 2, p. 91–113, 1997.
- 32

- 1 SONG, Luxia *et al.* Chinese Herbal Medicines and Active Metabolites: Potential
2 Antioxidant Treatments for Atherosclerosis. **Frontiers in Pharmacology**, [s. l.], v. 12,
3 2021.
- 4
- 5 SOUSSI-YANICOSTAS, Nadia. Zebrafish as a Model for Neurological Disorders.
6 **International Journal of Molecular Sciences**, [s. l.], v. 23, n. 8, p. 4321, 2022.
- 7
- 8 SPARVOLI, Francesca *et al.* Cloning and molecular analysis of structural genes
9 involved in flavonoid and stilbene biosynthesis in grape (*Vitis vinifera* L.). **Plant**
10 **Molecular Biology**, [s. l.], v. 24, n. 5, p. 743–755, 1994.
- 11
- 12 SPENCER, Jeremy P. E. *et al.* Biomarkers of the intake of dietary polyphenols:
13 strengths, limitations and application in nutrition research. **British Journal of**
14 **Nutrition**, [s. l.], v. 99, n. 1, p. 12–22, 2008.
- 15
- 16 SPINAZZI, Marco *et al.* Assessment of mitochondrial respiratory chain enzymatic
17 activities on tissues and cultured cells. **Nature Protocols**, [s. l.], v. 7, n. 6, p. 1235–
18 1246, 2012.
- 19
- 20 SRIVASTAVA, Garima *et al.* Resveratrol potentiates cytochrome P450 2d22-mediated
21 neuroprotection in maneb- and paraquat-induced parkinsonism in the mouse. **Free**
22 **Radical Biology and Medicine**, [s. l.], v. 52, n. 8, p. 1294–1306, 2012.
- 23
- 24 STERN, Y. *et al.* Cognitive change following MPTP exposure. **Neurology**, [s. l.], v. 40,
25 n. 2, p. 261–261, 1990.
- 26
- 27 STOJANOVIĆ, Sandra; SPRINZ, Helmut; BREDE, Ortwin. Efficiency and Mechanism
28 of the Antioxidant Action of trans-Resveratrol and Its Analogues in the Radical
29 Liposome Oxidation. **Archives of Biochemistry and Biophysics**, [s. l.], v. 391, n. 1, p.
30 79–89, 2001.
- 31
- 32 SU, Xiaomin *et al.* Synuclein activates microglia in a model of Parkinson's disease.
33 **Neurobiology of Aging**, [s. l.], v. 29, n. 11, p. 1690–1701, 2008.
- 34

- 1 SUN, Duxin *et al.* Why 90% of clinical drug development fails and how to improve it?
2 **Acta Pharmaceutica Sinica B**, [s. l.], v. 12, n. 7, p. 3049–3062, 2022.
3
- 4 SZYMKOWIAK, Izabela; KUCINSKA, Malgorzata; MURIAS, Marek. Between the
5 Devil and the Deep Blue Sea—Resveratrol, Sulfotransferases and Sulfatases—A Long
6 and Turbulent Journey from Intestinal Absorption to Target Cells. **Molecules**, [s. l.], v.
7 28, n. 8, p. 3297, 2023.
8
- 9 TAN, Charlotte *et al.* Daunomycin, an antitumor antibiotic, in the treatment of
10 neoplastic disease. Clinical evaluation with special reference to childhood leukemia.
11 **Cancer**, [s. l.], v. 20, n. 3, p. 333–353, 1967. Disponível em:
12 [https://onlinelibrary.wiley.com/doi/10.1002/1097-0142\(1967\)20:3%3C333::AID-](https://onlinelibrary.wiley.com/doi/10.1002/1097-0142(1967)20:3%3C333::AID-CNCR2820200302%3E3.0.CO;2-K)
13 [CNCR2820200302%3E3.0.CO;2-K](https://onlinelibrary.wiley.com/doi/10.1002/1097-0142(1967)20:3%3C333::AID-CNCR2820200302%3E3.0.CO;2-K).
14
- 15 THORSON, Jon S. *et al.* Structure-Based Enzyme Engineering and Its Impact on In
16 Vitro Glycorandomization. **ChemBioChem**, [s. l.], v. 5, n. 1, p. 16–25, 2004.
17 Disponível em: <http://doi.wiley.com/10.1002/cbic.200300620>.
18
- 19 TRAN, Thi Tuyet Van *et al.* Artificial Intelligence in Drug Toxicity Prediction: Recent
20 Advances, Challenges, and Future Perspectives. **Journal of Chemical Information**
21 **and Modeling**, [s. l.], v. 63, n. 9, p. 2628–2643, 2023.
22
- 23 TRONCOSO-ESCUADERO, Paulina *et al.* Outside in: Unraveling the Role of
24 Neuroinflammation in the Progression of Parkinson’s Disease. **Frontiers in Neurology**,
25 [s. l.], v. 9, 2018.
26
- 27 TSAI, Shu-Huei; LIN-SHIAU, Shoei-Yn; LIN, Jen-Kun. Suppression of nitric oxide
28 synthase and the down-regulation of the activation of NFκB in macrophages by
29 resveratrol. **British Journal of Pharmacology**, [s. l.], v. 126, n. 3, p. 673–680, 1999.
30
- 31 TURNER, R. Scott *et al.* A randomized, double-blind, placebo-controlled trial of
32 resveratrol for Alzheimer disease. **Neurology**, [s. l.], v. 85, n. 16, p. 1383–1391, 2015.
33

TURRENS, J. F. Mitochondrial formation of reactive oxygen species. **The Journal of Physiology**, [s. l.], v. 552, n. 2, p. 335–344, 2003.

TYSNES, Ole-Bjørn; STORSTEIN, Anette. Epidemiology of Parkinson's disease. **Journal of Neural Transmission**, [s. l.], v. 124, n. 8, p. 901–905, 2017.

UTPAL, Biswajit Kumar *et al.* Resveratrol: A Natural Compound Targeting the PI3K/Akt/mTOR Pathway in Neurological Diseases. **Molecular Neurobiology**, [s. l.], 2024a.

UTPAL, Biswajit Kumar *et al.* Resveratrol: A Natural Compound Targeting the PI3K/Akt/mTOR Pathway in Neurological Diseases. **Molecular Neurobiology**, [s. l.], 2024b.

VALENTE, Enza Maria *et al.* Hereditary Early-Onset Parkinson's Disease Caused by Mutations in PINK1. **Science**, [s. l.], v. 304, n. 5674, p. 1158–1160, 2004.

VAN DER MARK, Marianne *et al.* Is Pesticide Use Related to Parkinson Disease? Some Clues to Heterogeneity in Study Results. **Environmental Health Perspectives**, [s. l.], v. 120, n. 3, p. 340–347, 2012.

VÁRADI, Csaba. Clinical Features of Parkinson's Disease: The Evolution of Critical Symptoms. **Biology**, [s. l.], v. 9, n. 5, p. 103, 2020.

VAZ, Rita L.; OUTEIRO, Tiago F.; FERREIRA, Joaquim J. Zebrafish as an Animal Model for Drug Discovery in Parkinson's Disease and Other Movement Disorders: A Systematic Review. **Frontiers in Neurology**, [s. l.], v. 9, 2018.

VESTERGAARD, Martin; INGMER, Hanne. Antibacterial and antifungal properties of resveratrol. **International Journal of Antimicrobial Agents**, [s. l.], v. 53, n. 6, p. 716–723, 2019.

- 1 WADSWORTH, Teri L; KOOP, Dennis R. Effects of the wine polyphenolics quercetin
2 and resveratrol on pro-inflammatory cytokine expression in RAW 264.7 macrophages.
3 **Biochemical Pharmacology**, [s. l.], v. 57, n. 8, p. 941–949, 1999.
4
- 5 WAHID, Muqet *et al.* Pharmacological exploration of traditional plants for the
6 treatment of neurodegenerative disorders. **Phytotherapy Research**, [s. l.], v. 34, n. 12,
7 p. 3089–3112, 2020. Disponível em:
8 <https://onlinelibrary.wiley.com/doi/10.1002/ptr.6742>.
9
- 10 WALLE, Thomas. Bioavailability of resveratrol. **Annals of the New York Academy of**
11 **Sciences**, [s. l.], v. 1215, n. 1, p. 9–15, 2011.
12
- 13 WALLE, Thomas *et al.* High absorption but very low bioavailability of oral resveratrol
14 in humans. **Drug Metabolism and Disposition**, [s. l.], v. 32, n. 12, p. 1377–1382, 2004.
15 Disponível em: <http://dmd.aspetjournals.org/lookup/doi/10.1124/dmd.104.000885>.
16
- 17 WANG, Xiaobo *et al.* Advances of Zebrafish in Neurodegenerative Disease: From
18 Models to Drug Discovery. **Frontiers in Pharmacology**, [s. l.], v. 12, 2021.
19
- 20 WANG, Hui-Lin *et al.* Comparative studies of polydatin and resveratrol on mutual
21 transformation and antioxidative effect in vivo. **Phytomedicine**, [s. l.], v. 22, n. 5, p.
22 553–559, 2015. Disponível em:
23 <https://linkinghub.elsevier.com/retrieve/pii/S094471131500080X>.
24
- 25 WANG, Sijiao *et al.* Resveratrol attenuates inflammation and apoptosis through
26 alleviating endoplasmic reticulum stress via Akt/mTOR pathway in fungus-induced
27 allergic airways inflammation. **International Immunopharmacology**, [s. l.], v. 103, p.
28 108489, 2022.
29
- 30 WILLIAMS, Gavin J; ZHANG, Changsheng; THORSON, Jon S. Expanding the
31 promiscuity of a natural-product glycosyltransferase by directed evolution. **Nature**
32 **Chemical Biology**, [s. l.], v. 3, n. 10, p. 657–662, 2007.
33

WILLIAMS-GRAY, Caroline H. *et al.* Serum immune markers and disease progression in an incident Parkinson's disease cohort (ICICLE-PD). **Movement Disorders**, [s. l.], v. 31, n. 7, p. 995–1003, 2016.

WIRDEFELDT, Karin *et al.* Epidemiology and etiology of Parkinson's disease: a review of the evidence. **European Journal of Epidemiology**, [s. l.], v. 26, n. S1, p. 1–58, 2011.

WOOD, Stephen J. *et al.* α -Synuclein Fibrillogenesis Is Nucleation-dependent. **Journal of Biological Chemistry**, [s. l.], v. 274, n. 28, p. 19509–19512, 1999.

XIA, Yuanyuan *et al.* Development of thermostable sucrose phosphorylase by semi-rational design for efficient biosynthesis of alpha-D-glucosylglycerol. **Applied Microbiology and Biotechnology**, [s. l.], v. 105, n. 19, p. 7309–7319, 2021.

XIAO, Jianbo; MUZASHVILI, Tamar S.; GEORGIEV, Milen I. Advances in the biotechnological glycosylation of valuable flavonoids. **Biotechnology Advances**, [s. l.], v. 32, n. 6, p. 1145–1156, 2014.

XU, Luyao *et al.* Inhibition of NF- κ B Signaling Pathway by Resveratrol Improves Spinal Cord Injury. **Frontiers in Neuroscience**, [s. l.], v. 12, 2018.

XU, Lie Qiang *et al.* Polydatin attenuates d-galactose-induced liver and brain damage through its anti-oxidative, anti-inflammatory and anti-apoptotic effects in mice. **Food and Function**, [s. l.], v. 7, n. 11, p. 4545–4555, 2016.

XU, Bing *et al.* Protective effect of polydatin on a PC12 cell model of oxygen-glucose deprivation. **Nan fang yi ke da xue xue bao = Journal of Southern Medical University**, [s. l.], v. 30, n. 5, p. 1041–3, 2010.

YANG, Xuhao *et al.* The Role and Mechanism of SIRT1 in Resveratrol-regulated Osteoblast Autophagy in Osteoporosis Rats. **Scientific Reports**, [s. l.], v. 9, n. 1, p. 18424, 2019.

YAO, Li *et al.* Unexpected Neuroprotective Effects of Loganin on 1-Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine-Induced Neurotoxicity and Cell Death in Zebrafish. **Journal of Cellular Biochemistry**, [s. l.], v. 118, n. 3, p. 615–628, 2017.

YU, Biao; SUN, Jiansong; YANG, Xiaoyu. Assembly of Naturally Occurring Glycosides, Evolved Tactics, and Glycosylation Methods. **Accounts of Chemical Research**, [s. l.], v. 45, n. 8, p. 1227–1236, 2012.

ZAHOOR, Insha; SHAFI, Amrina; HAQ, Ehtishamul. Pharmacological Treatment of Parkinson's Disease. *In: PARKINSON'S DISEASE: PATHOGENESIS AND CLINICAL ASPECTS*. [S. l.]: Codon Publications, 2018. p. 129–144. Disponível em: <https://exonpublications.com/index.php/exon/article/view/192>.

ZHAN, Jiheng *et al.* Polydatin promotes the neuronal differentiation of bone marrow mesenchymal stem cells in vitro and in vivo: Involvement of Nrf2 signalling pathway. **Journal of Cellular and Molecular Medicine**, [s. l.], v. 24, n. 9, p. 5317–5329, 2020.

ZHANG, Shaozhi *et al.* Polydatin alleviates parkinsonism in MPTP-model mice by enhancing glycolysis in dopaminergic neurons. **Neurochemistry International**, [s. l.], v. 139, n. 104815, 2020. Disponível em: <https://doi.org/10.1016/j.neuint.2020.104815>.

ZHANG, Li-fan *et al.* Resveratrol alleviates motor and cognitive deficits and neuropathology in the A53T α -synuclein mouse model of Parkinson's disease. **Food & Function**, [s. l.], v. 9, n. 12, p. 6414–6426, 2018.

ZHAO, Yue *et al.* Rosmarinic acid protects against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced dopaminergic neurotoxicity in zebrafish embryos. **Toxicology in Vitro**, [s. l.], v. 65, p. 104823, 2020.

ZHONG, Michael *et al.* The relationship between specific cognitive defects and burden of care in Parkinson's disease. **International Psychogeriatrics**, [s. l.], v. 28, n. 2, p. 275–281, 2016.

1 ZHOU, Zhong-Xin *et al.* Anti-inflammatory activity of resveratrol prevents
2 inflammation by inhibiting NF- κ B in animal models of acute pharyngitis. **Molecular**
3 **Medicine Reports**, [s. l.], 2017.

4
5 ZHU, Lin *et al.* Interaction between resveratrol and SIRT1: role in neurodegenerative
6 diseases. **Naunyn-Schmiedeberg's Archives of Pharmacology**, [s. l.], v. 398, n. 1, p.
7 89–101, 2025.

8
9 ZYKOVA, Tatyana A. *et al.* Resveratrol directly targets COX-2 to inhibit
10 carcinogenesis. **Molecular Carcinogenesis**, [s. l.], v. 47, n. 10, p. 797–805, 2008.

CAPÍTULO 1 – MANUSCRITO 1**“Glycosylation matters: network pharmacology-based and molecular docking analysis of glycosylated resveratrol derivatives in Parkinson’s disease”**

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Abstract

Resveratrol, a natural polyphenol, has been extensively studied for its potential neuroprotective effects, particularly in neurodegenerative diseases such as Parkinson's Disease (PD). However, its clinical application is limited due to its low bioavailability and rapid metabolism. Glycosylated derivatives of resveratrol have emerged as a promising strategy to enhance its solubility, stability, and bioavailability. This study evaluates the pharmacokinetic profiles of resveratrol and its glycosylated derivatives, polydatin and resveratrol-3- α -glucoside, as well as their molecular interactions with PD-related targets. Using the ADMETLab platform, we found that, while glycosylation enhances solubility and stability, resveratrol itself exhibits a more favorable overall pharmacokinetic profile compared to its glycosylated forms. Pharmacodynamic tools were used to predict potential targets of resveratrol, polydatin, and resveratrol-3- α -glucoside in relation to PD. After identifying key potential targets, Gene Ontology (GO) functional enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were performed using the DAVID database. To further explore the molecular mechanisms, a protein-protein interaction (PPI) network was constructed, identifying 50 key targets linked to both resveratrol and its glycosylated derivatives in PD. Topological analysis of the PPI network revealed 11 core targets, which were subsequently analyzed through molecular docking to evaluate their interactions with resveratrol, polydatin, and resveratrol-3- α -glucoside. Resveratrol and its glycosylated derivatives exert their effects on PD through a multitarget protein network and associated pathways. The glycosylated derivatives exhibited higher binding affinity values for all analyzed targets compared to resveratrol aglycone. Among them, TNF- α , PPAR γ , and ERBB2 showed the better docking scores, suggesting their potential as key therapeutic targets in PD. These findings indicate that glycosylated derivatives of resveratrol may offer enhanced therapeutic potential for PD treatment by exhibiting stronger molecular interactions with critical targets. However, since efficacy is influenced by multiple pharmacokinetic parameters further studies are needed to determine whether these derivatives translate into improved neuroprotective effects *in vivo*.

Keywords: In silico, polydatin, piceid, transglycosylation, polyphenol

1. Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by the loss of dopaminergic neurons in the substantia nigra, leading to motor symptoms such as bradykinesia, tremor, rigidity, and postural instability, as well as non-motor complications, including neuropsychiatric and sensory disturbances, cognitive decline, autonomic dysfunction, sleep disorders, and pain^{1,2}. The World Health Organization estimates that the prevalence of PD has doubled over the past decades. As the disease progresses, clinical symptoms and complications significantly affect patients' quality of life, resulting in disability and an increased need for long-term care^{3,4}.

While several approved pharmacological therapies effectively alleviate motor symptoms and improve the mobility of PD patients, no available therapy currently slows disease progression or promotes relief for the wide spectrum of clinical manifestations. The multifactorial nature of PD makes the development of new potential drugs even more complicated, considering the multiple pathological mechanisms, involving α -synuclein aggregation, oxidative stress, mitochondrial dysfunction, and neuroinflammation, as well as heterogeneous disease progression^{5,6}.

Natural compounds are being explored as a potential source of therapeutic alternatives for PD due to their multitarget characteristics. While currently available conventional treatments focus on controlling symptoms, natural molecules have demonstrated promise in managing the underlying neurodegenerative processes mainly as a result of their different neuroprotective properties^{7,8}. Resveratrol (3,4',5-*trans*-trihydroxystilbene) is a naturally occurring polyphenol widely recognized for its biological activities such as antioxidant, anti-inflammatory, and anti-apoptotic, showing potential therapeutic effects in experimental models of PD⁹⁻¹¹.

Exploring natural molecules for therapeutic use experiences difficulties, particularly related to pharmacokinetics and bioavailability. The development of glycosylated analogues via biosynthetic pathways can enhance pharmacological properties, potentially improving pharmacokinetics while reducing toxicity and increasing target-specific activity^{12,13}. Polydatin (resveratrol-3-*O*- β -D-glucoside) and resveratrol-3- α -glucoside (resveratrol-3-*O*- α -D-glucoside) are glycosylated derivatives of resveratrol that have demonstrated improvements in stability and solubility compared to resveratrol, leading to better absorption and prolonged systemic circulation¹⁴(Fig. 1). Polydatin, a natural precursor of resveratrol, was originally isolated from *Polygonum cuspidatum*¹⁵, whereas resveratrol-3- α -glucoside was synthesized via enzymatic

glycosylation using sucrose phosphorylases from *Bifidobacterium adolescentis* (BaSP) with Q345F mutation, which enlarges the active site entry to accommodate polyphenols into the catalytic pocket of BaSP^{16,17}. While biological activities of polydatin largely overlap with those of resveratrol, its glycosylated precursor have been investigated for its antioxidant^{18,19} and anti-inflammatory²⁰ properties that may surpass those of its aglycone counterpart^{21,22}. Although not *in vitro* or *in vivo* studies have been conducted on the biological activities of resveratrol-3- α -glucoside, Akash and colleagues²³ recently evaluated its anti-colorectal cancer potential through *in silico* methods.

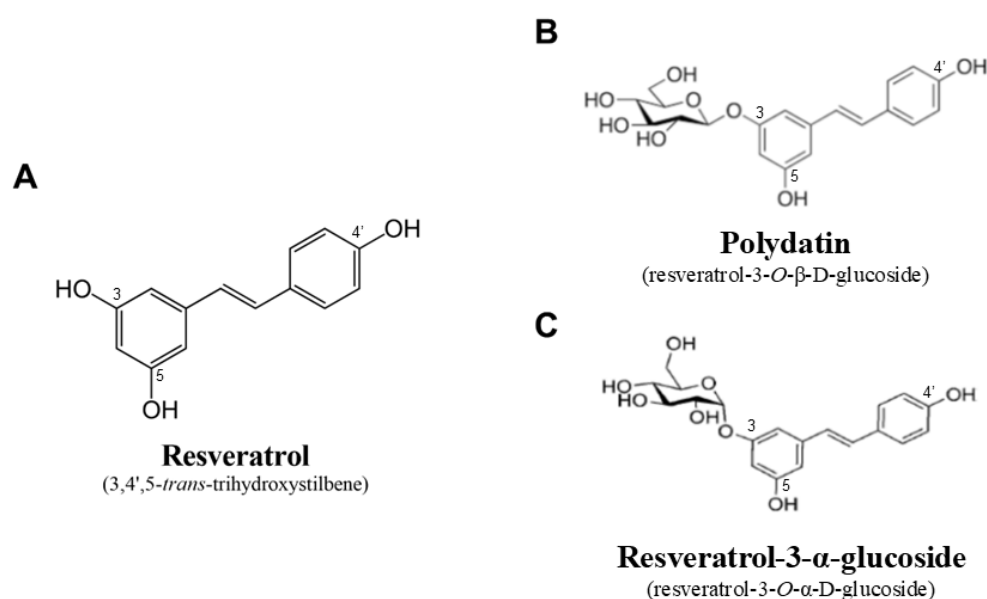


Figure 1. Chemical structure of (A) resveratrol, (B) polydatin, and (C) resveratrol-3- α -glucoside.

In silico approaches have become powerful tools in drug discovery and development processes, offering several advantages by increasing cost effectiveness, reducing time investment, and minimizing animal testing, especially on initial analyses²⁴. Network pharmacology tools provide innovative insights into drug candidate identification, operating in opposite to the traditional “one drug, one target” models and allowing a more comprehensive approach based on modulation of multiple targets in disease progression and potential therapeutic effects²⁵. Using computational analyses, we assessed the impact of resveratrol glycosylation and the glycosidic bond type on the pharmacological interactions of the glycosylated derivatives polydatin and resveratrol-3- α -glucoside with key molecular targets associated with PD, comparing their binding affinities and potential neuroprotective effects.

2. Materials and methods

2.1. Prediction of pharmacokinetic and pharmacodynamic profiles

The absorption, distribution, metabolism and excretion (ADME) and toxicity parameters of resveratrol, polydatin, and resveratrol-3- α -glucoside were predicted using the free online platforms ADMETLab 3.0 (<https://admetlab3.scbdd.com/>) and ProTox 3.0 (<https://tox.charite.de/>). Potential molecular targets of the three compounds were identified through searches on the SwissTargetPrediction (<http://www.swisstargetprediction.ch/>) and Comparative Toxicogenomics Database (CTD) (<https://ctdbase.org/>). Results were acquired by importing the SMILES (Simplified molecular-input line-entry system) code of the compounds into the platforms. The resulting three target datasets were merged, and duplicate entries were removed.

2.2. Protein-protein interaction (PPI) network

Targets related to PD were retrieved from The Human Gene Database (GeneCards) (<https://www.genecards.org/>) using the keyword “Parkinson’s disease”. The common predicted targets of resveratrol, polydatin and resveratrol-3- α -glucoside were compared with the PD-related targets and the final cluster of genes was then imported into the STRING platform (<https://string-db.org/>) to construct a PPI network. The screening analysis conditions were set to *Homo sapiens* as the organism, confidence score of 0.4, and all active interaction sources selected. The resulting data from STRING were imported into Cytoscape 3.8.2 software where the PPI network was built and analyzed using the “Network Analyzer” tool. The network diagram illustrating the interactions between the three polyphenols candidates and PD was obtained, with the color intensity of the nodes representing degree values, while the edge thickness indicated combined score values. Degree value refers to the number of direct connections a node has with other nodes in the network, the higher the degree value, the more essential the protein is for the biological interaction. Betweenness centrality is related to the frequency with which node appears on the shortest connection between two other nodes. Elevated betweenness centrality value indicates a protein that plays a key role in facilitating network connections. Closeness centrality measures the distance of a node to all the other nodes in the network, calculating the average of the shortest distance to each of them. This distance can be directly related to the speed of signal transmission between nodes²⁶.

2.3. Gene ontology enrichment and KEGG pathways analyses

To identify the biological functions, molecular interactions and biochemical pathways of the potential targets of resveratrol and its glycosylated forms in relation to

the pathology of PD, gene ontology (GO) enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways analyses were conducted using DAVID database (<https://davidbioinformatics.nih.gov/>). Species were set as *Homo sapiens* and the cutoff value set as $p \leq 0.05$ ²⁷. Through GO analysis it is possible to identify the biological process (BP), cell component (CC), and molecular function (MF) of genes involved in the network. KEGG pathway analysis focuses on identifying the complex networks of molecular interactions and biochemical processes, connecting genes and proteins within the context of a biological system as the disease development and progression^{28,29}. By analyzing these pathways, insights can show how specific proteins or gene products contribute to the development of pathology and identify key molecular targets and signaling networks that may be modulated with a view to therapeutic interventions. The top 15 genes of GO and the top 20 pathways of KEGG analyses with significant p value were selected to draw the diagrams using SRplot platform (<https://www.bioinformatics.com.cn/en>).

2.4. Molecular docking

The main objective of the molecular docking analysis was to investigate the underlying mechanisms and interactions between resveratrol, its glycosylated derivatives, and the key PD targets identified through PPI network analysis. The 3D structures of resveratrol and polydatin were downloaded from PubChem (<https://pubchem.ncbi.nlm.nih.gov/>), while the structure of resveratrol-3- α -glucoside was manually drawn using Maestro-GUI software by Schrödinger, Inc. (<https://www.schrodinger.com/platform/products/maestro/>). Receptor structures were selected from Protein Data Bank (PDB) (<http://www.rcsb.org/pdb/>) and downloaded in PDB format. Ligand and receptor files were prepared using UCSF Chimera v1.18 software (<https://www.cgl.ucsf.edu/chimera/>). Standard processing steps included water removal, structural minimization, addition of hydrogen atoms, and assignment of Gasteiger charges. For cases where protein structures required corrections (e.g.: missing or broken chain regions), Chimera's loop modeling and structure refinement tools were applied. Docking parameters were defined as follows: 1) the centroid of each co-crystallized ligand was used to define the grid center coordinates (x/y/z), in order to define the location of the receptor's active site; 2) grid box size was set as 20x20x20 Å³ (x/y/z) to ensure complete ligand coverage; 3) advanced docking parameters included number of binding modes set as 9, exhaustiveness level of 8, and a maximum energy difference of 3

kcal/mol. Grid box coordinates and dimensions can be found in Table S1 in the supplementary material. Molecular docking simulations were performed using executable file of AutoDock Vina v1.2.x (<https://vina.scripps.edu/>) integrated into Chimera software. For docking validation, each receptor was re-docked with its original co-crystallized ligand. Results were expressed as binding affinity (kcal/mol). The resulting PDBQT files were converted to PDB format using PyMOL (<https://www.pymol.org/>), and receptor-ligand complexes from the first poses were uploaded to Discovery Studio (BIOVIA) (<https://www.3ds.com/products/biovia/discovery-studio/visualization>) for visualization and interactions analyses.

3. Results

3.1. Predicted physicochemical, pharmacokinetic, and toxicological profiles of resveratrol and its glycosylated derivatives

The predicted physicochemical and ADME properties of resveratrol, polydatin and resveratrol-3- α -glucoside are summarized in Table 1. All three molecules exhibit high gastrointestinal absorption. According to the ADMETLab platform, none of the compounds act as P-glycoprotein (P-gp) substrates or inhibitors. Additionally, ADMETLab predicts that resveratrol has a probability of over 70% of achieving at least 30% oral bioavailability. In contrast, the glycosylated derivatives exhibit a high probability (>70%) of having bioavailability below 30%, indicating poor oral absorption.

All three compounds show a high probability of plasma proteins binding. The predicted volume of distribution (VD) for resveratrol is 1.262 L/kg, while polydatin and resveratrol-3- α -glucoside display lower values of 0.848 and 0.717 L/kg, respectively. ADMETLab also suggests that resveratrol can cross the blood-brain barrier (BBB). Meanwhile, glycosylated derivatives do not possess the capability to penetrate the CNS.

Resveratrol exhibits a high predicted probability of interacting with specific cytochrome P450 (CYP450) enzymes, particularly as an inhibitor of CYP1A2 and CYP3A4, and CYP2C8 isoforms, and as substrate of CYP2D6. Resveratrol-3- α -glucoside predicted to act exclusively as substrates for CYP2C9 and CYP2D6 isoforms. Polydatin showed no interaction with CYP450 enzymes.

Excretion predictions indicate that resveratrol is cleared more rapidly from the system than polydatin and resveratrol-3- α -glucoside, showing a clearance rate of 9 mL/min/kg, approximately more than the double of glycosylated derivatives clearance rate. Both clearance rate and volume of distribution are critical factors influencing drug

half-life and dosage frequency. Consequently, all three compounds exhibit less than 3 hours of half-life, considered low. Notably, resveratrol seems to have the shortest predicted half-life, around 1.5 hours, reflecting its rapid clearance from the bloodstream.

Resveratrol seems to be less toxic when compared to its glycosylated forms, exhibiting a predicted LD₅₀ of 1560 mg/kg against 1380 mg/kg of both polydatin and resveratrol-3- α -glucoside (prediction accuracy of 68.07%). These values indicate that all three compounds have been predicted with toxicity classification 4 according to the Globally Harmonized System of Classification of Labelling of Chemicals (GHS), with potential to cause acute oral toxicity. Although resveratrol showed to be safer than its glycosylated forms, the aglycone demonstrates high probability of causing toxicity to androgen and estrogen nuclear receptor signaling pathways, as well as to cause disturbance of mitochondrial membrane potential and DNA replication. Glycosylated derivatives exhibited a probability of 73% to be nephrotoxic, in addition to showing potential to cause immunotoxicity and chemical induced BBB toxicity.

Table 1. Summary of resveratrol, polydatin, and resveratrol-3- α -glucoside physicochemical and predicted ADMET properties.

	Resveratrol	Polydatin	RESV α -glucoside
Physicochemical properties			
Formula	C ₁₄ H ₁₂ O ₃	C ₂₀ H ₂₂ O ₈	C ₂₀ H ₂₂ O ₈
Molecular weight	228.24 g/mol	390.38 g/mol	390.38 g/mol
Number H-bond acceptors	3	8	8
Number H-bond donors	3	6	6
TPSA	60.69 Å ²	139.84 Å ²	139.84 Å ²
Lipophilicity			
LogP	2.895	1.479	0.937
Water Solubility			
LogS	-3.607	-3.313	-3.342
Class	Moderately soluble	Soluble	Soluble
Pharmacokinetics			
Absorption			
GI absorption	High	High	High
P-gp substrate	No	No	No
Bioavailability (F30%)*	>70%	<70%	<70%
Distribution			
PP binding	Yes (88.6%)	Yes (83.7%)	Yes (82.7%)
VD	1.262 L/kg	0.848 L/kg	0.717 L/kg
BBB permeant	Yes	No	No
Metabolism			
CYP1A2 inhibitor	Yes	No	No
CYP1A2 substrate	No	No	No
CYP2C19 inhibitor	No	No	No

CYP2C19 substrate	No	No	No
CYP2C9 inhibitor	No	No	No
CYP2C9 substrate	No	No	Yes
CYP2D6 inhibitor	No	No	No
CYP2D6 substrate	Yes	No	Yes
CYP3A4 inhibitor	Yes	No	No
CYP3A4 substrate	No	No	No
Excretion			
Clearance rate	9 mL/min/kg	2.7 mL/min/kg	3.5 mL/min/kg
Half-life ($T_{1/2}$)	~1.5 hours	~3.1 hours	~2.8 hours
Toxicity			
LD ₅₀	1560 mg/kg	1380 mg/kg	1380 mg/kg
Organ toxicity	No	Yes (Kidneys)	Yes (Kidneys)
Toxicity endpoints	No	Yes (Immunotoxicity; BBB-toxicity)	Yes (Immunotoxicity; BBB-toxicity)
NR signaling pathways toxicity	Yes (Androgen and estrogen receptors)	No	No
Stress response pathways toxicity	Yes (MMP and ATAD5)	No	No
Molecular events toxicity	No	No	No
Druglikeness			
Lipinski	Accepted; 0 violation	Accepted; 1 violation: NHorOH>5	Accepted; 1 violation: NHorOH>5

*Probability of showing at least 30% of bioavailability (F30%). BBB: blood-brain barrier; MMP: mitochondrial membrane potential; NR: nuclear receptor; ATAD5: ATPase family AAA domain containing protein 5; NHorOH: Number H-bond donors.

3.2. Target identification and functional enrichment analysis of resveratrol and its glycosylated derivatives in PD

A total of 7250 resveratrol targets and 69 polydatin targets were obtained from the CTD database, while SwissTargetPrediction identified 100 targets for all three compounds, resveratrol, polydatin, and resveratrol-3- α -glucoside. No targets for resveratrol-3- α -glucoside were found in the CTD database. After removing duplicates, the final dataset consisted of 7275 resveratrol targets, 163 polydatin targets, and 100 resveratrol-3- α -glucoside, resulting in 67 common targets shared by all three molecules (Fig. 2A). Additionally, a total of 4870 PD-related targets were collected from the GeneCards database. Both final target sets were intersected, revealing 51 potential common targets of resveratrol and its glycosylated derivatives in the context of PD (Fig. 2B), including catalytic proteins, signaling molecules, and transporters (Fig. 2C).

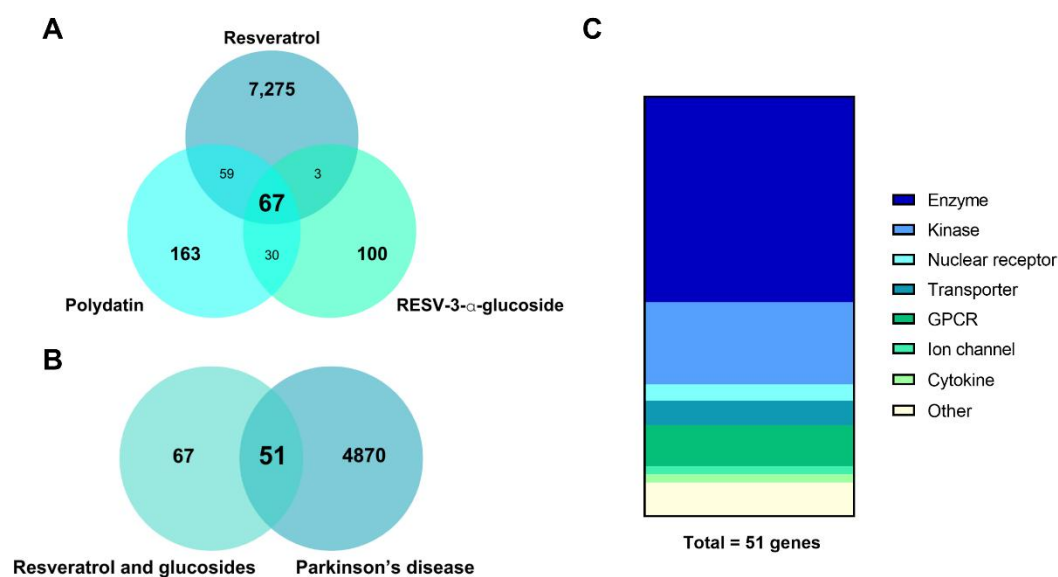


Figure 2. Target identification and intersection analysis of resveratrol, polydatin, and resveratrol-3- α -glucoside in the context of PD. (A) Venn diagram showing the number of predicted targets for each compound, obtained from CTD and SwissTargetPrediction databases. (B) Intersection between compound targets and PD-related targets retrieved from the GeneCards database, identifying 51 common targets. (C) Classification of the identified common targets based on their molecular functions, including catalytic proteins, signaling molecules, and transporters.

GO enrichment analysis identified a total of 186 biological functions and cellular roles significantly associated ($p \leq 0.05$) with the 51 genes set of resveratrol, polydatin, and resveratrol-3- α -glucoside in PD. Among these, 127 biological processes, 27 cellular components, and 32 molecular functions were annotated. Biological processes included those related to apoptosis and cell survival, immunity and inflammation, endocrine functions, cell signaling, development and cell differentiation, metabolic and catabolic processes, cardiovascular and vascular functions, transcriptional and post-transcriptional regulation, as well as responses to environmental and toxic stimuli. Cellular components involved in the set gene of targets included membrane structures, intracellular organelles, extracellular structures, protein complexes, and vesicles. Molecular functions prevalent in the dataset included enzymatic activities of kinases and phosphorylation, peptidases and proteolysis, and oxidoreductases, as well as their regulation and interactions with structural proteins. Functions such as glucose transport, metal ion and nucleotide binding, and cellular interaction and signaling were also identified. To provide deeper insight into the GO enrichment of these 51 target genes, the 15 most significant entries for each component, based on p-values, are present in Fig. 3A.

KEGG pathway enrichment analysis revealed a list of 113 signaling pathways. Of these, 53 pathways were significantly enriched from the initial set of 51 target genes ($p \leq$

1 0.05). The top 20 pathways with the most significant p-values were plotted in a bubble
 2 chart (Fig. 3B).

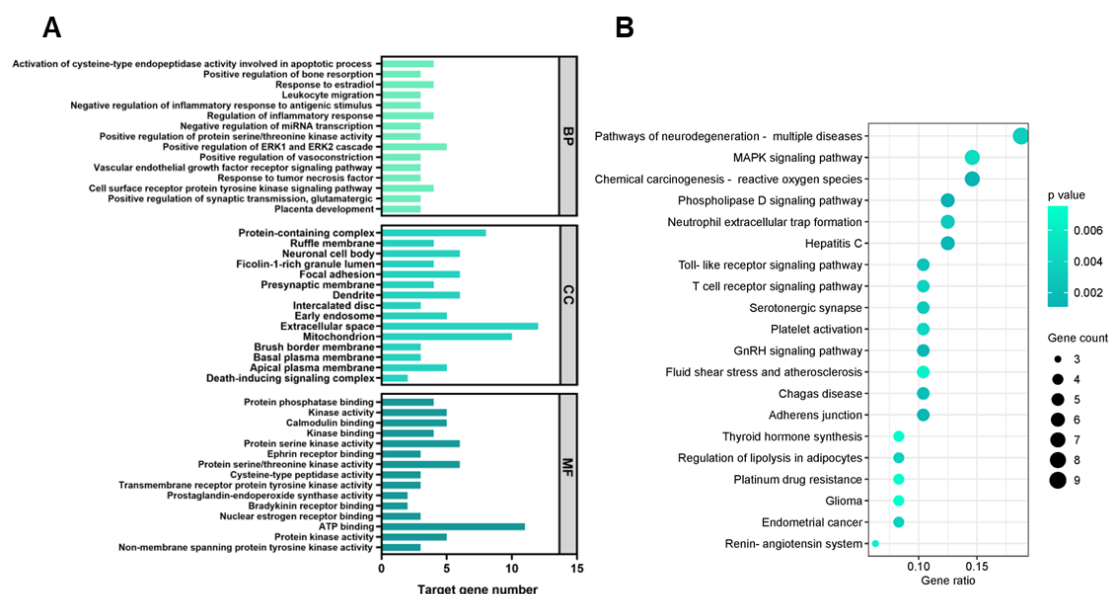


Figure 3. GO and KEGG pathway enrichment analyses of resveratrol, polydatin, and resveratrol-3- α -glucoside targets in PD. (A) GO enrichment analysis highlighting the 15 most significant biological processes, cellular components, and molecular functions associated with the 51 common target genes. (B) KEGG pathway enrichment analysis displaying the top 20 most significantly enriched pathways, providing insights into the molecular mechanisms through which resveratrol, polydatin, and resveratrol-3- α -glucoside may exert their effects in PD.

3.3. PPI network and key target identification

When the list of common targets was uploaded into the STRING platform, a PPI network was generated, consisting of 50 nodes and 326 edges, representing the proteins and their predicted functional interactions, respectively. The blue intensity of the node reflects the degree value, with nodes at the center indicating higher degree values. The edge thickness corresponds to the combined score, indicating the predicted confidence level of the interactions (Fig. 4). No direct connections were identified for SLC28A2 and DYRK1A genes, which were positioned at the left extremity of the network. Topological analysis of the PPI network revealed an average node degree of 13. By combining the average degree (≥ 13), betweenness centrality (≥ 0.03), and closeness centrality values (≥ 0.4), the 50 targets were screened for key interactions. Eleven targets were identified as potential key targets that may play a role in functions related to the molecular mechanisms of resveratrol and its glycosylated derivatives in PD (Table 2).

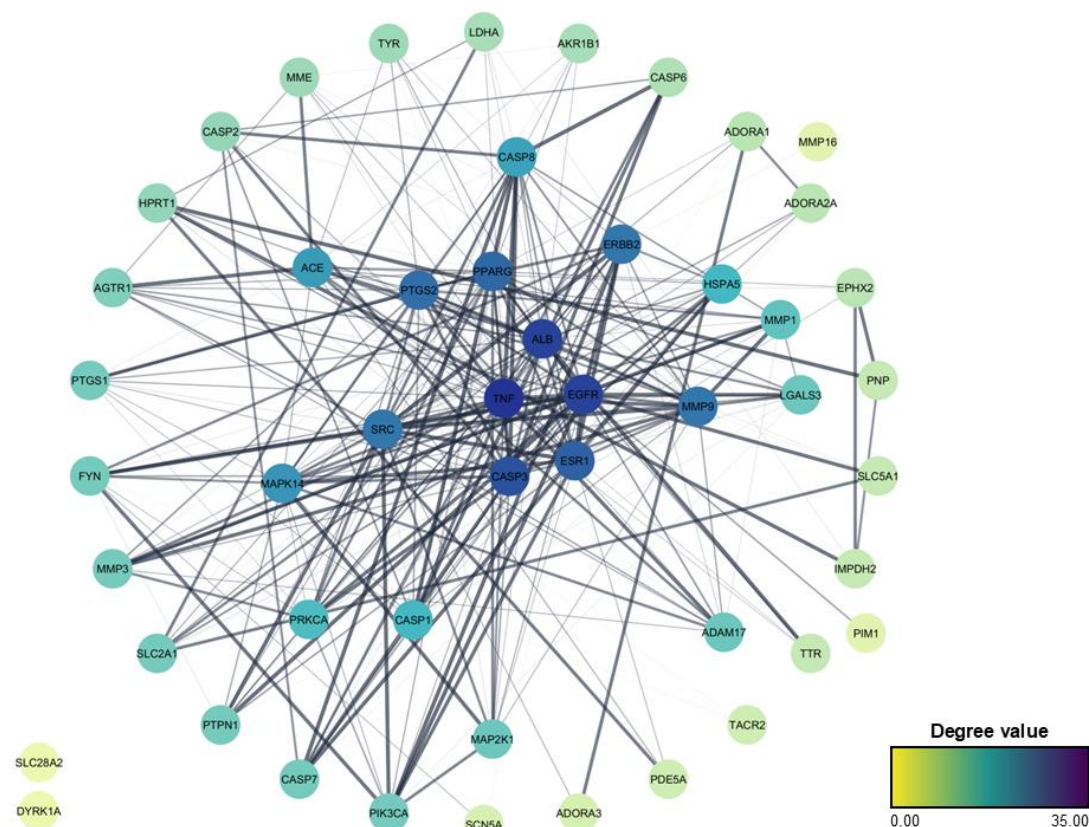


Figure 4. PPI network of resveratrol, polydatin, and resveratrol-3- α -glucoside targets in PD. The network includes 50 proteins and 326 functional interactions, with node color intensity indicating connectivity and edge thickness representing interaction confidence. SLC28A2 and DYRK1A showed no direct connections.

Table 2. Protein-protein interaction network results with the potential key targets of resveratrol, polydatin, and resveratrol-3- α -glucoside in Parkinson's disease.

Gene	Degree value	Betweenness centrality	Closeness centrality
TNF	35	0.092	0.797
EGFR	33	0.110	0.770
ALB	33	0.069	0.770
CASP3	31	0.054	0.746
ESR1	29	0.096	0.723
PPARG	28	0.051	0.712
PTGS2	27	0.061	0.701
ERBB2	26	0.062	0.681
SRC	26	0.063	0.681
ACE	21	0.033	0.627
MAP2K1	13	3.802	0.573

*Screening parameters: degree value (≥ 13); betweenness centrality (≥ 0.03); closeness centrality (≥ 0.4).

3.4. Molecular docking analysis of resveratrol and glycosylated derivatives with PD-related targets

Molecular docking analysis plays an important role in the drug discovery process by predicting the interactions between small molecules (ligands) and target proteins associated with diseases. This computational technique elucidates binding affinity, molecular mechanisms, and potential therapeutic targets.

To investigate if glycosylation influences the affinity and binding characteristics of resveratrol, polydatin and resveratrol-3- α -glucoside with target proteins involved in PD, molecular docking simulations were performed. Binding affinity between resveratrol and its glycosylated derivatives and the eleven core target proteins identified through PPI network analysis are summarized in Figure 5 and Table S2. The lower the docking score (more negative), the stronger the affinity between the ligand molecule and the target protein. The compounds investigated showed good affinity for tumor necrosis factor- α (TNF- α), peroxisome proliferator-activated receptor gamma (PPAR γ), and human epidermal growth factor receptor 2 (ERBB2) receptors, with docking scores ranging from -9.1 to -9.6 kcal/mol. Detailed diagrams of ligand-receptor interactions for these targets are provided. Further details of the other targets are available in the supplementary material (Figures S1-8). Hydrogen bonds and hydrophobic interactions were the main observed interaction types. Glycosylated forms showed higher values of docking score compared to the aglycone form of resveratrol. Polydatin demonstrated the best binding activity for most targets, suggesting potentially superior performance compared to resveratrol and resveratrol-3- α -glucoside.

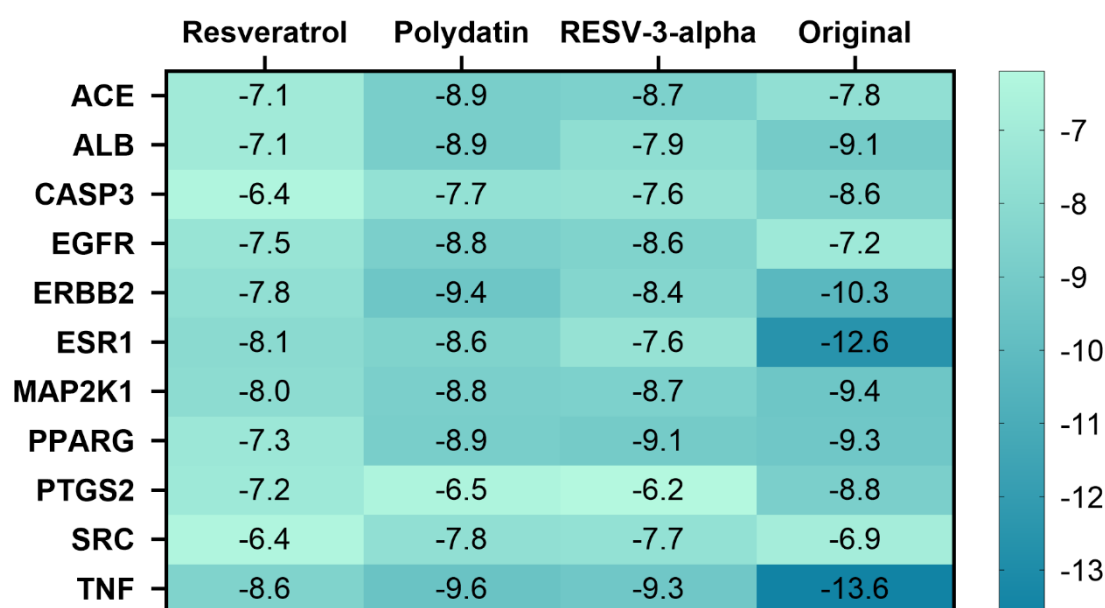


Figure 5. Binding energies of resveratrol, polydatin, and resveratrol-3- α -glucoside with the 11 core target proteins identified in the PPI network analysis. The lower binding energy values, stronger are the molecular interactions.

Figure 6 shows the binding interactions between resveratrol, polydatin and resveratrol-3- α -glucoside in the active site of TNF- α receptor, with docking scores of -8.6, -9.6, and -9.3 kcal/mol, respectively. Resveratrol and resveratrol-3- α -glucoside both

form conventional hydrogen bonds with LeuA233 and LeuC112 residues. In general, resveratrol forms three hydrogen bonds (LeuA233, LeuC112, SerC52) (Fig. 6B), while polydatin presents two (AlaA232, LeuB52) (Fig. 6C) and resveratrol-3- α -glucoside forms four (LeuA233, TyrA195, TyrB114, LeuC112) (Fig. 6D). Resveratrol-3- α -glucoside also showed a sterically unfavorable interaction with SerC52 residue, which may impact its binding affinity to TNF- α receptor (Fig. 6D). Additionally, all three molecules exhibit hydrophobic interactions in various amino acid residues in TNF- α active site, including Pi-Alkyl, Pi-Sigma, and Pi-Pi Stacked interactions that often enhance the stability of ligand-receptor complexes (Fig. 7).

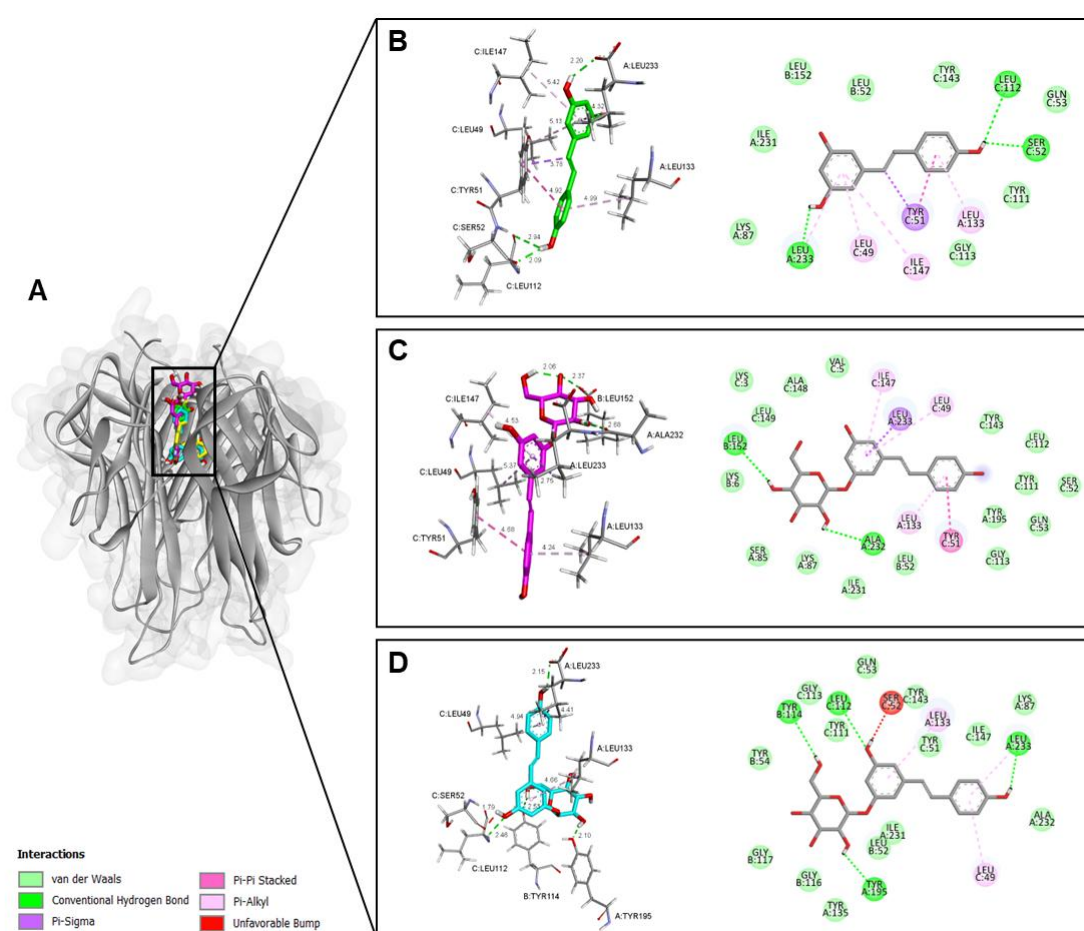


Figure 6. (A) Binding interactions of resveratrol, polydatin, and resveratrol-3- α -glucoside within the TNF- α receptor active site. Resveratrol (B), polydatin (C), and resveratrol-3- α -glucoside (D) molecular interactions.

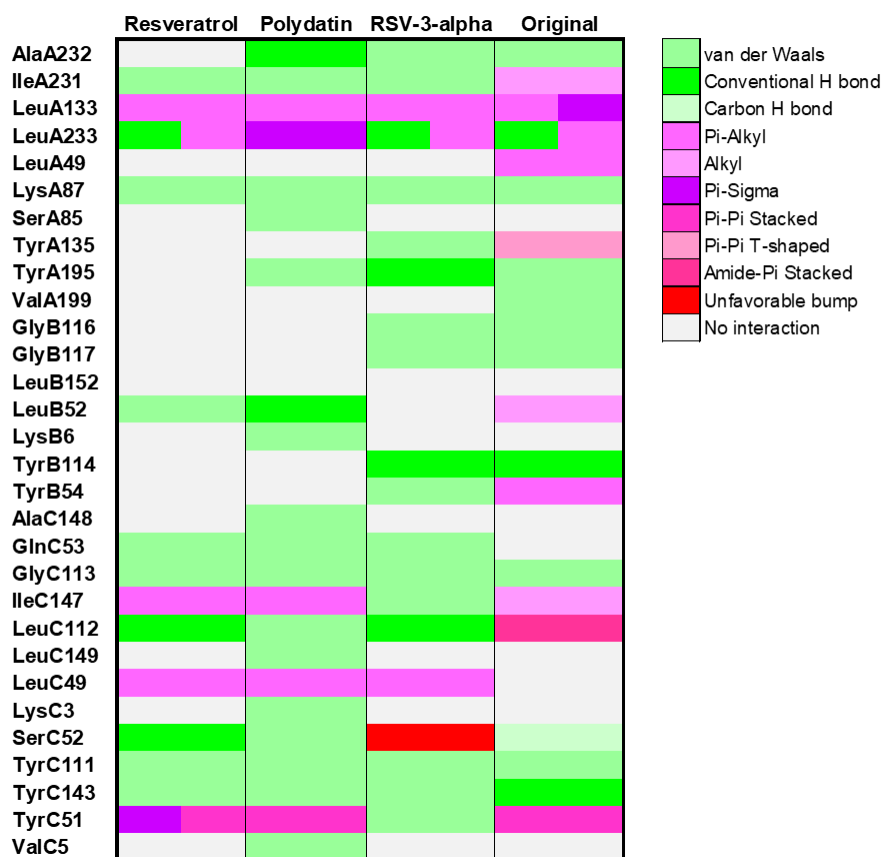


Figure 7. Heatmap illustrating the interactions between TNF- α protein residues and resveratrol, polydatin, and resveratrol-3- α -glucoside. The color gradient represents different types of interactions, as detailed in the right panel.

Binding interactions of all three compounds and PPAR γ target (Fig. 8A) revealed the formation of hydrogen bonds, notably polydatin showed three hydrogen interactions (Fig. 8C). Alternatively, resveratrol and resveratrol-3- α -glucoside both established Pi-Cation interactions with Arg288 residue (Fig. 8B; 8D). Similar hydrophobic contacts near the resveratrol scaffold, including Ile341, Cys285, and Leu330 amino acid residues, were observed for all three compounds (Fig. 9). Out of these compounds, resveratrol-3- α -glucoside presented the best docking score at -9.1 kcal/mol.

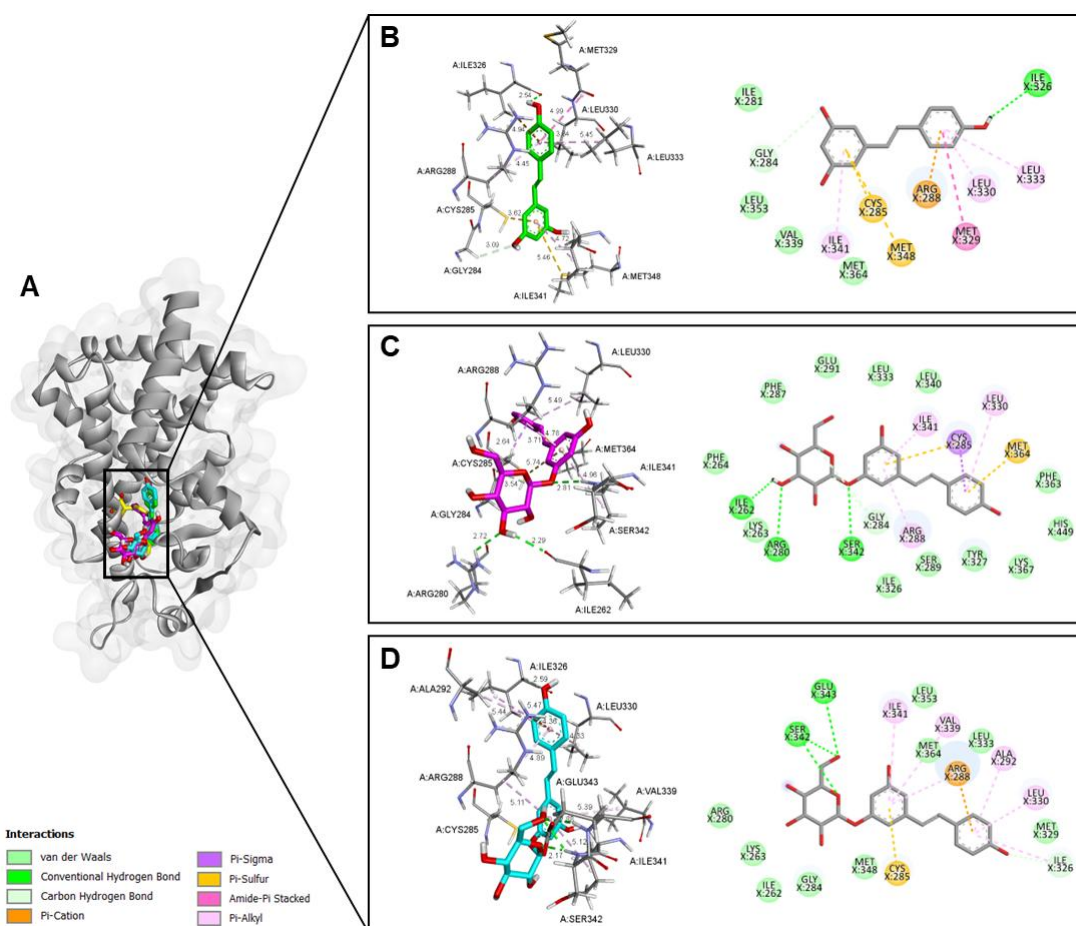


Figure 8. (A) Binding interactions of resveratrol, polydatin, and resveratrol-3- α -glucoside within the PPAR γ receptor active site. Resveratrol (B), polydatin (C), and resveratrol-3- α -glucoside (D) molecular interactions.

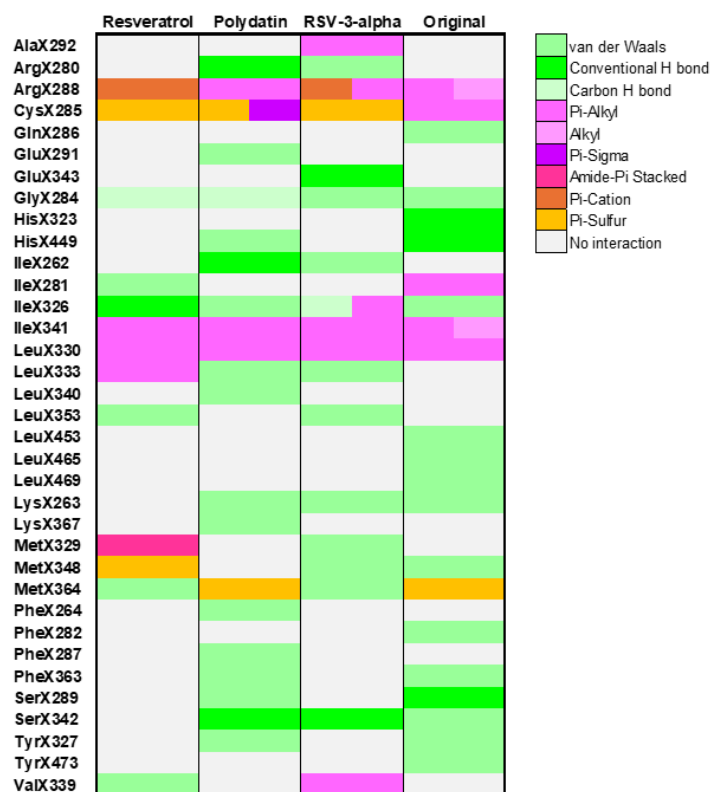


Figure 9. Heatmap illustrating the interactions between PPAR γ protein residues and resveratrol, polydatin, and resveratrol-3- α -glucoside. The color gradient represents different types of interactions, as detailed in the right panel.

Considerable differences were observed in the interactions with ERBB2 receptor (Fig. 10). Polydatin achieved the greatest binding affinity, with a docking score of -9.4 kcal/mol. In contrast, the polydatin arrangement is likely unstable given the acceptor-acceptor interaction involving the Asp154 residue (Fig. 10C). There was also a similar trend in hydrogen bonds count, where only one hydrogen interaction was formed by polydatin, compared with two hydrogen bonds by both resveratrol and resveratrol-3- α -glucoside (Fig. 11).

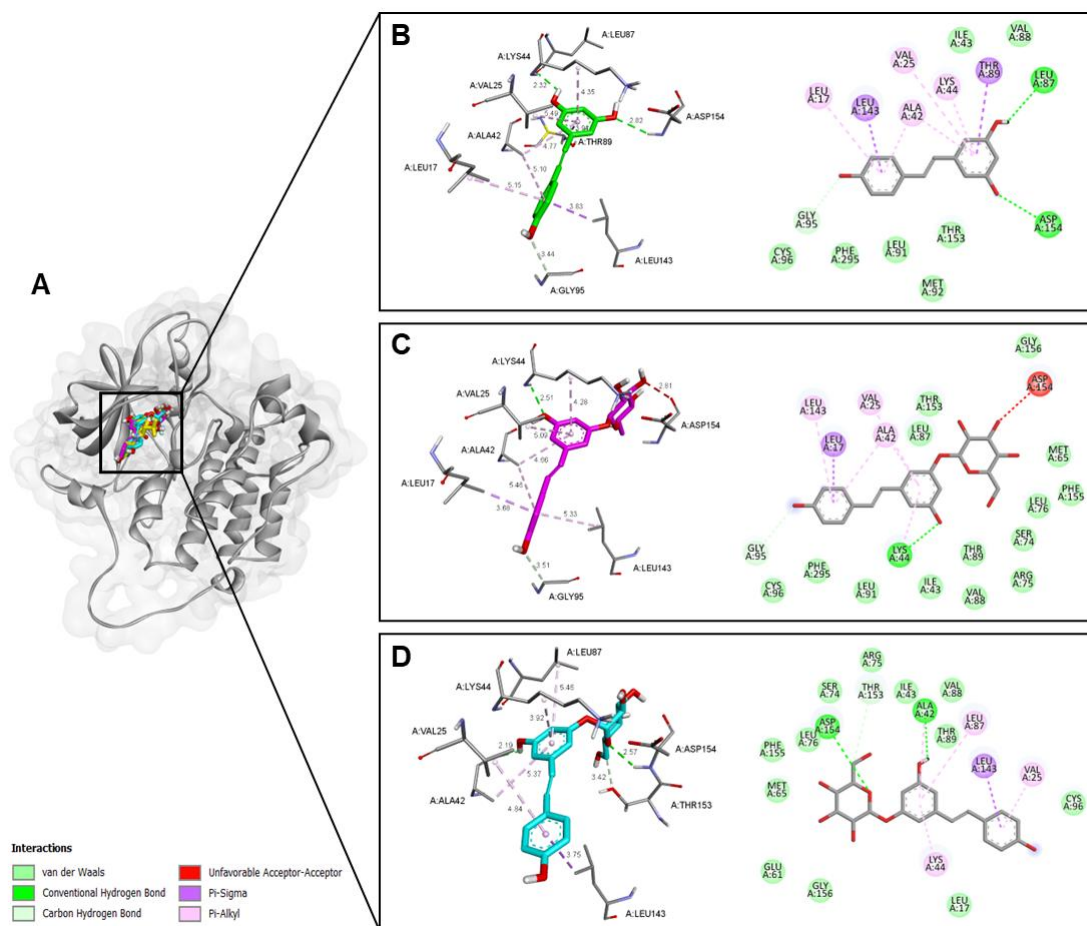


Figure 10. (A) Binding interactions of resveratrol, polydatin, and resveratrol-3- α -glucoside within the ERBB2 receptor active site. Resveratrol (B), polydatin (C), and resveratrol-3- α -glucoside (D) molecular interactions.



Figure 11. Heatmap illustrating the interactions between ERBB2 protein residues and resveratrol, polydatin, and resveratrol-3- α -glucoside. The color gradient represents different types of interactions, as detailed in the right panel.

4. Discussion

Understanding the molecular mechanisms involved in the interaction of bioactive compounds with therapeutic targets is essential for the development of new therapeutic strategies. In this study, we employed *in silico* approaches to investigate the impact of resveratrol glycosylation on its pharmacokinetic properties and molecular interactions with proteins associated with PD. Our findings indicate that the introduction of glycosyl residues can modulate binding affinity and the stability of ligand-receptor complexes, influencing potential neuroprotective effects. Additionally, we explored how the type of glycosidic bond (α or β) may impact these interactions, providing insights into the structural role of glycosylation in the bioactivity of resveratrol derivatives. In the following sections, we discuss the results obtained and their implications in the context of optimizing natural compounds for PD treatment.

Our study found that glycosylation appears to decrease the predicted bioavailability of glycosylated derivatives compared to resveratrol aglycone. Experimental findings have already shown that polydatin exhibits 3-4 times higher bioavailability than resveratrol³⁰. Although *in silico* methods are valuable tools for

1 predicting the properties of compounds, their limitations primarily arise from the
2 dependence on statistical correlations and machine learning, which may not capture the
3 full complexity of *in vivo* absorption and metabolism³¹. This is particularly relevant for
4 compounds with structural modifications, such as glycosylation, which can alter
5 bioavailability in a complex manner. Glycosylation can facilitate absorption through
6 specific transporters, but it can also hinder it by increasing the polarity of the compound
7³². Bioavailability can be also influenced by factors such as the volume of distribution,
8 clearance rate, and half-life of a compound. Resveratrol's high volume of distribution and
9 clearance rate, combined with its shorter half-life compared to glycosylated derivatives
10 may reflect its hydrophobic structure, which facilitates penetration through lipidic cell
11 membranes but makes it more vulnerable to enzymatic metabolism and rapid elimination.
12 Conversely, the glycosidic moiety in polydatin and resveratrol-3- α -glucoside increases
13 their polarity, suggesting that the glycosylated derivatives remain longer in plasma by
14 reducing distribution, which combined with better absorption and lower susceptibility to
15 enzymatic oxidation can contribute to a more stable bioavailability^{19,22,33}.

16 The ability to cross the BBB is a determining factor for potential compound
17 efficacy in the treatment of neurodegenerative diseases, such as PD³⁴. Our *in silico*
18 predictions suggest that polydatin and resveratrol-3- α -glucoside exhibited significant
19 differences in their BBB permeability. While resveratrol, which possesses a hydrophobic
20 structure and low molecular weight, showed to cross the BBB, its glycosylated derivatives
21 tend to be more hydro soluble, which could compromise their passive diffusion.
22 Nevertheless, glucose transporters expressed in brain tissue, such as GLUT1, may
23 mediate the passage of these glycoconjugate compounds across the BBB^{35,36}.

24 Resveratrol showed a higher LD₅₀ compared to glycosylated derivatives, being
25 generally less toxic. Differently, *in vivo* studies have shown that polydatin has lower
26 toxicity compared to the aglycone form^{19,37,38}. *In silico* tools generally are based on
27 computational models and databases of chemical structures, where the toxicity prediction
28 can mainly consider the compound chemical structure and its physicochemical properties,
29 without taking into account the interactions with transporter proteins, metabolism
30 enzymes, and pharmacokinetic factors such as distribution and excretion, as mentioned
31 above^{39,40}. These facts highlight the importance of checking literature, when available,
32 in parallel to prediction *in silico* platforms.

The enzymes of the CYP450 system are responsible for the biotransformation of multiple drugs. Investigating the possible interactions with drug-metabolizing enzymes is crucial during the early stages of drug discovery and development. These isoenzymes can be inhibited or induced by compounds, the main reason for unanticipated adverse effects occurring due to drug-drug interactions ^{41,42}. Our results show that resveratrol can inhibit CYP450 enzymes, which is in line with the literature already published ^{43–45}. Inhibiting CYP450 enzymes can decrease the metabolism of other drugs that depend on the same pathway to be metabolized, increasing this drug concentration and leading to important adverse drug interactions ⁴². Resveratrol and glycosylated derivatives can also act as substrates of CYP2D6 and CYP2C9, respectively. Being metabolized by these CYP450 enzymes can result in individual variations of biological responses when compounds are administered ⁴⁶. When co-administered with an inhibitor (e.g. fluoxetine or valproic acid), resveratrol and glycosylated derivatives can be slowly metabolized, increasing their half-life and bioavailability, which may consequently potentiate their therapeutic effects. However, if resveratrol and its glycosylated forms are co-administered with CYP2D6 and CYP2C9 inducers (e.g. dexamethasone or corticosterone, and phenobarbital or carbamazepine, respectively) they would be metabolized even faster, reducing their therapeutic efficacy ^{47,48}.

To be considered a promising drug candidate, the compound should present some essential pharmacokinetic characteristics such as high absorption rate, adequate distribution among tissues and organs reaching the therapeutic target, controlled metabolism, medium half-life, efficient excretion and good bioavailability values ⁴⁹. Taking into account this aimed pharmacokinetic profile, resveratrol exhibited a promising predicted pharmacokinetic profile compared to its glycosylated derivatives. As mentioned before, resveratrol also shows the ability to cross the BBB, a crucial factor for addressing therapeutic effects on PD. Moreover, resveratrol's relatively low predicted toxicity profile, when compared to its glycosylated derivatives, further enhances its potential as a promising candidate for development into a therapeutic agent for PD.

For a deeper understanding of the molecular mechanisms of resveratrol and its glycosylated derivatives, it is important to investigate their interactions with specific biological targets, especially in the context of pathways related to neuroprotection and neurodegeneration on PD. Resveratrol and glycosylated derivatives neuroprotective activities seem to be related to the modulation of apoptotic processes as well as cell proliferation and survival, as indicated in GO enrichment analysis. The resveratrol

scaffold can act as a phytoestrogen, promoting responses linked to estrogen receptors that can potentialize protective effects of ERK signaling pathway^{50–52}. These processes appear to occur in extracellular space, mitochondrion, and protein-containing complexes, possibly related to molecular functions such as phosphorylation of kinase cascades. KEGG analysis suggests modulation of pathways involved in neurodegeneration – across multiple diseases, damage induced by ROS, and phospholipase D signaling pathway which may play a key role in resveratrol, polydatin, and resveratrol-3- α -glucoside therapeutic actions. These findings align with PD physiopathology pathways already well described in the literature such as the involvement of oxidative stress, neuroinflammation, and mitochondrial dysfunction⁵³.

Through network pharmacology analysis, we identified 11 core proteins that might be key targets of resveratrol, polydatin, and resveratrol-3- α -glucoside in PD. After molecular docking verifications, glycosylation appears to enhance binding affinity compared to the aglycone form of resveratrol, showing higher binding scores in ten of the eleven receptors analyzed. Furthermore, TNF- α , PPAR γ , and ERBB2 showed the best degree of binding with resveratrol and glycosylated derivatives.

TNF- α is a pro-inflammatory cytokine implicated in the pathogenesis and progression of PD. Elevated levels of TNF- α have been found in the serum, cerebrospinal fluid, and brain tissue of Parkinson's disease patients^{54,55}. As a key inflammatory mediator, targeting neuroinflammation through the modulation of TNF- α has emerged as a potential therapeutic alternative for mitigating neurodegeneration⁵⁶. Inhibition of TNF- α by resveratrol and polydatin is already described in the literature^{57–62}. Different experimental studies have investigated anti-inflammatory activity and neuroprotective effects of resveratrol and polydatin in the context of Parkinson's disease through decreasing TNF- α levels and expression^{63–67}.

Our molecular docking analysis results revealed that resveratrol, polydatin and resveratrol-3- α -glucoside exhibit good affinity for TNF- α . Among these compounds, glycosylated derivatives showed the lowest binding energy, suggesting greater complex stability. This increase in stability is possibly attributed to the presence of additional hydroxyl groups in the glucose moiety, which favored additional interactions through the formation of hydrogen bonds and hydrophobic contacts^{68,69}. However, subtle differences in molecular conformation and accessibility to the active site suggest that glycosylation could have an influence on complex stability. Additionally, the type of glycosidic binding (α or β) might significantly impact on the positioning and accommodation of the molecule

1 inside the active site of the receptor. These differences occur due to the spatial orientation
2 of the glucose unit in relation to the resveratrol core. The α -glycosidic bond orients the
3 glucose moiety in the same direction as the resveratrol aromatic ring, creating a more
4 compact molecular profile. Differently, the β -glycosidic bond (as in polydatin) orients the
5 glucose unit in the opposite direction, which might interfere in how the molecule fits in
6 the binding site ⁷⁰. This becomes clear when comparing the opposite orientations of the
7 glycosylated derivatives within the active site, evidencing the hypothesis that molecular
8 orientation is influenced by the stereochemistry of the glycosidic bond.

9 Despite these conformational differences, the glycosylated derivatives binding
10 affinity for TNF- α was not significantly compromised, with both showing higher binding
11 energy values compared to resveratrol. Interactions with LeuA233 and LeuC112 of TNF-
12 α may be important for complex stabilization, as leucine, being an apolar amino acid,
13 promotes hydrophobic interactions and hydrogen bonding, especially with compounds
14 that contain aromatic rings, such as resveratrol and its glycosylated derivatives. Although
15 resveratrol-3- α -glucoside formed favorable interactions with LeuA233 and LeuC112, its
16 binding affinity could be slightly decreased due to the conformational adjustments
17 required to accommodate these interactions as well as the presence of an unfavorable
18 bump with SerC52 residue, located near LeuC112, could impact complex stability and
19 cause a minor decrease in binding affinity of resveratrol-3- α -glucoside compared to
20 polydatin. Polydatin, on the other hand, established more stable hydrophobic interactions,
21 demonstrating a slight energy advantage. Thus, the position and type of interactions with
22 relevant residues seem to influence the complex stability more than just the
23 stereochemistry of glycosylation⁷¹. Resveratrol, being a smaller and more flexible
24 molecule, efficiently interacted with both critical Leu residues, but its overall binding
25 energy might be lower due to its inability to establish additional interactions with other
26 relevant residues.

27 PPAR γ is a ligand-activated transcription factor able to influence the expression
28 and activity of a variety of targets in different signaling networks. It regulates
29 mitochondrial function, immune responses, redox balance, and the metabolism of sugar
30 and lipids ^{72,73}. Resveratrol is a well-known activator of SIRT1, a major pathway with
31 bidirectional activity that can indirectly modulate PPAR γ . SIRT1 acts deacetylating PGC-
32 1 α , a coactivator of PPAR γ , thereby stimulating the transcription of genes related to
33 mitochondrial biogenesis, energy metabolism, and antioxidant defense, including Nrf2
34 (Nuclear factor erythroid 2-related factor 2) ^{74,75}. Additionally, PPAR γ activation can

inhibit the NF- κ B (Nuclear Factor kappa-light-chain-enhancer of activated B cells), a key regulator of pro-inflammatory gene expression. Its inhibition reduces the production of inflammatory mediators such as TNF- α , interleukin 6 (IL-6), and cyclooxygenase-2 (COX-2), and decreases microglia activation, containing neuroinflammation⁷⁶. Consequently, targeting PPAR γ could be a promising therapeutic strategy for PD, as its activation may modulate important pathways involved in mitochondrial function, antioxidant defense, and neuroinflammation, potentially protecting dopaminergic neurons from degeneration.

Resveratrol-3- α -glucoside exhibited the best binding affinity for the PPAR γ receptor (-9.1 kcal/mol), followed by polydatin (-8.9 kcal/mol) and resveratrol (-7.3 kcal/mol). As observed in molecular docking results of the TNF- α target, glycosylation may also contribute to better accommodation of glycosylated derivatives in the active site of PPAR γ receptors. Additional hydroxyl groups in the glucose moiety facilitate the formation of hydrogen bond interactions, which stabilize the complex. All three ligands were positioned near the β -sheet, a well-described binding region for PPAR γ partial agonists^{77,78}. However, only the glycosylated derivatives were able to form H-bond with Ser342 critical residue. Hydrophobic contacts were observed for all three compounds, especially due to the presence of aromatic rings of the resveratrol scaffold. Among them, we can highlight interactions with Cys285, a key residue in the PPAR γ active site⁷⁹. Resveratrol and resveratrol-3- α -glucoside formed Pi-Sulfur interactions with the aromatic ring A, while polydatin exhibited similar interactions with Cys285 residue, establishing a Pi-Sulfur interaction in ring A and an additional Pi-Sigma interaction in ring B. These non-covalent interactions contribute to a more flexible and dynamic ligand attachment, allowing reversible binding and dissociation. Resveratrol and resveratrol-3- α -glucoside also formed a Pi-Cation interaction with Arg288. This type of contact may enhance ligand-receptor affinity by combining electrostatic forces and stabilizing interactions within the active site⁸⁰.

Consistent with these findings, glycosylated derivatives exhibited stronger binding affinities for ERBB2, with polydatin showing the highest score of -9.4 kcal/mol, followed by resveratrol-3- α -glucoside (-8.4 kcal/mol) and aglycone resveratrol (-7.8 kcal/mol). Both resveratrol and resveratrol-3- α -glucoside formed H-bonds with Asp154. Meanwhile, an unfavorable acceptor-acceptor interaction was observed between polydatin and Asp154 residue. Since both have hydrogen-acceptors groups, without an adequate donor to stabilize the interaction, this probably resulted in repulsion and an

1 unfavorable conformational adjustment ⁸¹. Nevertheless, polydatin established multiple
2 hydrophobic contacts, van der Waals and carbon-based interactions, which enhance
3 complex stabilization, implicating in the highest binding energy among the three
4 compounds.

5 The involvement of the ERBB2 receptor in PD pathogenesis is still largely
6 unexplored. PD patient's brains have shown decreased levels of ERBB in postmortem
7 analysis ⁸². ERBB2 primary functions in amplifying signals are mainly linked to MAPK
8 and PI3K-Akt pathways, which modulate cell proliferation, differentiation, and survival,
9 especially in glial cells. PI3K/Akt pathway activation can promote neural survival and
10 decrease dopaminergic neurons apoptosis ⁸³. Contrarily, inhibition of MAPK-related
11 protein phosphorylation, especially p38 and JNK, has been associated with anti-apoptotic
12 and anti-inflammatory effects ⁸⁴. Experimental research indicates that resveratrol could
13 exert neuroprotective effects by activating the PI3K/Akt pathway, thereby reducing
14 neuronal death ⁸⁵⁻⁸⁷. Moreover, inhibition of the MAPK pathway by resveratrol has been
15 shown to increase the neuronal survival in rodent models of neurological diseases ⁸⁸.

16 Our results suggest that glycosylation plays a key role in modulating the binding
17 affinity and interaction profiles of resveratrol glycosylated derivatives with proteins
18 involved in PD pathogenesis. The increased binding stability exhibited by polydatin and
19 resveratrol-3- α -glucoside with the multiple target proteins analyzed and particularly with
20 TNF- α and PPAR γ , supports the hypothesis that additional hydrogen groups introduced
21 through the glycosylation enhance hydrogen bonding and hydrophobic interactions.
22 Moreover, glycosylation not only improves molecular interactions but also seems to
23 enhance stability and selectivity in ligand-receptor binding. Still, structural factors such
24 as the glycosidic bond type and its steric effects appear to influence the ligand orientation
25 and accessibility to active sites, potentially influencing receptor affinity. Polydatin
26 exhibited the highest affinity for ERBB2 protein, a receptor with emerging implications
27 in neurodegenerative processes whose activation shows complex functional roles. These
28 results provide understanding of the molecular determinants underlying the mechanisms
29 that may be involved in the bioactivity of resveratrol and its glycosylated derivatives, yet
30 experimental validation is necessary to fully elucidate their pharmacological potential in
31 PD.

32 5. Conclusion

Considering these findings collectively, our data highlight the potential of glycosylated resveratrol derivatives as promising candidates for targeting key molecular pathways associated with Parkinson's disease pathogenesis. The increased binding affinities observed for polydatin and resveratrol-3- α -glucoside suggest that structural optimization via glycosylation may improve molecular stability and interaction profiles with multiple PD targets, especially with TNF- α , PPARG, and ERBB2. These interactions align with already established neuroprotective mechanisms, including antioxidant, anti-inflammatory, and anti-apoptotic effects. Despite these promising *in silico* results, further experimental studies are required to complete preclinical drug verification, assessing their pharmacokinetic properties, bioavailability, and therapeutic potential of these compounds in relevant biological models. Future studies should investigate whether these compounds can cross the BBB, their metabolic stability, and their efficacy in preclinical models of neurodegeneration. Understanding these aspects will be essential for translating these findings into clinical therapeutic applications for glycosylated resveratrol derivatives in PD as well as other neurodegenerative disorders.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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1 **References**

- 2 (1) Bloem, B. R.; Okun, M. S.; Klein, C. Parkinson's Disease. *The Lancet* 2021, 397
- 3 (10291), 2284–2303. [https://doi.org/10.1016/S0140-6736\(21\)00218-X](https://doi.org/10.1016/S0140-6736(21)00218-X).
- 4 (2) Kalia, L. V.; Lang, A. E. Parkinson's Disease. *The Lancet* 2015, 386 (9996), 896–
- 5 912. [https://doi.org/10.1016/S0140-6736\(14\)61393-3](https://doi.org/10.1016/S0140-6736(14)61393-3).
- 6 (3) Luo, Y.; Qiao, L.; Li, M.; Wen, X.; Zhang, W.; Li, X. Global, Regional, National
- 7 Epidemiology and Trends of Parkinson's Disease from 1990 to 2021: Findings from the
- 8 Global Burden of Disease Study 2021. *Front Aging Neurosci* 2025, 16, 1498756.
- 9 <https://doi.org/10.3389/fnagi.2024.1498756>.
- 10 (4) Ferrari, A. J.; Santomauro, D. F.; Aali, A.; Abate, Y. H.; Abbafati, C.; Abbastabar,
- 11 H.; Abd ElHafeez, S.; Abdelmasseh, M.; Abd-Elsalam, S.; Abdollahi, A.; Abdullahi, A.;
- 12 Abegaz, K. H.; Abeldaño Zuñiga, R. A.; Aboagye, R. G.; Abolhassani, H.; Abreu, L. G.;
- 13 Abualruz, H.; Abu-Gharbieh, E.; Abu-Rmeileh, N. M.; Ackerman, I. N.; Addo, I. Y.;
- 14 Addolorato, G.; Adebisi, A. O.; Adepoju, A. V.; Adewuyi, H. O.; Afyouni, S.; Afzal, S.;
- 15 Afzal, S.; Agodi, A.; Ahmad, A.; Ahmad, D.; Ahmad, F.; Ahmad, S.; Ahmed, A.; Ahmed,
- 16 L. A.; Ahmed, M. B.; Ajami, M.; Akinosoglou, K.; Akkaif, M. A.; Al Hasan, S. M.;
- 17 Alalalmeh, S. O.; Al-Aly, Z.; Albashtawy, M.; Aldridge, R. W.; Alemu, M. D.; Alemu, Y.
- 18 M.; Alene, K. A.; Al-Gheethi, A. A. S.; Alharrasi, M.; Alhassan, R. K.; Ali, M. U.; Ali, R.;
- 19 Ali, S. S. S.; Alif, S. M.; Aljunid, S. M.; Al-Marwani, S.; Almazan, J. U.; Alomari, M. A.;
- 20 Al-Omari, B.; Altaany, Z.; Alvis-Guzman, N.; Alvis-Zakzuk, N. J.; Alwafi, H.; Al-Wardat,
- 21 M. S.; Al-Worafi, Y. M.; Aly, S.; Alzoubi, K. H.; Amare, A. T.; Amegbor, P. M.; Ameyaw,
- 22 E. K.; Amin, T. T.; Amindarolzarbi, A.; Amiri, S.; Amugsi, D. A.; Ancuceanu, R.;
- 23 Anderlini, D.; Anderson, D. B.; Andrade, P. P.; Andrei, C. L.; Ansari, H.; Antony, C. M.;
- 24 Anwar, S.; Anwar, S. L.; Anwer, R.; Anyanwu, P. E.; Arab, J. P.; Arabloo, J.; Arafat, M.;
- 25 Araki, D. T.; Aravkin, A. Y.; Arkew, M.; Armocida, B.; Arndt, M. B.; Arooj, M.;
- 26 Artamonov, A. A.; Aruleba, R. T.; Arumugam, A.; Ashbaugh, C.; Ashemo, M. Y.; Ashraf,
- 27 M.; Asika, M. O.; Askari, E.; Astell-Burt, T.; Athari, S. S.; Atorkey, P.; Atout, M. M. W.;
- 28 Atreya, A.; Aujayeb, A.; Ausloos, M.; Avan, A.; Awotidebe, A. W.; Awuviry-Newton, K.;
- 29 Ayala Quintanilla, B. P.; Ayuso-Mateos, J. L.; Azadnajafabad, S.; Azevedo, R. M. S.;
- 30 Babu, A. S.; Badar, M.; Badiye, A. D.; Baghdadi, S.; Bagheri, N.; Bah, S.; Bai, R.; Baker,
- 31 J. L.; Bakkannavar, S. M.; Bako, A. T.; Balakrishnan, S.; Bam, K.; Banik, P. C.; Barchitta,
- 32 M.; Bardhan, M.; Bardideh, E.; Barker-Collo, S. L.; Barqawi, H. J.; Barrow, A.; Barteit,

1 S.; Barua, L.; Bashiri Aliabadi, S.; Basiru, A.; Basu, S.; Basu, S.; Bathini, P. P.; Batra, K.;
 2 Baune, B. T.; Bayileyegn, N. S.; Behnam, B.; Behnouch, A. H.; Beiranvand, M.; Bejarano
 3 Ramirez, D. F.; Bell, M. L.; Bello, O. O.; Beloukas, A.; Bensenor, I. M.; Berezvai, Z.;
 4 Bernabe, E.; Bernstein, R. S.; Bettencourt, P. J. G.; Bhagavathula, A. S.; Bhala, N.;
 5 Bhandari, D.; Bhargava, A.; Bhaskar, S.; Bhat, V.; Bhatti, G. K.; Bhatti, J. S.; Bhatti, M.
 6 S.; Bhatti, R.; Bhutta, Z. A.; Bikbov, B.; Bishai, J. D.; Bisignano, C.; Bitra, V. R.; Bjørge,
 7 T.; Bodolica, V.; Bodunrin, A. O.; Bogale, E. K.; Bonakdar Hashemi, M.; Bonny, A.; Bora
 8 Basara, B.; Borhany, H.; Boxe, C.; Brady, O. J.; Bragazzi, N. L.; Braithwaite, D.; Brant,
 9 L. C.; Brauer, M.; Breitner, S.; Brenner, H.; Brown, J.; Brugha, T.; Bulamu, N. B.;
 10 Buonsenso, D.; Burkart, K.; Burns, R. A.; Busse, R.; Bustanji, Y.; Butt, Z. A.; Byun, J.;
 11 Caetano dos Santos, F. L.; Calina, D.; Cámara, L. A.; Campos-Nonato, I. R.; Cao, C.;
 12 Capodici, A.; Carr, S.; Carreras, G.; Carugno, A.; Carvalho, M.; Castaldelli-Maia, J. M.;
 13 Castañeda-Orjuela, C. A.; Castelpietra, G.; Catapano, A. L.; Cattaruzza, M. S.; Caye, A.;
 14 Cegolon, L.; Cembranel, F.; Cenderadewi, M.; Cerin, E.; Chakraborty, P. A.; Chan, J. S.
 15 K.; Chan, R. N. C.; Chandika, R. M.; Chandrasekar, E. K.; Charalampous, P.; Chattu, V.
 16 K.; Chatzimavridou-Grigoriadou, V.; Chen, A. W.; Chen, A.-T.; Chen, C. S.; Chen, H.;
 17 Chen, N. M.; Cheng, E. T. W.; Chimed-Ochir, O.; Chimoriya, R.; Ching, P. R.; Cho, W.
 18 C. S.; Choi, S.; Chong, B.; Chong, Y. Y.; Choudhari, S. G.; Chowdhury, R.; Christensen,
 19 S. W. M.; Chu, D.-T.; Chukwu, I. S.; Chung, E.; Chung, E.; Chutiyami, M.; Claassens,
 20 M. M.; Cogen, R. M.; Columbus, A.; Conde, J.; Cortesi, P. A.; Cousin, E.; Criqui, M. H.;
 21 Cruz-Martins, N.; Dadras, O.; Dai, S.; Dai, X.; Dai, Z.; Dalaba, M. A.; Damiani, G.; Das,
 22 J. K.; Das, S.; Dashti, M.; Dávila-Cervantes, C. A.; Davletov, K.; De Leo, D.; Debele, A.
 23 T.; Debopadhaya, S.; DeCleene, N. K.; Deebe, F.; Degenhardt, L.; Del Bo', C.; Delgado-
 24 Enciso, I.; Demetriades, A. K.; Denova-Gutiérrez, E.; Dervenis, N.; Desai, H. D.; Desai,
 25 R.; Deuba, K.; Dhama, K.; Dharmaratne, S. D.; Dhingra, S.; Dias da Silva, D.; Diaz, D.;
 26 Diaz, L. A.; Diaz, M. J.; Dima, A.; Ding, D. D.; Dirac, M. A.; Do, T. H. P.; do Prado, C.
 27 B.; Dohare, S.; Dominguez, R.-M. V.; Dong, W.; Dongarwar, D.; D'Oria, M.; Dorsey, E.
 28 R.; Doshmangir, L.; Dowou, R. K.; Driscoll, T. R.; Dsouza, H. L.; Dsouza, V.; Dube, J.;
 29 Dumith, S. C.; Duncan, B. B.; Duraes, A. R.; Duraisamy, S.; Durojaiye, O. C.; Dzianach,
 30 P. A.; Dziedzic, A. M.; Eboreime, E.; Ebrahimi, A.; Edinur, H. A.; Edvardsson, D.;
 31 Eikemo, T. A.; Eini, E.; Ekholuenetale, M.; Ekundayo, T. C.; El Sayed, I.; El Tantawi, M.;
 32 Elbarazi, I.; Elemam, N. M.; ElGohary, G. M. T.; Elhadi, M.; Elmeligy, O. A. A.;
 33 ELNahas, G.; Elshaer, M.; Elsohaby, I.; Engelbert Bain, L.; Erkhembayar, R.; Eshrati, B.;
 34 Estep, K.; Fabin, N.; Fagbamigbe, A. F.; Falzone, L.; Fareed, M.; Farinha, C. S. e S.;

1 Faris, M. E. M.; Faro, A.; Farrokhi, P.; Fatehizadeh, A.; Fauk, N. K.; Feigin, V. L.; Feng,
 2 X.; Fereshtehnejad, S.-M.; Feroze, A. H.; Ferreira, N.; Ferreira, P. H.; Fischer, F.; Flavel,
 3 J.; Flood, D.; Flor, L. S.; Foigt, N. A.; Folayan, M. O.; Force, L. M.; Fortuna, D.; Foschi,
 4 M.; Franklin, R. C.; Freitas, A.; Fukumoto, T.; Furtado, J. M.; Gaal, P. A.; Gadanya, M.
 5 A.; Gaidhane, A. M.; Gaihre, S.; Galali, Y.; Ganbat, M.; Gandhi, A. P.; Ganesan, B.;
 6 Ganie, M. A.; Ganiyani, M. A.; Gardner, W. M.; Gebi, T. G.; Gebregergis, M. W.;
 7 Gebrehiwot, M.; Gebremariam, T. B. B.; Gebremeskel, T. G.; Gela, Y. Y.; Georgescu, S.
 8 R.; Getachew Obsa, A.; Gething, P. W.; Getie, M.; Ghadiri, K.; Ghadirian, F.; Ghailan, K.
 9 Y.; Ghajar, A.; Ghasemi, M.; Ghasempour Dabaghi, G.; Ghasemzadeh, A.; Ghazy, R. M.;
 10 Gholamrezanezhad, A.; Ghorbani, M.; Ghotbi, E.; Gibson, R. M.; Gill, T. K.; Ginindza,
 11 T. G.; Girmay, A.; Glasbey, J. C.; Göbölös, L.; Godinho, M. A.; Goharinezhad, S.;
 12 Goldust, M.; Golechha, M.; Goleij, P.; Gona, P. N.; Gorini, G.; Goulart, A. C.; Grada, A.;
 13 Grivna, M.; Guan, S.-Y.; Guarducci, G.; Gubari, M. I. M.; Gudeta, M. D.; Guha, A.;
 14 Guicciardi, S.; Gulati, S.; Gulisashvili, D.; Gunawardane, D. A.; Guo, C.; Gupta, A. K.;
 15 Gupta, B.; Gupta, I.; Gupta, M.; Gupta, R.; Gupta, V. B.; Gupta, V. K.; Gupta, V. K.;
 16 Gutiérrez, R. A.; Habibzadeh, F.; Habibzadeh, P.; Haddadi, R.; Hadi, N. R.; Haep, N.;
 17 Hafezi-Nejad, N.; Hafiz, A.; Hagins, H.; Halboub, E. S.; Halimi, A.; Haller, S.; Halwani,
 18 R.; Hamilton, E. B.; Hankey, G. J.; Hannan, M. A.; Haque, M. N.; Harapan, H.; Haro, J.
 19 M.; Hartvigsen, J.; Hasaballah, A. I.; Hasan, I.; Hasanian, M.; Hasnain, M. S.; Hassan,
 20 A.; Haubold, J.; Havmoeller, R. J.; Hay, S. I.; Hayat, K.; Hebert, J. J.; Hegazi, O. E.;
 21 Heidari, G.; Helfer, B.; Hemmati, M.; Hendrie, D.; Henson, C. A.; Hezam, K.; Hiraike,
 22 Y.; Hoan, N. Q.; Holla, R.; Hon, J.; Hossain, M. M.; Hosseinzadeh, H.; Hosseinzadeh,
 23 M.; Hostiuc, M.; Hostiuc, S.; Hsu, J. M.; Huang, J.; Hugo, F. N.; Hushmandi, K.; Hussain,
 24 J.; Hussein, N. R.; Huynh, C. K.; Huynh, H.-H.; Hwang, B.-F.; Iannucci, V. C.; Ihler, A.
 25 L.; Ikiroma, A. I.; Ikuta, K. S.; Ilesanmi, O. S.; Ilic, I. M.; Ilic, M. D.; Imam, M. T.;
 26 Immurana, M.; Irham, L. M.; Islam, M. R.; Islam, S. M. S.; Islami, F.; Ismail, F.; Ismail,
 27 N. E.; Isola, G.; Iwagami, M.; Iwu, C. C. D.; Iyer, M.; Jaafari, J.; Jacobsen, K. H.; Jadidi-
 28 Niaragh, F.; Jafarinia, M.; Jaggi, K.; Jahankhani, K.; Jahanmehr, N.; Jahrami, H.; Jain,
 29 A.; Jain, N.; Jairoun, A. A.; Jaiswal, A.; Jakovljevic, M.; Jatau, A. I.; Javadov, S.; Javaheri,
 30 T.; Jayapal, S. K.; Jayaram, S.; Jee, S. H.; Jeganathan, J.; Jeyakumar, A.; Jha, A. K.; Jiang,
 31 H.; Jin, Y.; Jonas, J. B.; Joo, T.; Joseph, A.; Joseph, N.; Joshua, C. E.; Jozwiak, J. J.;
 32 Jürisson, M.; K, V.; Kaambwa, B.; Kabir, A.; Kabir, Z.; Kadashetti, V.; Kalani, R.;
 33 Kalankesh, L. R.; Kaliyadan, F.; Kalra, S.; Kamenov, K.; Kamyari, N.; Kanagasabai, T.;
 34 Kandel, H.; Kanmanthareddy, A. R.; Kanmodi, K. K.; Kantar, R. S.; Karaye, I. M.; Karim,

1 A.; Karimi, S. E.; Karimi, Y.; Kasraei, H.; Kassel, M. B.; Kauppila, J. H.; Kawakami, N.;
 2 Kayode, G. A.; Kazemi, F.; Kazemian, S.; Keikavoosi-Arani, L.; Keller, C.; Kempen, J.
 3 H.; Kerr, J. A.; Keshtkar, K.; Kesse-Guyot, E.; Keykhaei, M.; Khajuria, H.; Khalaji, A.;
 4 Khalid, A.; Khalid, N.; Khalilian, A.; Khamesipour, F.; Khan, A.; Khan, I.; Khan, M.;
 5 Khan, M. A.; Khanmohammadi, S.; Khatab, K.; Khatami, F.; Khatatbeh, M. M.; Khater,
 6 A. M.; Khayat Kashani, H. R.; Khidri, F. F.; Khodadoust, E.; Khormali, M.; Khorrami,
 7 Z.; Kifle, Z. D.; Kim, M. S.; Kimokoti, R. W.; Kisa, A.; Kisa, S.; Knudsen, A. K. S.;
 8 Kocarnik, J. M.; Kochhar, S.; Koh, H. Y.; Kolahi, A.-A.; Kompani, F.; Koren, G.; Korzh,
 9 O.; Kosen, S.; Koulmane Laxminarayana, S. L.; Krishan, K.; Krishna, V.;
 10 Krishnamoorthy, V.; Kuate Defo, B.; Kuddus, M. A.; Kuddus, M.; Kuitunen, I.; Kulkarni,
 11 V.; Kumar, M.; Kumar, N.; Kumar, R.; Kurmi, O. P.; Kusuma, D.; Kyu, H. H.; La Vecchia,
 12 C.; Lacey, B.; Ladan, M. A.; Laflamme, L.; Lafranconi, A.; Lahariya, C.; Lai, D. T. C.;
 13 Lal, D. K.; Lalloo, R.; Lallukka, T.; Lám, J.; Lan, Q.; Lan, T.; Landires, I.; Lanfranchi, F.;
 14 Langguth, B.; Laplante-Lévesque, A.; Larijani, B.; Larsson, A. O.; Lasrado, S.; Lauriola,
 15 P.; Le, H.-H.; Le, L. K. D.; Le, N. H. H.; Le, T. D. T.; Leasher, J. L.; Ledda, C.; Lee, M.;
 16 Lee, P. H.; Lee, S.; Lee, S. W.; Lee, W.-C.; Lee, Y. H.; LeGrand, K. E.; Lenzi, J.; Leong,
 17 E.; Leung, J.; Li, M.-C.; Li, W.; Li, X.; Li, Y.; Li, Y.; Lim, L.-L.; Lim, S. S.; Lindstrom,
 18 M.; Linn, S.; Liu, G.; Liu, R.; Liu, S.; Liu, W.; Liu, X.; Liu, X.; Llanaj, E.; Lo, C.-H.;
 19 López-Bueno, R.; Loreche, A. M.; Lorenzovici, L.; Lozano, R.; Lubinda, J.; Lucchetti,
 20 G.; Lunevicius, R.; Lusk, J. B.; Lv, hengliang; Ma, Z. F.; Machairas, N.; Madureira-
 21 Carvalho, Á. M.; Magaña Gómez, J. A.; Maghazachi, A. A.; Maharjan, P.; Mahasha, P.
 22 W.; Maheri, M.; Mahjoub, S.; Mahmoud, M. A.; Mahmoudi, E.; Majeed, A.; Makris, K.
 23 C.; Malakan Rad, E.; Malhotra, K.; Malik, A. A.; Malik, I.; Malta, D. C.; Manla, Y.;
 24 Mansour, A.; Mansouri, P.; Mansournia, M. A.; Mantilla Herrera, A. M.; Mantovani, L.
 25 G.; Manu, E.; Marateb, H. R.; Mardi, P.; Martinez, G.; Martinez-Piedra, R.; Martini, D.;
 26 Martins-Melo, F. R.; Martorell, M.; Marx, W.; Maryam, S.; Marzo, R. R.;
 27 Mathangasinghe, Y.; Mathieson, S.; Mathioudakis, A. G.; Mattumpuram, J.; Maugeri, A.;
 28 Mayeli, M.; Mazidi, M.; Mazzotti, A.; McGrath, J. J.; McKee, M.; McKowen, A. L. W.;
 29 McPhail, M. A.; Mehrabani-Zeinabad, K.; Mehrabi Nasab, E.; Mekene Meto, T.;
 30 Mendoza, W.; Menezes, R. G.; Mensah, G. A.; Mentis, A.-F. A.; Meo, S. A.; Meresa, H.
 31 A.; Meretoja, A.; Meretoja, T. J.; Mersha, A. M.; Mestrovic, T.; Mettananda, K. C. D.;
 32 Mettananda, S.; Michalek, I. M.; Miller, P. A.; Miller, T. R.; Mills, E. J.; Minh, L. H. N.;
 33 Mirijello, A.; Mirrakhimov, E. M.; Mirutse, M. K.; Mirza-Aghazadeh-Attari, M.; Mirzaei,
 34 M.; Mirzaei, R.; Misganaw, A.; Mishra, A. K.; Mitchell, P. B.; Mittal, C.; Moazen, B.;

- 1 Moberg, M. E.; Mohamed, J.; Mohamed, M. F. H.; Mohamed, N. S.; Mohammadi, E.;
- 2 Mohammadi, S.; Mohammed, H.; Mohammed, S.; Mohammed, S.; Mohr, R. M.;
- 3 Mokdad, A. H.; Molinaro, S.; Momtazmanesh, S.; Monasta, L.; Mondello, S.; Moodi
- 4 Ghalibaf, A.; Moradi, M.; Moradi, Y.; Moradi-Lakeh, M.; Moraga, P.; Morawska, L.;
- 5 Moreira, R. S.; Morovatdar, N.; Morrison, S. D.; Morze, J.; Mosapour, A.; Mosser, J. F.;
- 6 Mossialos, E.; Motappa, R.; Mougin, V.; Mouodi, S.; Mrejen, M.; Msherghi, A.; Mubarik,
- 7 S.; Mueller, U. O.; Mulita, F.; Munjal, K.; Murillo-Zamora, E.; Murlimanju, B.; Mustafa,
- 8 G.; Muthu, S.; Muzaffar, M.; Myung, W.; Nagarajan, A. J.; Naghavi, P.; Naik, G. R.;
- 9 Nainu, F.; Nair, S.; Najmuldeen, H. H. R.; Nangia, V.; Naqvi, A. A.; Narayana, A. I.;
- 10 Nargus, S.; Nascimento, G. G.; Nashwan, A. J.; Nasrollahizadeh, A.; Nasrollahizadeh, A.;
- 11 Natto, Z. S.; Nayak, B. P.; Nayak, V. C.; Nduaguba, S. O.; Negash, H.; Negoï, I.; Negoï,
- 12 R. I.; Nejadghaderi, S. A.; Nesbit, O. D.; Netsere, H. B.; Ng, M.; Nguefack-Tsague, G.;
- 13 Ngunjiri, J. W.; Nguyen, D. H.; Nguyen, H. Q.; Niazi, R. K.; Nikolouzakakis, T. K.;
- 14 Nikoobar, A.; Nikoomanesh, F.; Nikpoor, A. R.; Nnaji, C. A.; Nnyanzi, L. A.; Noman, E.
- 15 A.; Nomura, S.; Norrving, B.; Nri-Ezedi, C. A.; Ntaios, G.; Ntsekhe, M.; Nurrika, D.;
- 16 Nzoputam, C. I.; Nzoputam, O. J.; Oancea, B.; Odetokun, I. A.; O'Donnell, M. J.;
- 17 Oguntade, A. S.; Oguta, J. O.; Okati-Aliabad, H.; Okeke, S. R.; Okekunle, A. P.; Okonji,
- 18 O. C.; Olagunju, A. T.; Olasupo, O. O.; Olatubi, M. I.; Oliveira, G. M. M.; Olufadewa, I.
- 19 I.; Olusanya, B. O.; Olusanya, J. O.; Omar, H. A.; Omer, G. L.; Omonisi, A. E. E.; Onie,
- 20 S.; Onwujekwe, O. E.; Ordak, M.; Orish, V. N.; Ortega-Altamirano, D. V.; Ortiz, A.; Ortiz-
- 21 Brizuela, E.; Osman, W. M. S.; Ostroff, S. M.; Osuagwu, U. L.; Otoiu, A.; Otstavnov, N.;
- 22 Otstavnov, S. S.; Ouyahia, A.; Ouyang, G.; Owolabi, M. O.; P A, M. P.; Padron-
- 23 Monedero, A.; Padubidri, J. R.; Palicz, T.; Palladino, C.; Pan, F.; Pandi-Perumal, S. R.;
- 24 Pangaribuan, H. U.; Panos, G. D.; Panos, L. D.; Pantea Stoian, A. M.; Pardhan, S.; Parikh,
- 25 R. R.; Pashaei, A.; Pasovic, M.; Passera, R.; Patel, J.; Patel, S. K.; Patil, S.; Patoulias, D.;
- 26 Patthipati, V. S.; Pawar, S.; Pazoki Toroudi, H.; Pease, S. A.; Peden, A. E.; Pedersini, P.;
- 27 Peng, M.; Pensato, U.; Pepito, V. C. F.; Peprah, E. K.; Peprah, P.; Perdigão, J.; Pereira, M.
- 28 O.; Perianayagam, A.; Perico, N.; Pesudovs, K.; Petermann-Rocha, F. E.; Petri, W. A.;
- 29 Pham, H. T.; Philip, A. K.; Phillips, M. R.; Pigeolet, M.; Pigott, D. M.; Pillay, J. D.;
- 30 Piracha, Z. Z.; Pirouzpanah, S.; Plass, D.; Plotnikov, E.; Poddighe, D.; Polinder, S.;
- 31 Postma, M. J.; Pourtaheri, N.; Prada, S. I.; Pradhan, P. M. S.; Prakash, V.; Prasad, M.;
- 32 Prates, E. J. S.; Priscilla, T.; Pritchett, N.; Puri, P.; Puvvula, J.; Qasim, N. H.; Qattea, I.;
- 33 Qazi, A. S.; Qian, G.; Rabiee Rad, M.; Radhakrishnan, R. A.; Radhakrishnan, V.; Raeisi
- 34 Shahraki, H.; Rafferty, Q.; Raggi, A.; Raghav, P. R.; Rahim, M. J.; Rahman, M. M.;

- 1 Rahman, M. H. U.; Rahman, M.; Rahman, M. A.; Rahmani, S.; Rahmanian, M.;
- 2 Rahmawaty, S.; Rajaa, S.; Ramadan, M. M.; Ramasamy, S. K.; Ramasubramani, P.;
- 3 Ramazanu, S.; Rana, K.; Ranabhat, C. L.; Rancic, N.; Rane, A.; Rao, C. R.; Rao, K.; Rao,
- 4 M.; Rao, S. J.; Rashidi, M.-M.; Rathnaiah Babu, G.; Rauniyar, S. K.; Rawaf, D. L.;
- 5 Rawaf, S.; Razo, C.; Reddy, M. M. R. K.; Redwan, E. M. M.; Reifels, L.; Reiner Jr, R.
- 6 C.; Remuzzi, G.; Renzaho, A. M. N.; Reshmi, B.; Reyes, L. F.; Rezaei, N.; Rezaei, N.;
- 7 Rezaei, N.; Rezaei Hachesu, P.; Rezaeian, M.; Rickard, J.; Rodrigues, C. F.; Rodriguez,
- 8 J. A. B.; Roever, L.; Ronfani, L.; Roshandel, G.; Rotimi, K.; Rout, H. S.; Roy, B.; Roy,
- 9 N.; Roy, P.; Rubagotti, E.; S N, C.; Saad, A. M. A.; Saber-Ayad, M. M.; Sabour, S.; Sacco,
- 10 S.; Sachdev, P. S.; Saddik, B.; Saddler, A.; Sadee, B. A.; Sadeghi, E.; Sadeghi, M.; Saeb,
- 11 M. R.; Saeed, U.; Safi, S. Z.; Sagar, R.; Sagoe, D.; Saif, Z.; Sajid, M. R.; Sakshaug, J. W.;
- 12 Salam, N.; Salami, A. A.; Salaroli, L. B.; Saleh, M. A.; Salem, M. R.; Salem, M. Z. Y.;
- 13 Sallam, M.; Samadzadeh, S.; Samargandy, S.; Samodra, Y. L.; Samy, A. M.; Sanabria, J.;
- 14 Sanna, F.; Santos, I. S.; Santric-Milicevic, M. M.; Sarasmita, M. A.; Sarikhani, Y.;
- 15 Sarmiento-Suárez, R.; Sarode, G. S.; Sarode, S. C.; Sarveazad, A.; Sathian, B.;
- 16 Sathyanarayan, A.; Satpathy, M.; Sawhney, M.; Scarneas, N.; Schaarschmidt, B. M.;
- 17 Schmidt, M. I.; Schneider, I. J. C.; Schumacher, A. E.; Schwebel, D. C.; Schwendicke, F.;
- 18 Sedighi, M.; Senapati, S.; Senthilkumaran, S.; Sepanlou, S. G.; Sethi, Y.; Setoguchi, S.;
- 19 Seylani, A.; Shadid, J.; Shafie, M.; Shah, H.; Shah, N. S.; Shah, P. A.; Shahbandi, A.;
- 20 Shahid, S.; Shahid, W.; Shahwan, M. J.; Shaikh, M. A.; Shakeri, A.; Shalash, A. S.; Sham,
- 21 S.; Shamim, M. A.; Shamshirgaran, M. A.; Shamsi, M. A.; Shanawaz, M.; Shankar, A.;
- 22 Shannawaz, M.; Sharath, M.; Sharifan, A.; Sharifi-Rad, J.; Sharma, M.; Sharma, R.;
- 23 Sharma, S.; Sharma, U.; Sharma, V.; Shastri, R. P.; Shavandi, A.; Shayan, A. M.; Shayan,
- 24 M.; Shehabeldine, A. M. E.; Shetty, P. H.; Shibuya, K.; Shifa, J. E.; Shiferaw, D.;
- 25 Shiferaw, W. S.; Shigematsu, M.; Shiri, R.; Shitaye, N. A.; Shittu, A.; Shivakumar, K. M.;
- 26 Shivarov, V.; Shokati Eshkiki, Z.; Shool, S.; Shrestha, S.; Shuval, K.; Sibhat, M. M.;
- 27 Siddig, E. E.; Sigfusdottir, I. D.; Silva, D. A. S.; Silva, J. P.; Silva, L. M. L. R.; Silva, S.;
- 28 Simpson, C. R.; Singal, A.; Singh, A.; Singh, B. B.; Singh, H.; Singh, J. A.; Singh, M.;
- 29 Singh, P.; Skou, S. T.; Sleet, D. A.; Slepak, E. L. N.; Solanki, R.; Soliman, S. S. M.; Song,
- 30 S.; Song, Y.; Sorensen, R. J. D.; Soriano, J. B.; Soyiri, I. N.; Spartalis, M.; Sreeramareddy,
- 31 C. T.; Stark, B. A.; Starodubova, A. V.; Stein, C.; Stein, D. J.; Steiner, C.; Steiner, T. J.;
- 32 Steinmetz, J. D.; Steiropoulos, P.; Stockfelt, L.; Stokes, M. A.; Subedi, N. S.;
- 33 Subramaniyan, V.; Suemoto, C. K.; Suleman, M.; Suliankatchi Abdulkader, R.; Sultana,
- 34 A.; Sundström, J.; Swain, C. K.; Szarpak, L.; Tabae Damavandi, P.; Tabarés-Seisdedos,

1 R.; Tabatabaei Malazy, O.; Tabatabaeizadeh, S.-A.; Tabatabai, S.; Tabche, C.; Tabish, M.;
 2 Tadakamadla, S. K.; Taheri Abkenar, Y.; Taheri Soodejani, M.; Taherkhani, A.; Taiba, J.;
 3 Talaat, I. M.; Talukder, A.; Tampa, M.; Tamuzi, J. L.; Tan, K.-K.; Tandukar, S.; Tang, H.;
 4 Tavakoli Oliaee, R.; Tavangar, S. M.; Teimoori, M.; Temsah, M.-H.; Teramoto, M.;
 5 Thangaraju, P.; Thankappan, K. R.; Thapar, R.; Thayakaran, R.; Thirunavukkarasu, S.;
 6 Thomas, N.; Thomas, N. K.; Thum, C. C. C.; Tichopad, A.; Ticoalu, J. H. V.; Tillawi, T.;
 7 Tiruye, T. Y.; Tobe-Gai, R.; Tonelli, M.; Topor-Madry, R.; Torre, A. E.; Touvier, M.;
 8 Tovani-Palone, M. R.; Tran, J. T.; Tran, M. T. N.; Tran, N. M.; Tran, N.-H.; Trico, D.;
 9 Tromans, S. J.; Truyen, T. T. T. T.; Tsatsakis, A.; Tsegay, G. M.; Tsermpini, E. E.;
 10 Tumurkhuu, M.; Tyrovolas, S.; Udoh, A.; Umair, M.; Umakanthan, S.; Umar, T. P.;
 11 Undurraga, E. A.; Unim, B.; Unnikrishnan, B.; Unsworth, C. A.; Upadhyay, E.; Urso, D.;
 12 Usman, J. S.; Vahabi, S. M.; Vaithinathan, A. G.; Van den Eynde, J.; Varga, O.; Varma, R.
 13 P.; Vart, P.; Vasankari, T. J.; Vasic, M.; Vaziri, S.; Vellingiri, B.; Venketasubramanian, N.;
 14 Veroux, M.; Verras, G.-I.; Vervoort, D.; Villafañe, J. H.; Violante, F. S.; Vlassov, V.;
 15 Vollset, S. E.; Volovat, S. R.; Vongpradith, A.; Waheed, Y.; Wang, C.; Wang, F.; Wang,
 16 N.; Wang, S.; Wang, Y.; Wang, Y.-P.; Ward, P.; Wassie, E. G.; Weaver, M. R.; Weerakoon,
 17 K. G.; Weintraub, R. G.; Weiss, D. J.; Weldemariam, A. H.; Wells, K. M.; Wen, Y. F.;
 18 Whisnant, J. L.; Whiteford, H. A.; Wiangkham, T.; Wickramasinghe, D. P.;
 19 Wickramasinghe, N. D.; Wilandika, A.; Wilkerson, C.; Willeit, P.; Wimo, A.;
 20 Woldegebreale, D. H.; Wolf, A. W.; Wong, Y. J.; Woolf, A. D.; Wu, C.; Wu, F.; Wu, X.;
 21 Wu, Z.; Wulf Hanson, S.; Xia, Y.; Xiao, H.; Xu, X.; Xu, Y. Y.; Yadav, L.; Yadollahpour,
 22 A.; Yaghoubi, S.; Yamagishi, K.; Yang, L.; Yano, Y.; Yao, Y.; Yaribeygi, H.; Yazdanpanah,
 23 M. H.; Ye, P.; Yehualashet, S. S.; Yesuf, S. A.; Yezli, S.; Yiğit, A.; Yiğit, V.; Yigzaw, Z.
 24 A.; Yismaw, Y.; Yon, D. K.; Yonemoto, N.; Younis, M. Z.; Yu, C.; Yu, Y.; Yusuf, H.; Zahid,
 25 M. H.; Zakham, F.; Zaki, L.; Zaki, N.; Zaman, B. A.; Zamora, N.; Zand, R.; Zandieh, G.
 26 G. Z.; Zar, H. J.; Zarrintan, A.; Zastrozhin, M. S.; Zhang, H.; Zhang, N.; Zhang, Y.; Zhao,
 27 H.; Zhong, C.; Zhong, P.; Zhou, J.; Zhu, Z.; Ziafati, M.; Zielińska, M.; Zimsen, S. R. M.;
 28 Zoladl, M.; Zumla, A.; Zyoud, S. H.; Vos, T.; Murray, C. J. L. Global Incidence,
 29 Prevalence, Years Lived with Disability (YLDs), Disability-Adjusted Life-Years
 30 (DALYs), and Healthy Life Expectancy (HALE) for 371 Diseases and Injuries in 204
 31 Countries and Territories and 811 Subnational Locations, 1990–2021: A Systematic
 32 Analysis for the Global Burden of Disease Study 2021. *The Lancet* 2024, 403 (10440),
 33 2133–2161. [https://doi.org/10.1016/S0140-6736\(24\)00757-8](https://doi.org/10.1016/S0140-6736(24)00757-8).

- 1 (5) Connolly, B. S.; Lang, A. E. Pharmacological Treatment of Parkinson Disease: A
2 Review. *JAMA - Journal of the American Medical Association* 2014, *311* (16), 1670–
3 1683. <https://doi.org/10.1001/jama.2014.3654>.
- 4 (6) Kabra, A.; Sharma, R.; Kabra, R.; Baghel, U. S. Emerging and Alternative
5 Therapies For Parkinson Disease: An Updated Review. *Curr Pharm Des* 2018, *24* (22),
6 2573–2582. <https://doi.org/10.2174/1381612824666180820150150>.
- 7 (7) Pohl, F.; Kong Thoo Lin, P. The Potential Use of Plant Natural Products and Plant
8 Extracts with Antioxidant Properties for the Prevention/Treatment of Neurodegenerative
9 Diseases: In Vitro, In Vivo and Clinical Trials. *Molecules* 2018, *23* (12), 3283.
10 <https://doi.org/10.3390/molecules23123283>.
- 11 (8) Rehman, M. U.; Wali, A. F.; Ahmad, A.; Shakeel, S.; Rasool, S.; Ali, R.; Rashid,
12 S. M.; Madkhali, H.; Ganaie, M. A.; Khan, R. Neuroprotective Strategies for Neurological
13 Disorders by Natural Products: An Update. *Curr Neuroparmacol* 2019, *17* (3), 247–267.
14 <https://doi.org/10.2174/1570159X16666180911124605>.
- 15 (9) Arbo, B. D.; André-Miral, C.; Nasre-Nasser, R. G.; Schimith, L. E.; Santos, M.
16 G.; Costa-Silva, D.; Muccillo-Baisch, A. L.; Hort, M. A. Resveratrol Derivatives as
17 Potential Treatments for Alzheimer's and Parkinson's Disease. *Front Aging Neurosci*
18 2020, *12* (103). <https://doi.org/10.3389/fnagi.2020.00103>.
- 19 (10) dos Santos, M. G.; da Luz, D. B.; de Miranda, F. B.; de Aguiar, R. F.; Siebel, A.
20 M.; Arbo, B. D.; Hort, M. A. Resveratrol and Neuroinflammation: Total-Scale Analysis
21 of the Scientific Literature. *Nutraceuticals* 2024, *4* (2), 165–180.
22 <https://doi.org/10.3390/nutraceuticals4020011>.
- 23 (11) dos Santos, M. G.; Schimith, L. E.; André-Miral, C.; Muccillo-Baisch, A. L.;
24 Arbo, B. D.; Hort, M. A. Neuroprotective Effects of Resveratrol in In Vivo and In Vitro
25 Experimental Models of Parkinson's Disease: A Systematic Review. *Neurotox Res* 2022,
26 *40* (1), 319–345. <https://doi.org/10.1007/s12640-021-00450-x>.
- 27 (12) Atanasov, A. G.; Zotchev, S. B.; Dirsch, V. M.; Orhan, I. E.; Banach, M.;
28 Rollinger, J. M.; Barreca, D.; Weckwerth, W.; Bauer, R.; Bayer, E. A.; Majeed, M.;
29 Bishayee, A.; Bochkov, V.; Bonn, G. K.; Braidy, N.; Bucar, F.; Cifuentes, A.; D'Onofrio,
30 G.; Bodkin, M.; Diederich, M.; Dinkova-Kostova, A. T.; Efferth, T.; El Bairi, K.; Arkells,
31 N.; Fan, T.-P.; Fiebich, B. L.; Freissmuth, M.; Georgiev, M. I.; Gibbons, S.; Godfrey, K.

- 1 M.; Gruber, C. W.; Heer, J.; Huber, L. A.; Ibanez, E.; Kijjoo, A.; Kiss, A. K.; Lu, A.;
2 Macias, F. A.; Miller, M. J. S.; Mocan, A.; Müller, R.; Nicoletti, F.; Perry, G.; Pittalà, V.;
3 Rastrelli, L.; Ristow, M.; Russo, G. L.; Silva, A. S.; Schuster, D.; Sheridan, H.; Skalicka-
4 Woźniak, K.; Skaltsounis, L.; Sobarzo-Sánchez, E.; Bredt, D. S.; Stuppner, H.; Sureda,
5 A.; Tzvetkov, N. T.; Vacca, R. A.; Aggarwal, B. B.; Battino, M.; Giampieri, F.; Wink, M.;
6 Wolfender, J.-L.; Xiao, J.; Yeung, A. W. K.; Lizard, G.; Popp, M. A.; Heinrich, M.;
7 Berindan-Neagoe, I.; Stadler, M.; Daglia, M.; Verpoorte, R.; Supuran, C. T. Natural
8 Products in Drug Discovery: Advances and Opportunities. *Nat Rev Drug Discov* 2021,
9 20 (3), 200–216. <https://doi.org/10.1038/s41573-020-00114-z>.
- 10 (13) Ancajas, C. M. F.; Oyedele, A. S.; Butt, C. M.; Walker, A. S. Advances,
11 Opportunities, and Challenges in Methods for Interrogating the Structure Activity
12 Relationships of Natural Products. *Nat Prod Rep* 2024, 41 (10), 1543–1578.
13 <https://doi.org/10.1039/D4NP00009A>.
- 14 (14) Torres, P.; Poveda, A.; Jimenez-Barbero, J.; Parra, J. L.; Comelles, F.; Ballesteros,
15 A. O.; Plou, F. J. Enzymatic Synthesis of A-Glucosides of Resveratrol with Surfactant
16 Activity. *Adv Synth Catal* 2011, 353 (7), 1077–1086.
17 <https://doi.org/10.1002/adsc.201000968>.
- 18 (15) Du, Q. H.; Peng, C.; Zhang, H. Polydatin: A Review of Pharmacology and
19 Pharmacokinetics. *Pharm Biol* 2013, 51 (11), 1347–1354.
20 <https://doi.org/10.3109/13880209.2013.792849>.
- 21 (16) Kraus, M.; Grimm, C.; Seibel, J. Switching Enzyme Specificity from Phosphate
22 to Resveratrol Glucosylation. *Chemical Communications* 2017, 53 (90), 12181–12184.
23 <https://doi.org/10.1039/C7CC05993K>.
- 24 (17) Febres-Molina, C.; Sánchez, L.; Prat-Resina, X.; Jaña, G. A. Glucosylation
25 Mechanism of Resveratrol through the Mutant Q345F Sucrose Phosphorylase from the
26 Organism *Bifidobacterium Adolescentis*: A Computational Study. *Org Biomol Chem*
27 2022, 20 (26), 5270–5283. <https://doi.org/10.1039/D2OB00821A>.
- 28 (18) Zhao, G.; Yang, L.; Zhong, W.; Hu, Y.; Tan, Y.; Ren, Z.; Ban, Q.; Yang, C. S.;
29 Wang, Y.; Wang, Z. Polydatin, A Glycoside of Resveratrol, Is Better Than Resveratrol in
30 Alleviating Non-Alcoholic Fatty Liver Disease in Mice Fed a High-Fructose Diet. *Front*
31 *Nutr* 2022, 9. <https://doi.org/10.3389/fnut.2022.857879>.

- 1 (19) Wang, H.-L.; Gao, J.-P.; Han, Y.-L.; Xu, X.; Wu, R.; Gao, Y.; Cui, X.-H.
2 Comparative Studies of Polydatin and Resveratrol on Mutual Transformation and
3 Antioxidative Effect in Vivo. *Phytomedicine* 2015, 22 (5), 553–559.
4 <https://doi.org/10.1016/j.phymed.2015.03.014>.
- 5 (20) Lanzilli, G.; Cottarelli, A.; Nicotera, G.; Guida, S.; Ravagnan, G.; Fuggetta, M. P.
6 Anti-Inflammatory Effect of Resveratrol and Polydatin by In Vitro IL-17 Modulation.
7 *Inflammation* 2012, 35 (1), 240–248. <https://doi.org/10.1007/s10753-011-9310-z>.
- 8 (21) Karami, A.; Fakhri, S.; Kooshki, L.; Khan, H. Polydatin: Pharmacological
9 Mechanisms, Therapeutic Targets, Biological Activities, and Health Benefits. *Molecules*
10 2022, 27 (19), 6474. <https://doi.org/10.3390/molecules27196474>.
- 11 (22) Schimith, L. E.; dos Santos, M. G.; Arbo, B. D.; André-Miral, C.; Muccillo-
12 Baisch, A. L.; Hort, M. A. Polydatin as a Therapeutic Alternative for Central Nervous
13 System Disorders: A Systematic Review of Animal Studies. *Phytotherapy Research* 2022,
14 36 (7), 2852–2877. <https://doi.org/10.1002/ptr.7497>.
- 15 (23) Akash, S.; Islam, Md. R.; Bhuiyan, A. A.; Islam, M. N.; Bayıl, I.; Saleem, R. M.;
16 Albadrani, G. M.; Al-Ghadi, M. Q.; Abdel-Daim, M. M. In Silico Evaluation of Anti-
17 Colorectal Cancer Inhibitors by Resveratrol Derivatives Targeting Armadillo Repeats
18 Domain of APC: Molecular Docking and Molecular Dynamics Simulation. *Front Oncol*
19 2024, 14. <https://doi.org/10.3389/fonc.2024.1360745>.
- 20 (24) Sadybekov, A. V.; Katritch, V. Computational Approaches Streamlining Drug
21 Discovery. *Nature* 2023, 616 (7958), 673–685. [https://doi.org/10.1038/s41586-023-](https://doi.org/10.1038/s41586-023-05905-z)
22 05905-z.
- 23 (25) Hopkins, A. L. Network Pharmacology: The next Paradigm in Drug Discovery.
24 *Nat Chem Biol* 2008, 4 (11), 682–690. <https://doi.org/10.1038/nchembio.118>.
- 25 (26) Safari-Alighiarloo, N.; Taghizadeh, M.; Rezaei-Tavirani, M.; Goliaei, B.;
26 Peyvandi, A. A. Protein-Protein Interaction Networks (PPI) and Complex Diseases.
27 *Gastroenterol Hepatol Bed Bench* 2014, 7 (1), 17–31.
- 28 (27) Dennis, G.; Sherman, B. T.; Hosack, D. A.; Yang, J.; Gao, W.; Lane, H. C.;
29 Lempicki, R. A. DAVID: Database for Annotation, Visualization, and Integrated
30 Discovery. *Genome Biol* 2003, 4 (5), P3.

- 1 (28) Reimand, J.; Isserlin, R.; Voisin, V.; Kucera, M.; Tannus-Lopes, C.; Rostamianfar,
2 A.; Wadi, L.; Meyer, M.; Wong, J.; Xu, C.; Merico, D.; Bader, G. D. Pathway Enrichment
3 Analysis and Visualization of Omics Data Using g:Profiler, GSEA, Cytoscape and
4 EnrichmentMap. *Nat Protoc* 2019, *14* (2), 482–517. [https://doi.org/10.1038/s41596-018-](https://doi.org/10.1038/s41596-018-0103-9)
5 0103-9.
- 6 (29) Huang, D. W.; Sherman, B. T.; Lempicki, R. A. Systematic and Integrative
7 Analysis of Large Gene Lists Using DAVID Bioinformatics Resources. *Nat Protoc* 2009,
8 *4* (1), 44–57. <https://doi.org/10.1038/nprot.2008.211>.
- 9 (30) Zhou, S.; Yang, R.; Teng, Z.; Zhang, B.; Hu, Y.; Yang, Z.; Huan, M.; Zhang, X.;
10 Mei, Q. Dose-Dependent Absorption and Metabolism of Trans-Polydatin in Rats. *J Agric*
11 *Food Chem* 2009, *57* (11), 4572–4579. <https://doi.org/10.1021/jf803948g>.
- 12 (31) Kirchmair, J.; Göller, A. H.; Lang, D.; Kunze, J.; Testa, B.; Wilson, I. D.; Glen, R.
13 C.; Schneider, G. Predicting Drug Metabolism: Experiment and/or Computation? *Nat Rev*
14 *Drug Discov* 2015, *14* (6), 387–404. <https://doi.org/10.1038/nrd4581>.
- 15 (32) Lee, U.-J.; Sohng, J. K.; Kim, B.-G.; Choi, K.-Y. Recent Trends in the
16 Modification of Polyphenolic Compounds Using Hydroxylation and Glycosylation. *Curr*
17 *Opin Biotechnol* 2023, *80*, 102914. <https://doi.org/10.1016/j.copbio.2023.102914>.
- 18 (33) Koc, T. Y.; Dogan, S.; Karadayi, M. Potential Using of Resveratrol and Its
19 Derivatives in Medicine. *Eurasian J Med* 2024, *56* (2), 136–141.
20 <https://doi.org/10.5152/eurasianjmed.2024.24392>.
- 21 (34) Pardridge, W. M. Treatment of Parkinson’s Disease with Biologics That Penetrate
22 the Blood–Brain Barrier via Receptor-Mediated Transport. *Front Aging Neurosci* 2023,
23 *15*. <https://doi.org/10.3389/fnagi.2023.1276376>.
- 24 (35) Devraj, K.; Klinger, M. E.; Myers, R. L.; Mokashi, A.; Hawkins, R. A.; Simpson,
25 I. A. GLUT-1 Glucose Transporters in the Blood–Brain Barrier: Differential
26 Phosphorylation. *J Neurosci Res* 2011, *89* (12), 1913–1925.
27 <https://doi.org/10.1002/jnr.22738>.
- 28 (36) Zeller, K.; Rahner-Welsch, S.; Kuschinsky, W. Distribution of Glut1 Glucose
29 Transporters in Different Brain Structures Compared to Glucose Utilization and Capillary
30 Density of Adult Rat Brains. *Journal of Cerebral Blood Flow & Metabolism* 1997, *17* (2),
31 204–209. <https://doi.org/10.1097/00004647-199702000-00010>.

- 1 (37) Zhang, Z.; Sun, Z.; Jia, R.; Jiang, D.; Xu, Z.; Zhang, Y.; Wu, Y.-Q.; Wang, X.
2 Protective Effects of Polydatin against Bone and Joint Disorders: The in Vitro and in Vivo
3 Evidence so Far. *Nutr Res Rev* 2024, 37 (1), 96–107.
4 <https://doi.org/10.1017/S0954422423000082>.
- 5 (38) Schimith, L. E.; Machado da Silva, V.; Costa-Silva, D. G. da; Seregni Monteiro,
6 L. K.; Muccillo-Baisch, A. L.; André-Miral, C.; Hort, M. A. Preclinical Toxicological
7 Assessment of Polydatin in Zebrafish Model. *Drug Chem Toxicol* 2024, 47 (6), 923–932.
8 <https://doi.org/10.1080/01480545.2024.2311287>.
- 9 (39) Yang, H.; Sun, L.; Li, W.; Liu, G.; Tang, Y. Corrigendum: In Silico Prediction of
10 Chemical Toxicity for Drug Design Using Machine Learning Methods and Structural
11 Alerts. *Front Chem* 2018, 6. <https://doi.org/10.3389/fchem.2018.00129>.
- 12 (40) Bhat, V.; Chatterjee, J. The Use of In Silico Tools for the Toxicity Prediction of
13 Potential Inhibitors of SARS-CoV-2. *Alternatives to Laboratory Animals* 2021, 49 (1–2),
14 22–32. <https://doi.org/10.1177/02611929211008196>.
- 15 (41) Zhao, M.; Ma, J.; Li, M.; Zhang, Y.; Jiang, B.; Zhao, X.; Huai, C.; Shen, L.; Zhang,
16 N.; He, L.; Qin, S. Cytochrome P450 Enzymes and Drug Metabolism in Humans. *Int J*
17 *Mol Sci* 2021, 22 (23), 12808. <https://doi.org/10.3390/ijms222312808>.
- 18 (42) Lynch, T.; Price, A. The Effect of Cytochrome P450 Metabolism on Drug
19 Response, Interactions, and Adverse Effects. *Am Fam Physician* 2007, 76 (3), 391–396.
- 20 (43) Hyrsova, L.; Vanduchova, A.; Dusek, J.; Smutny, T.; Carazo, A.; Maresova, V.;
21 Trejtnar, F.; Barta, P.; Anzenbacher, P.; Dvorak, Z.; Pavek, P. Trans-Resveratrol, but Not
22 Other Natural Stilbenes Occurring in Food, Carries the Risk of Drug-Food Interaction via
23 Inhibition of Cytochrome P450 Enzymes or Interaction with Xenosensor Receptors.
24 *Toxicol Lett* 2019, 300, 81–91. <https://doi.org/10.1016/j.toxlet.2018.10.028>.
- 25 (44) Yu, C.; Shin, Y. G.; Kosmeder, J. W.; Pezzuto, J. M.; van Breemen, R. B. Liquid
26 Chromatography/Tandem Mass Spectrometric Determination of Inhibition of Human
27 Cytochrome P450 Isozymes by Resveratrol and Resveratrol-3-sulfate. *Rapid*
28 *Communications in Mass Spectrometry* 2003, 17 (4), 307–313.
29 <https://doi.org/10.1002/rcm.918>.

- 1 (45) Chun, Y. J.; Kim, M. Y.; Guengerich, F. P. Resveratrol Is a Selective Human
2 Cytochrome P450 1A1 Inhibitor. *Biochem Biophys Res Commun* 1999, 262 (1), 20–24.
3 <https://doi.org/10.1006/bbrc.1999.1152>.
- 4 (46) Iacopetta, D.; Ceramella, J.; Catalano, A.; Scali, E.; Scumaci, D.; Pellegrino, M.;
5 Aquaro, S.; Saturnino, C.; Sinicropi, M. S. Impact of Cytochrome P450 Enzymes on the
6 Phase I Metabolism of Drugs. *Applied Sciences* 2023, 13 (10), 6045.
7 <https://doi.org/10.3390/app13106045>.
- 8 (47) Mohos, V.; Bencsik, T.; Boda, G.; Fliszár-Nyúl, E.; Lemli, B.; Kunsági-Máté, S.;
9 Poór, M. Interactions of Casticin, Ipriflavone, and Resveratrol with Serum Albumin and
10 Their Inhibitory Effects on CYP2C9 and CYP3A4 Enzymes. *Biomedicine &*
11 *Pharmacotherapy* 2018, 107, 777–784. <https://doi.org/10.1016/j.biopha.2018.08.068>.
- 12 (48) Chow, H.-H. S.; Garland, L. L.; Hsu, C.-H.; Vining, D. R.; Chew, W. M.; Miller,
13 J. A.; Perloff, M.; Crowell, J. A.; Alberts, D. S. Resveratrol Modulates Drug- and
14 Carcinogen-Metabolizing Enzymes in a Healthy Volunteer Study. *Cancer Prevention*
15 *Research* 2010, 3 (9), 1168–1175. <https://doi.org/10.1158/1940-6207.CAPR-09-0155>.
- 16 (49) Walker, D. K. The Use of Pharmacokinetic and Pharmacodynamic Data in the
17 Assessment of Drug Safety in Early Drug Development. *Br J Clin Pharmacol* 2004, 58
18 (6), 601–608. <https://doi.org/10.1111/j.1365-2125.2004.02194.x>.
- 19 (50) Gehm, B. D.; McAndrews, J. M.; Chien, P.-Y.; Jameson, J. L. Resveratrol, a
20 Polyphenolic Compound Found in Grapes and Wine, Is an Agonist for the
21 Estrogen Receptor. *Proceedings of the National Academy of Sciences* 1997, 94 (25),
22 14138–14143. <https://doi.org/10.1073/pnas.94.25.14138>.
- 23 (51) Kobylka, P.; Kucinska, M.; Kujawski, J.; Lazewski, D.; Wierzbowski, M.;
24 Murias, M. Resveratrol Analogues as Selective Estrogen Signaling Pathway Modulators:
25 Structure–Activity Relationship. *Molecules* 2022, 27 (20), 6973.
26 <https://doi.org/10.3390/molecules27206973>.
- 27 (52) Dai, Z.; Li, Y.; Quarles, L. D.; Song, T.; Pan, W.; Zhou, H.; Xiao, Z. Resveratrol
28 Enhances Proliferation and Osteoblastic Differentiation in Human Mesenchymal Stem
29 Cells via ER-Dependent ERK1/2 Activation. *Phytomedicine* 2007, 14 (12), 806–814.
30 <https://doi.org/10.1016/j.phymed.2007.04.003>.

- 1 (53) Picca, A.; Calvani, R.; Coelho-Junior, H. J.; Landi, F.; Bernabei, R.; Marzetti, E.
2 Mitochondrial Dysfunction, Oxidative Stress, and Neuroinflammation: Intertwined
3 Roads to Neurodegeneration. *Antioxidants* 2020, 9 (8), 647.
4 <https://doi.org/10.3390/antiox9080647>.
- 5 (54) Kouchaki, E.; Kakhaki, R. D.; Tamtaji, O. R.; Dadgostar, E.; Behnam, M.;
6 Nikoueinejad, H.; Akbari, H. Increased Serum Levels of TNF- α and Decreased Serum
7 Levels of IL-27 in Patients with Parkinson Disease and Their Correlation with Disease
8 Severity. *Clin Neurol Neurosurg* 2018, 166, 76–79.
9 <https://doi.org/10.1016/j.clineuro.2018.01.022>.
- 10 (55) Barcia, C.; Ros, C. M.; Annese, V.; Gómez, A.; Ros-Bernal, F.; Aguado-Llera, D.;
11 Martínez-Pagán, M. E.; de Pablos, V.; Fernandez-Villalba, E.; Herrero, M. T. IFN- γ
12 Signaling, with the Synergistic Contribution of TNF- α , Mediates Cell Specific Microglial
13 and Astroglial Activation in Experimental Models of Parkinson's Disease. *Cell Death Dis*
14 2011, 2 (4), e142–e142. <https://doi.org/10.1038/cddis.2011.17>.
- 15 (56) Liu, T.-W.; Chen, C.-M.; Chang, K.-H. Biomarker of Neuroinflammation in
16 Parkinson's Disease. *Int J Mol Sci* 2022, 23 (8), 4148.
17 <https://doi.org/10.3390/ijms23084148>.
- 18 (57) Zhu, X.; Liu, Q.; Wang, M.; Liang, M.; Yang, X.; Xu, X.; Zou, H.; Qiu, J.
19 Activation of Sirt1 by Resveratrol Inhibits TNF- α Induced Inflammation in Fibroblasts.
20 *PLoS One* 2011, 6 (11), e27081. <https://doi.org/10.1371/journal.pone.0027081>.
- 21 (58) Silva, A. M.; Oliveira, M. I.; Sette, L.; Almeida, C. R.; Oliveira, M. J.; Barbosa,
22 M. A.; Santos, S. G. Resveratrol as a Natural Anti-Tumor Necrosis Factor- α Molecule:
23 Implications to Dendritic Cells and Their Crosstalk with Mesenchymal Stromal Cells.
24 *PLoS One* 2014, 9 (3), e91406. <https://doi.org/10.1371/journal.pone.0091406>.
- 25 (59) Zhang, H.; Yu, C.-H.; Jiang, Y.-P.; Peng, C.; He, K.; Tang, J.-Y.; Xin, H.-L.
26 Protective Effects of Polydatin from Polygonum Cuspidatum against Carbon
27 Tetrachloride-Induced Liver Injury in Mice. *PLoS One* 2012, 7 (9), e46574.
28 <https://doi.org/10.1371/journal.pone.0046574>.
- 29 (60) Martin, L. M.; Johnson, P. J.; Amorim, J. R.; DeClue, A. E. Effects of Orally
30 Administered Resveratrol on TNF, IL-1 β , Leukocyte Phagocytic Activity and Oxidative

- 1 Burst Function in Horses: A Prospective, Randomized, Double-Blinded, Placebo-
2 Controlled Study. *Int J Mol Sci* 2020, 21 (4), 1453. <https://doi.org/10.3390/ijms21041453>.
- 3 (61) Zhang, J.; Tan, Y.; Yao, F.; Zhang, Q. Polydatin Alleviates Non-Alcoholic Fatty
4 Liver Disease in Rats by Inhibiting the Expression of TNF- α and SREBP-1c. *Mol Med*
5 *Rep* 2012, 6 (4), 815–820. <https://doi.org/10.3892/mmr.2012.1015>.
- 6 (62) Guan, S. Y.; Zhang, K.; Wang, X. S.; Yang, L.; Feng, B.; Tian, D. D.; Gao, M. R.;
7 Liu, S. B.; Liu, A.; Zhao, M. G. Anxiolytic Effects of Polydatin through the Blockade of
8 Neuroinflammation in a Chronic Pain Mouse Model. *Mol Pain* 2020, 16, 1–13.
9 <https://doi.org/10.1177/1744806919900717>.
- 10 (63) Sun, W.; Li, H.; Shen, Y.; Xiao, H. Resveratrol Attenuates Rotenone-Induced
11 Inflammation and Oxidative Stress via STAT1 and Nrf2/Keap1/SLC7A11 Pathway in a
12 Microglia Cell Line. *Pathol Res Pract* 2021, 225, 153576.
13 <https://doi.org/10.1016/j.prp.2021.153576>.
- 14 (64) Lofrumento, D. D.; Nicolardi, G.; Cianciulli, A.; Nuccio, F. De; Pesa, V. La;
15 Carofiglio, V.; Dragone, T.; Calvello, R.; Panaro, M. A. Neuroprotective Effects of
16 Resveratrol in an MPTP Mouse Model of Parkinson's-like Disease: Possible Role of
17 SOCS-1 in Reducing pro-Inflammatory Responses. *Innate Immun* 2014, 20 (3), 249–260.
18 <https://doi.org/10.1177/1753425913488429>.
- 19 (65) Jin, F.; Wu, Q.; Lu, Y.-F.; Gong, Q.-H.; Shi, J.-S. Neuroprotective Effect of
20 Resveratrol on 6-OHDA-Induced Parkinson's Disease in Rats. *Eur J Pharmacol* 2008,
21 600 (1–3), 78–82. <https://doi.org/10.1016/j.ejphar.2008.10.005>.
- 22 (66) Zhang, L.; Yu, X.; Ji, M.; Liu, S.; Wu, X.; Wang, Y.; Liu, R. Resveratrol Alleviates
23 Motor and Cognitive Deficits and Neuropathology in the A53T α -Synuclein Mouse
24 Model of Parkinson's Disease. *Food Funct* 2018, 9 (12), 6414–6426.
25 <https://doi.org/10.1039/C8FO00964C>.
- 26 (67) Song, J.; Cheon, S.; Jung, W.; Lee, W.; Lee, J. Resveratrol Induces the Expression
27 of Interleukin-10 and Brain-Derived Neurotrophic Factor in BV2 Microglia under
28 Hypoxia. *Int J Mol Sci* 2014, 15 (9), 15512–15529.
29 <https://doi.org/10.3390/ijms150915512>.
- 30 (68) Hudait, A.; Qiu, Y.; Odendahl, N.; Molinero, V. Hydrogen-Bonding and
31 Hydrophobic Groups Contribute Equally to the Binding of Hyperactive Antifreeze and

- 1 Ice-Nucleating Proteins to Ice. *J Am Chem Soc* 2019, *141* (19), 7887–7898.
2 <https://doi.org/10.1021/jacs.9b02248>.
- 3 (69) Pace, C. N.; Fu, H.; Fryar, K. L.; Landua, J.; Trevino, S. R.; Shirley, B. A.;
4 Hendricks, M. M.; Iimura, S.; Gajiwala, K.; Scholtz, J. M.; Grimsley, G. R. Contribution
5 of Hydrophobic Interactions to Protein Stability. *J Mol Biol* 2011, *408* (3), 514–528.
6 <https://doi.org/10.1016/j.jmb.2011.02.053>.
- 7 (70) Thorn, C. R.; Howell, P. L.; Wozniak, D. J.; Prestidge, C. A.; Thomas, N.
8 Enhancing the Therapeutic Use of Biofilm-Dispersing Enzymes with Smart Drug
9 Delivery Systems. *Adv Drug Deliv Rev* 2021, *179*, 113916.
10 <https://doi.org/10.1016/j.addr.2021.113916>.
- 11 (71) Saddala, M. S.; Huang, H. Identification of Novel Inhibitors for TNF α , TNFR1
12 and TNF α -TNFR1 Complex Using Pharmacophore-Based Approaches. *J Transl Med*
13 2019, *17* (1), 215. <https://doi.org/10.1186/s12967-019-1965-5>.
- 14 (72) Corona, J. C.; Duchon, M. R. PPAR γ as a Therapeutic Target to Rescue
15 Mitochondrial Function in Neurological Disease. *Free Radic Biol Med* 2016, *100*, 153–
16 163. <https://doi.org/10.1016/j.freeradbiomed.2016.06.023>.
- 17 (73) Christofides, A.; Konstantinidou, E.; Jani, C.; Boussiotis, V. A. The Role of
18 Peroxisome Proliferator-Activated Receptors (PPAR) in Immune Responses. *Metabolism*
19 2021, *114*, 154338. <https://doi.org/10.1016/j.metabol.2020.154338>.
- 20 (74) Huang, Y.; Lu, J.; Zhan, L.; Wang, M.; Shi, R.; Yuan, X.; Gao, X.; Liu, X.; Zang,
21 J.; Liu, W.; Yao, X. Resveratrol-Induced Sirt1 Phosphorylation by LKB1 Mediates
22 Mitochondrial Metabolism. *Journal of Biological Chemistry* 2021, *297* (2), 100929.
23 <https://doi.org/10.1016/j.jbc.2021.100929>.
- 24 (75) Zhu, H.; Li, X.; Qiao, M.; Sun, X.; Li, G. Resveratrol Alleviates Inflammation and
25 ER Stress Through SIRT1/NRF2 to Delay Ovarian Aging in a Short-Lived Fish. *The*
26 *Journals of Gerontology: Series A* 2023, *78* (4), 596–602.
27 <https://doi.org/10.1093/gerona/glad009>.
- 28 (76) Villapol, S. Roles of Peroxisome Proliferator-Activated Receptor Gamma on
29 Brain and Peripheral Inflammation. *Cell Mol Neurobiol* 2018, *38* (1), 121–132.
30 <https://doi.org/10.1007/s10571-017-0554-5>.

- 1 (77) Montanari, R.; Saccoccia, F.; Scotti, E.; Crestani, M.; Godio, C.; Gilardi, F.;
2 Loiodice, F.; Fracchiolla, G.; Laghezza, A.; Tortorella, P.; Lavecchia, A.; Novellino, E.;
3 Mazza, F.; Aschi, M.; Pochetti, G. Crystal Structure of the Peroxisome Proliferator-
4 Activated Receptor γ (PPAR γ) Ligand Binding Domain Complexed with a Novel Partial
5 Agonist: A New Region of the Hydrophobic Pocket Could Be Exploited for Drug Design.
6 *J Med Chem* 2008, 51 (24), 7768–7776. <https://doi.org/10.1021/jm800733h>.
- 7 (78) Bruning, J. B.; Chalmers, M. J.; Prasad, S.; Busby, S. A.; Kamenecka, T. M.; He,
8 Y.; Nettles, K. W.; Griffin, P. R. Partial Agonists Activate PPAR γ Using a Helix 12
9 Independent Mechanism. *Structure* 2007, 15 (10), 1258–1271.
10 <https://doi.org/10.1016/j.str.2007.07.014>.
- 11 (79) Kroker, A. J.; Bruning, J. B. Review of the Structural and Dynamic Mechanisms
12 of PPAR γ Partial Agonism. *PPAR Res* 2015, 2015, 1–15.
13 <https://doi.org/10.1155/2015/816856>.
- 14 (80) Calleri, E.; Pochetti, G.; Dossou, K. S. S.; Laghezza, A.; Montanari, R.; Capelli,
15 D.; Prada, E.; Loiodice, F.; Massolini, G.; Bernier, M.; Moaddel, R. Resveratrol and Its
16 Metabolites Bind to PPARs. *ChemBioChem* 2014, 15 (8), 1154–1160.
17 <https://doi.org/10.1002/cbic.201300754>.
- 18 (81) Thorley, K. J.; Nielsen, C. B. Conformational Analysis of Conjugated Organic
19 Materials: What Are My Heteroatoms Really Doing? *Chempluschem* 2024, 89 (6).
20 <https://doi.org/10.1002/cplu.202300773>.
- 21 (82) Iwakura, Y.; Piao, Y.; Mizuno, M.; Takei, N.; Kakita, A.; Takahashi, H.; Nawa, H.
22 Influences of Dopaminergic Lesion on Epidermal Growth Factor-ErbB Signals in
23 Parkinson's Disease and Its Model: Neurotrophic Implication in Nigrostriatal Neurons. *J*
24 *Neurochem* 2005, 93 (4), 974–983. <https://doi.org/10.1111/j.1471-4159.2005.03073.x>.
- 25 (83) Zhong, H.; Zhou, Z.; Wang, H.; Wang, R.; Shen, K.; Huang, R.; Wang, Z. The
26 Biological Roles and Clinical Applications of the PI3K/AKT Pathway in Targeted
27 Therapy Resistance in HER2-Positive Breast Cancer: A Comprehensive Review. *Int J*
28 *Mol Sci* 2024, 25 (24), 13376. <https://doi.org/10.3390/ijms252413376>.
- 29 (84) Shi, A.; Liu, L.; Li, S.; Qi, B. Natural Products Targeting the MAPK-Signaling
30 Pathway in Cancer: Overview. *J Cancer Res Clin Oncol* 2024, 150 (1), 6.
31 <https://doi.org/10.1007/s00432-023-05572-7>.

(85) Simão, F.; Matté, A.; Pagnussat, A. S.; Netto, C. A.; Salbego, C. G. Resveratrol Prevents CA1 Neurons against Ischemic Injury by Parallel Modulation of Both GSK-3 β and CREB through PI3-K/Akt Pathways. *European Journal of Neuroscience* 2012, 36 (7), 2899–2905. <https://doi.org/10.1111/j.1460-9568.2012.08229.x>.

(86) Hou, Y.; Wang, K.; Wan, W.; Cheng, Y.; Pu, X.; Ye, X. Resveratrol Provides Neuroprotection by Regulating the JAK2/STAT3/PI3K/AKT/MTOR Pathway after Stroke in Rats. *Genes Dis* 2018, 5 (3), 245–255. <https://doi.org/10.1016/j.gendis.2018.06.001>.

(87) Ozpak, L.; Bağca, B. G. Neuroprotective Effects of Resveratrol through Modulation of $\text{PI3K}/\text{Akt}/\text{GSK-3}\beta$ Pathway and Metalloproteases. *IUBMB Life* 2024, 76 (12), 1199–1208. <https://doi.org/10.1002/iub.2902>.

(88) Kan, S.; Liu, C.; Zhao, X.; Feng, S.; Zhu, H.; Ma, B.; Zhou, M.; Fu, X.; Hu, W.; Zhu, R. Resveratrol Improves the Prognosis of Rats after Spinal Cord Injury by Inhibiting Mitogen-Activated Protein Kinases Signaling Pathway. *Sci Rep* 2023, 13 (1), 19723. <https://doi.org/10.1038/s41598-023-46541-x>.

Supplementary material

Table S1. Grid box coordinates and dimensions used in molecular docking simulations.

Receptor	PDB ID	Grid box coordinates (Å)		
		X	Y	Z
TNF	7JRA	-14.378	-2.467	-26.322
EGFR	1M17	22.014	0.253	52.794
ALB	1H9Z	32.092	13.756	9.434
CASP3	1NMS	-9.183	-3.963	23.945
ESR1	1XPC	31.616	-1.861	24.613
PPAR γ	1FM6	-1.722	69.521	35.872
PTGS2	5IKR	38.996	2.383	61.501
ERBB2	3RCD	13.415	2.748	27.535
SRC	8JN9	-17.822	75.904	106.807
ACE	1UZE	40.766	35.486	46.908
MAP2K1	3VVH	68.054	107.433	-32.097
Box dimension		20	20	20

Table S2. Molecular docking results of resveratrol, polydatin, and resveratrol-3- α -glucoside in key targets of Parkinson's disease.

Receptor	PDB ID	Binding energy (kcal/mol)		
		Resveratrol	Polydatin	Resveratrol-3- α -glucoside
TNF	7JRA	-8.6	-9.6	-9.3
EGFR	1M17	-7.5	-8.8	-8.6
ALB	1H9Z	-7.1	-8.9	-7.9
CASP3	1NMS	-6.4	-7.7	-7.6
ESR1	1XPC	-8.1	-8.6	-7.6
PPAR γ	1FM6	-7.3	-8.9	-9.1
PTGS2	5IKR	-7.2	-6.5	-6.2
ERBB2	3RCD	-7.8	-9.4	-8.4
SRC	8JN9	-6.4	-7.8	-7.7
ACE	1UZE	-7.1	-8.9	-8.7
MAP2K1	3VVH	-8.0	-8.8	-8.7

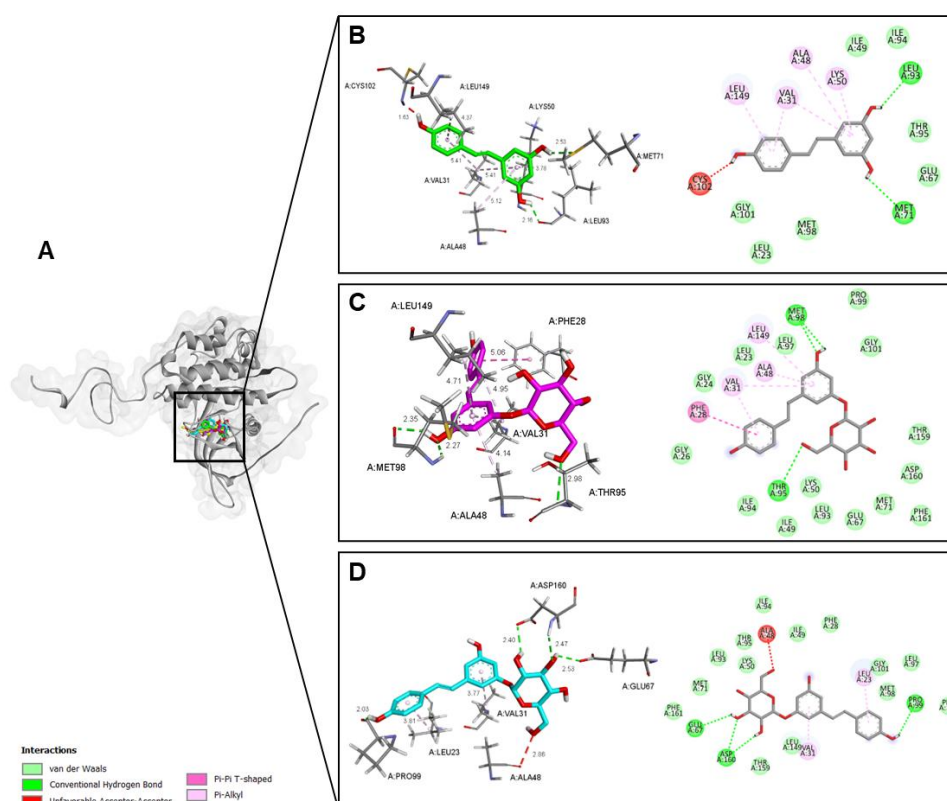


Figure S1. (A) Binding interactions of resveratrol, polydatin, and resveratrol-3- α -glucoside within the EGFR receptor active site. Resveratrol (B), polydatin (C), and resveratrol-3- α -glucoside (D) molecular interactions.

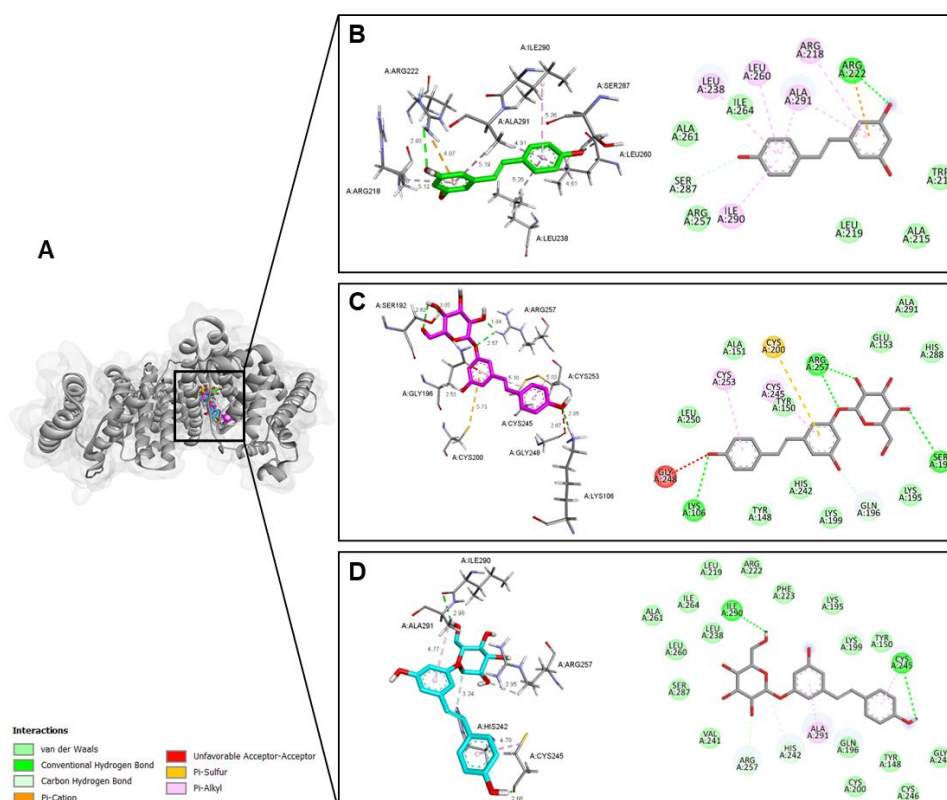


Figure S2. (A) Binding interactions of resveratrol, polydatin, and resveratrol-3- α -glucoside within the ALB receptor active site. Resveratrol (B), polydatin (C), and resveratrol-3- α -glucoside (D) molecular interactions.

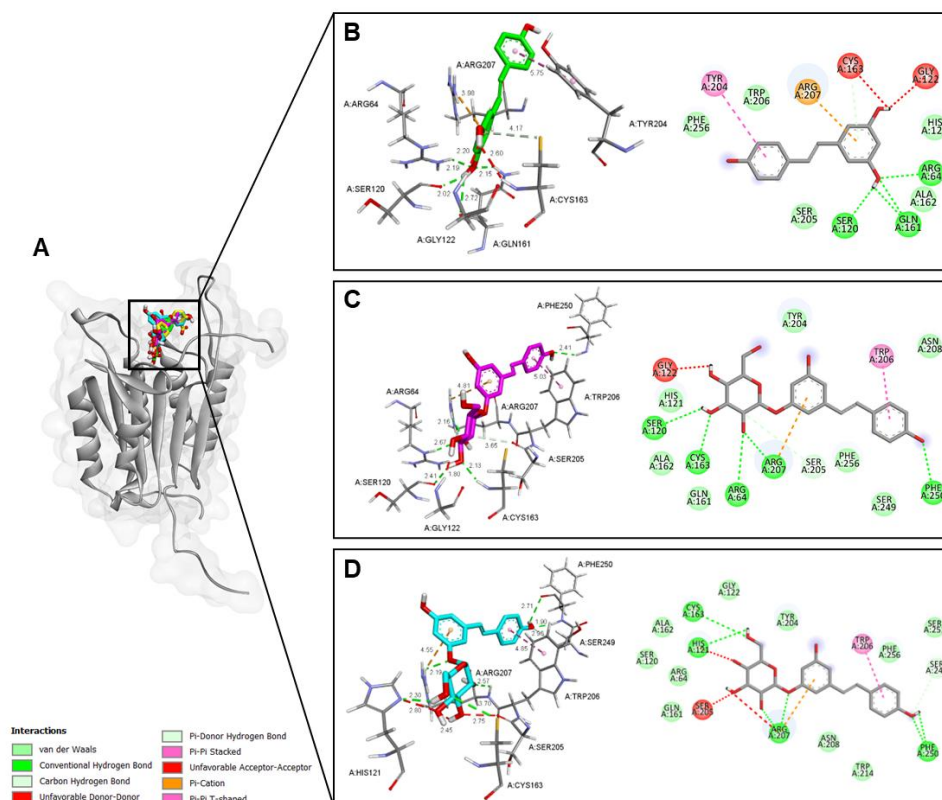


Figure S3. (A) Binding interactions of resveratrol, polydatin, and resveratrol-3- α -glucoside within the CASP3 receptor active site. Resveratrol (B), polydatin (C), and resveratrol-3- α -glucoside (D) molecular interactions.

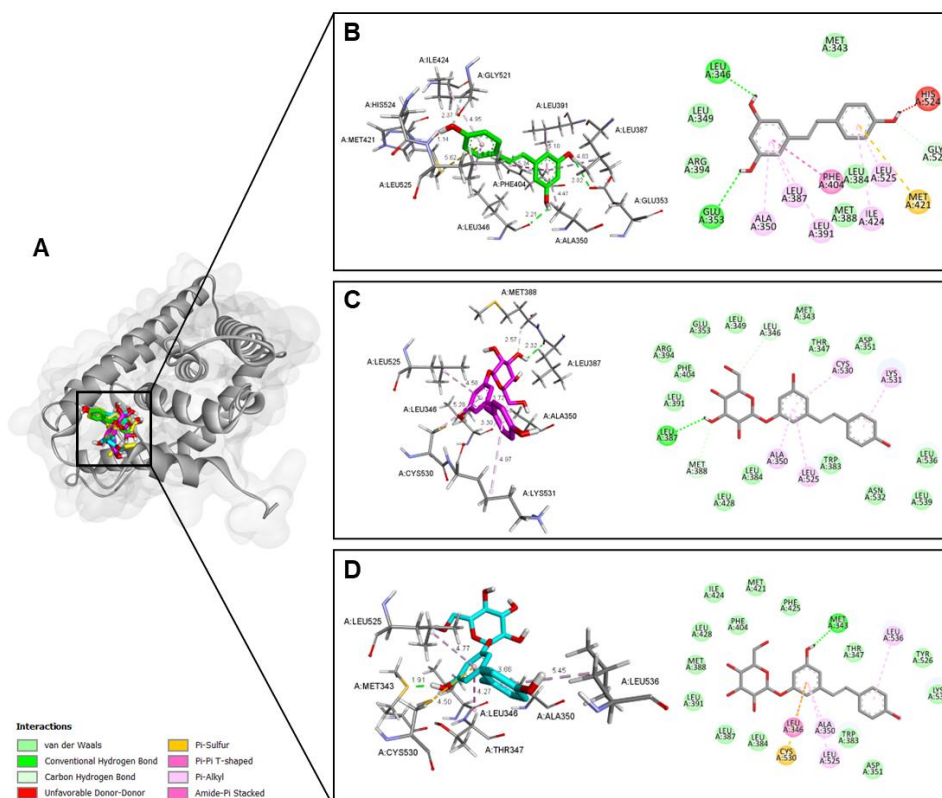


Figure S4. (A) Binding interactions of resveratrol, polydatin, and resveratrol-3- α -glucoside within the ESR1 receptor active site. Resveratrol (B), polydatin (C), and resveratrol-3- α -glucoside (D) molecular interactions.

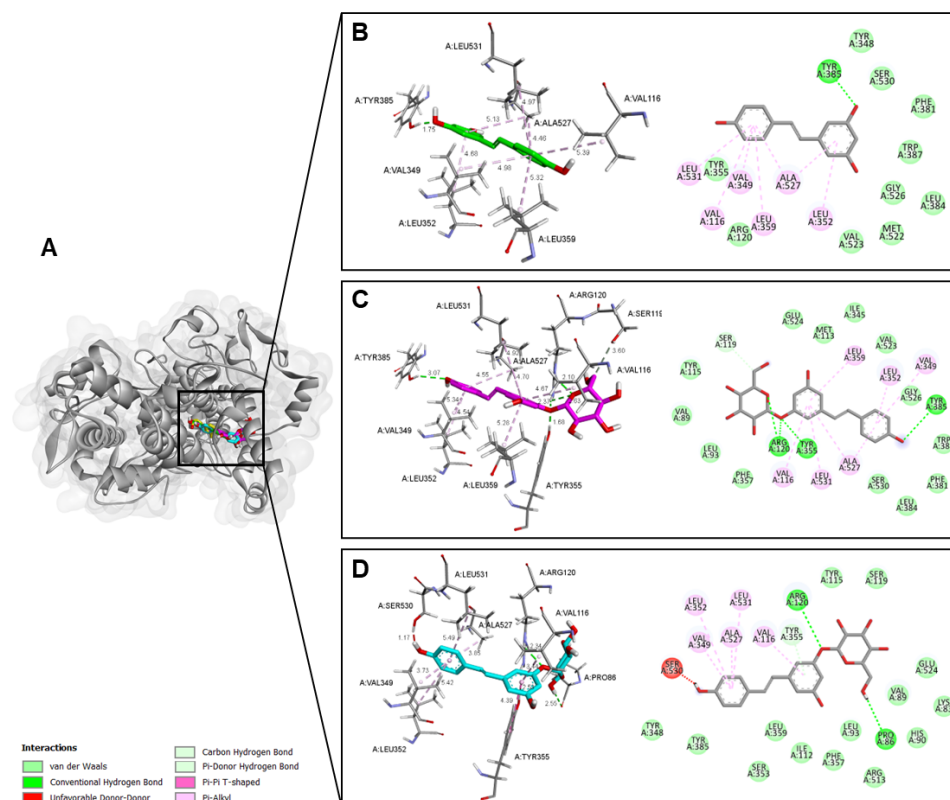


Figure S5. (A) Binding interactions of resveratrol, polydatin, and resveratrol-3- α -glucoside within the PTGS2 receptor active site. Resveratrol (B), polydatin (C), and resveratrol-3- α -glucoside (D) molecular interactions.

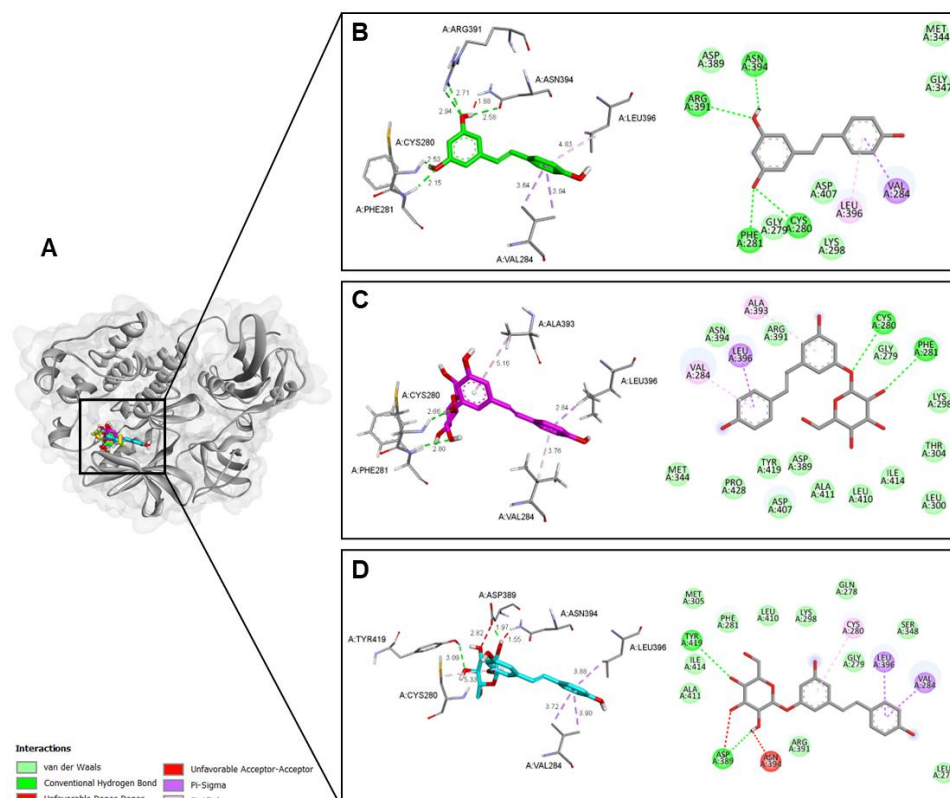


Figure S6. (A) Binding interactions of resveratrol, polydatin, and resveratrol-3- α -glucoside within the SRC receptor active site. Resveratrol (B), polydatin (C), and resveratrol-3- α -glucoside (D) molecular interactions.

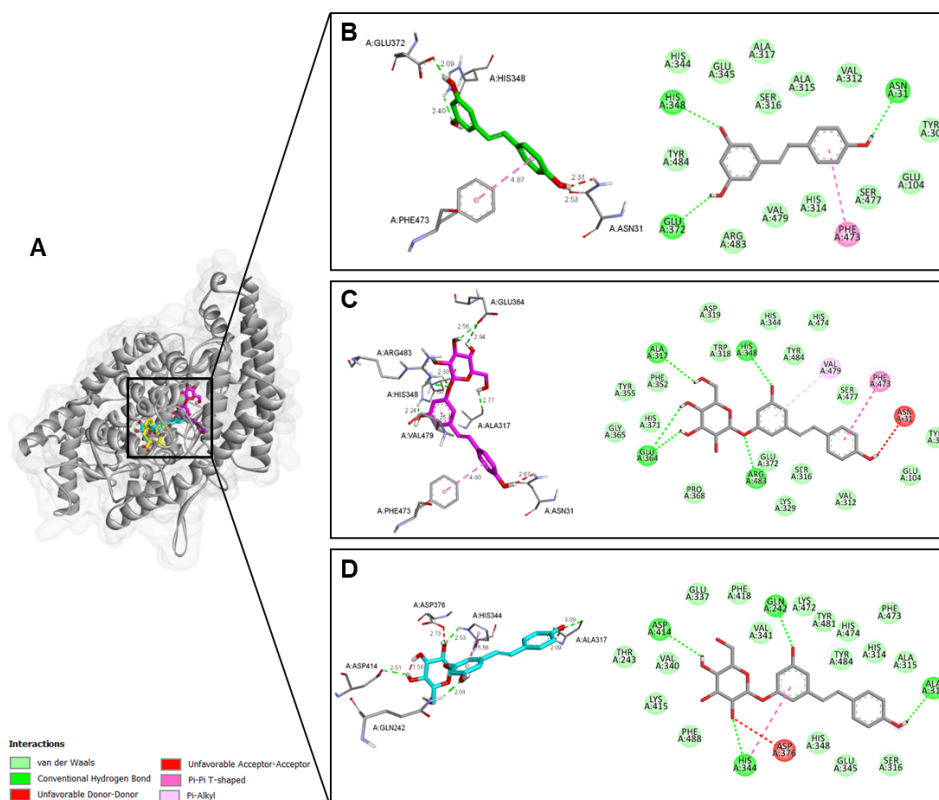


Figure S7. (A) Binding interactions of resveratrol, polydatin, and resveratrol-3- α -glucoside within the ACE receptor active site. Resveratrol (B), polydatin (C), and resveratrol-3- α -glucoside (D) molecular interactions.

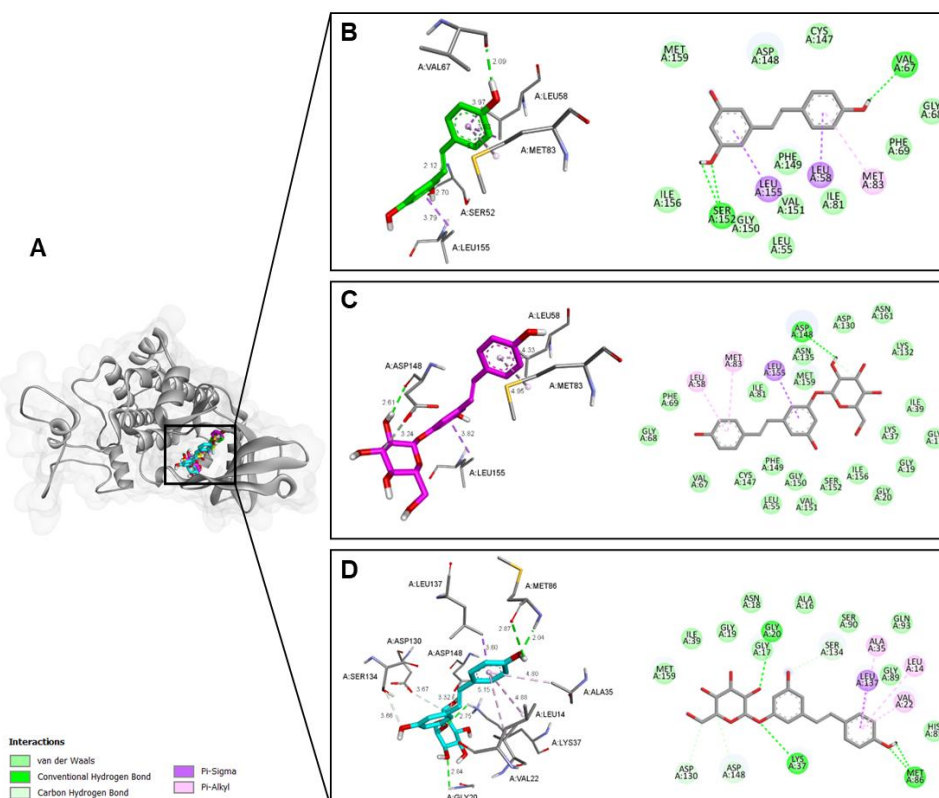


Figure S8. (A) Binding interactions of resveratrol, polydatin, and resveratrol-3- α -glucoside within the MAP2K1 receptor active site. Resveratrol (B), polydatin (C), and resveratrol-3- α -glucoside (D) molecular interactions.

CAPÍTULO 2 – MANUSCRITO 2**“Preclinical toxicological assessment of polydatin in zebrafish model”**

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Title: Preclinical toxicological assessment of polydatin in zebrafish model

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Abstract

Polydatin (3,4',5-trihydroxystilbene-3- β -D-glucoside, piceid), a natural stilbenoid found in different plant sources, has gained increasing attention for its potential health benefits. However, prior to its widespread adoption in human therapeutics and consumer products, a comprehensive investigation of its toxicological effects is crucial. In this study, the toxicity of polydatin was investigated in a developmental toxicity test using zebrafish (*Danio rerio*) as a valuable model for preclinical assessments. We employed the Fish Embryo Test (FET test – OECD n°236) to investigate the effects of polydatin on survival, hatchability, development, and behavior of zebrafish embryo-larval stage. Remarkably, the results demonstrated that polydatin up to 435 μ M showed no toxicity. Throughout the exposure period, zebrafish embryos exposed to polydatin exhibited normal development, with no significant mortality observed. Furthermore, hatching success and heartbeat rate were unaffected, and no morphological abnormalities were identified, signifying a lack of teratogenic effects and cardiotoxicity. Locomotion activity assessment revealed normal swimming patterns and response to stimuli, indicating no neurotoxic effects. Our study provides valuable insights into the toxicological profile of polydatin, suggesting that it may offer potential therapeutic benefits under a considerable concentration range. In addition, zebrafish model proves to be an efficient system for early-stage toxicological screening, guiding further investigations into the secure utilization of polydatin for human health and wellness.

Keywords: piceid; resveratrol; *Danio rerio*; fish embryo toxicity; behavior

1. Introduction

Polydatin (3,4',5-trihydroxystilbene-3- β -D-glucoside; Figure 1), also known as piceid, is commonly found in plants, particularly in grapes and their products (Du *et al.* 2013). The molecule is a stilbene glucoside, natural precursor of resveratrol and it has been studied for its high antioxidant activity due to its ability to scavenge free radicals and modulate antioxidant enzymes (Ma *et al.* 2016, Meng *et al.* 2016). Polydatin can also act as an anti-inflammatory agent through inhibition of the production of pro-inflammatory cytokines (Chen *et al.* 2021, Peritore *et al.* 2021), and to show anti-tumor property inducing apoptosis and preventing angiogenesis (Hu *et al.* 2019, Jiang *et al.* 2020).

In recent years, polydatin has attracted the attention of researchers for its potential therapeutic effects on various health conditions, such as cardiovascular disease (Zhang *et al.* 2015, Tan *et al.* 2020, Wu *et al.* 2020), neurodegenerative disorders (Chen *et al.* 2015, Gao *et al.* 2016, Schimith *et al.* 2022), metabolic syndrome (Wu *et al.* 2015, Luo *et al.* 2022, Tang *et al.* 2022), and cancer (Zou *et al.* 2018, Bai *et al.* 2021, Shah *et al.* 2022). Despite showing a wide range of potential pharmacological activities, there have been concerns about the toxicity of polydatin, particularly when consumed in high amounts. Therefore, there is a need for further research to determine the safe dosage of polydatin for human consumption, as well as to understand the potential risks associated with its use and take precautions to avoid adverse effects.

Several animal models have been used to study the toxicity of drug candidates and natural products. Rodents are the conventional models to study toxicity, pharmacology and behavior, however zebrafish (*Danio rerio*) has become a qualified animal model for conducting early biological screening of new molecules in laboratories (Kalueff *et al.* 2014, Stewart *et al.* 2014). Zebrafish is considered an alternative model, following the ethical principle of 3Rs (replacement, reduction, and refinement) especially in embryos and early larval stages. It has been suggested to perform hit-to-lead identification in high-throughput screenings in the process of drug discovery due its fast embryonic developmental, optical transparency, easy manipulation and behavior patterns well documented (Falcão *et al.* 2018, Bauer *et al.* 2021). Zebrafish larvae have the capability to exhibit a variety of behaviors, encompassing movement and responses to stimuli. These locomotor behaviors are observable and measurable, facilitating the

evaluation of the impacts of compounds on the development of neurological and behavioral functions (Rosa *et al.* 2022, Tan *et al.* 2022). Therefore, the *in vivo* toxicological characterization of these new drugs using zebrafish as an alternative method aims to supplement *in vitro* evaluations and mammalian rodent studies, besides providing insight into the potential toxicological effects and helping to identify safer alternatives (Basnet *et al.* 2019).

Thus, in this study we assessed the potential toxicological effects of polydatin on zebrafish embryo development, survival, teratogenicity, and locomotor ability, providing information about polydatin to guide future studies regarding its potential applications in pharmacology.

2. Materials and methods

2.1 Chemicals and solutions

Polydatin (purity $\geq 95\%$) and tricaine methanesulfonate (MS-222, 98% purity) were purchased from Sigma-Aldrich (St. Louis, USA). Polydatin stock solution was prepared in dimethyl sulfoxide (DMSO) and stored at -20°C . Test solutions (81, 113, 158, 221, 310, and 435 μM) were prepared by dissolving the volume required from the stock solution in E3 medium (5 mM NaCl, 0.17 mM KCl, 0.33 mM CaCl_2 , 0.33 mM MgSO_4 , pH 7.2, and dissolved oxygen (8 mg/L). Tricaine methanesulfonate solution was prepared in E3 medium and the pH was adjusted to 7.2 ± 0.2 .

2.2 Zebrafish maintenance

Adult wild-type zebrafish (*Danio rerio*) were maintained in an aquatic recirculating system (Altamar, Brazil), in 10 L tanks in a fish density of 1 fish/L, following standard conditions of pH 7.2 ± 0.5 , temperature of $28 \pm 1^{\circ}\text{C}$, conductivity 500 ± 50 $\mu\text{S}/\text{cm}$, water hardness 75-200 mg/L CaCO_3 , and 12/12h light/dark cycle. Adult fish were fed 2-3 times daily with brine shrimp (*Artemia salina*) and commercial dry flakes (Westerfield 2000, Avdesh *et al.* 2012, Aleström *et al.* 2020). For breeding, females and males were maintained at a ratio of 1:1 overnight, and to prevent the fish from eating their eggs, the bottom of the tanks was equipped with a net where only the eggs could pass through. The following morning, fertilized eggs were collected approximately 45 minutes after the lights were turned on.

2.3 Experimental design

The experimental procedure was based in OECD guideline 236 (FET test – Fish embryo toxicity test) (2013) with minor modifications as the extending of exposure period until 120 hours post-fertilization (hpf). Embryos up to 3 hpf were collected, sanitized, randomly placed into a 96-well plate, and assigned to treatment and control groups. A range of polydatin concentrations was tested (81, 113, 158, 221, 310, and 435 μM) based on previous study performed by Cavalcante (2017) using resveratrol due to the similarities between the molecules and the experimental design used. Animals of the vehicle group were exposed to DMSO (0.85%, concentration found at the 435 μM polydatin test solution) in the E3 medium, and the control group received exclusively E3 medium. Each well contained one embryo and 300 μL of test solution and only embryos that showed a normal cell division (blastula stage) were used (Kimmel *et al.* 1995). Animals were treated only once by immersion, through static condition, and polydatin exposure effects were evaluated at 24, 48, 72, 96, and 120 hpf. The 96-well plates were kept in a biochemical oxygen demand (B.O.D) incubator, under constant temperature ($28 \pm 0.5^\circ\text{C}$) and light/dark cycle of 12/12h until 120 hpf. Experiments were conducted in four independent replicates using 156 animals/group ($n = 39$ animals/group, replicate) (Figure 2).

All procedures followed the guidelines of the Brazilian Council of Animal Experimentation for Animal Experimentation (CONCEA). At the end of the experiments larvae were euthanized according to the Brazilian Guide to Euthanasia Practices of the CONCEA by immersion in cooling and freezing water (0 to 4°C) for 20 minutes until cessation of movements to ensure death by hypoxia (CONCEA 2018). Experimental procedures were approved by the Animal Use Ethics Committee of the Federal University of Rio Grande (Process number 23116.000500/2022-63. The ARRIVE guidelines was used for reporting *in vivo* experiments (Kilkenny *et al.* 2010).

2.4 Survival and hatch rates

Animals were monitored daily for survival rate and for the occurrence of malformations, such as pericardial and yolk sac edema, spine and tail deformities, and growth retardation. The death was determined through the lack of heartbeat (OECD 2006). Hatching rate was evaluated at 48, 72, and 96 hpf, and quantified based on the number of live individuals each time evaluated (Westerfield 2000).

2.5 Heartbeat rate

At 96 hpf, the larvae ($n = 21$ -38 animals/group) were anesthetized using MS-222 (0.02%) and immobilized with methylcellulose (3%) on microscope slides under a G1200 digital microscope (Mustool®) for video acquisition (Westerfield 2000, Brannen *et al.* 2010). The heartbeat of each animal was recorded for 20s and the frequency obtained was used to determine the rate of beats per minute (bpm) through DanioScope software version 1.1 (Noldus Information Technology®, Netherlands).

2.6 Body length measurement

Distance between larvae mouth and pigmented tip of the tail was measured using the software Image J 1.53t for Windows using a photographic registration taken at the same moment of the video acquisition for heartbeat register. The measure was made at 96 hpf of $n = 32$ -38 animals/group.

2.7 Locomotor activity

Only larvae without detectable morphological alterations were used for the locomotor activity assessment (see item 2.4). Photolocomotor response assay was performed between 11:00 am and 3:30 pm to avoid alterations in the metabolic basal rate/activity (Chiffre *et al.* 2016), under darkness (infrared light) or light conditions (~1000 lux) at 25 frames per second using DanioVision acquisition system (Noldus Information Technology®, Netherlands). At 120 hpf, the 96-well plate containing one larva and 300 μ L of test solution per well was placed in the DanioVision chamber, acclimatized at 28°C for 20 minutes in darkness ($n = 95$ -103 animals/group). Larvae locomotion was tracked following 25 minutes of alternating 5 minutes of light and dark conditions, totalizing 5 cycles of transition (Maeda *et al.* 2021). Parameters of distance moved (mm) and mean velocity (mm/s) were analyzed using Ethovision XT version 11.5 (Noldus Information Technology®, Netherlands).

2.8 Statistical analysis

Statistical analyses were performed using GraphPad Prism version 9 for Windows (GraphPad Software, USA). Survival rate was calculated using Mantel-Cox test. Outliers were identified by Grubbs' method ($\alpha = 0.05$) and the data result were checked for normal distribution with Shapiro-Wilk and Kolmogorov-Smirnov. When the requisites of normality were fulfilled, one-way analysis of variance (ANOVA) was applied and followed by post hoc comparisons using Tukey's test. In the cases where the requisites were not fulfilled, Kruskal-Wallis non-parametric test was applied and followed by Dunn's post hoc. Parametric data are presented as mean $\pm$ SEM whereas median $\pm$

interquartile range values for non-parametric data. The level of significance was set at $p \leq 0.05$.

3. Results

We investigated the effect of polydatin exposure on survival and hatching ability. Survival rate of animals exposed to increasing concentrations of polydatin was evaluated every 24 hours up to 120 hpf. Polydatin has not affected the survival rate when compared to the control group ($p = 0.9733$), whereas to those exposed to the higher concentration of 435 mM (Figure 3A). Zebrafish embryos hatching endpoint under normal conditions are expected from 48 up to 96 hpf (Kimmel *et al.* 1995, Westerfield 2000). There was also no significant difference in the hatching rate of embryos exposed to polydatin (Figure 3B). The possibility of exposure to polydatin causing teratogenic effects on the morphology of animals was evaluated at 96 hpf. The percentage of occurrences of malformations was less than 5% in all the experimental groups (Pype *et al.* 2015) (Figure 4A). No differences were observed in body length between the control group and polydatin-exposed groups (Figure 4B). Polydatin exposure has also showed no significant effect on the heartbeat rate compared to unexposed embryos (Figure 4C).

The effects of polydatin on locomotor activity were analyzed using the photolocomotor response assay at 120 hpf, where parameters of distance traveled, and mean velocity were evaluated. Animals exposed to 310 μ M of polydatin showed an increase in the distance moved compared to control group in the dark-light first transition (L1, 5-6 minutes; $p = 0.0238$) (Figure 5A). Mean velocity of larvae exposed to the different concentrations of polydatin was not affected (Figure 6B). Animals of the vehicle group exposed to DMSO (0.85%) exhibited decreased distance moved ($p = 0.0158$) and mean velocity ($p = 0.0003$) in the light-dark transitions (D2, 10-11 minutes and D3, 20-21 minutes) when compared to animals of the control group (Figure 5A and 6A).

4. Discussion

Polydatin, a natural compound, has been the subject of numerous studies due to its potential therapeutic applications in various fields (Karami *et al.* 2022, Luo *et al.* 2022, Schimith *et al.* 2022, Shah *et al.* 2022, Tang *et al.* 2022). However, comprehensive information regarding the safety and toxicity of polydatin remains limited. In this study, we evaluated the potential toxicity of polydatin during the early development of zebrafish,

1 using different concentrations (81, 113, 158, 221, 310, and 435 μM). Our findings
2 demonstrated that exposure to polydatin has not caused any toxicological effects on
3 embryo survival, development, hatching, morphology, or locomotor behavior. These
4 results suggest that polydatin is non-toxic for embryo-larval zebrafish, based on the
5 conducted tests.

6 In a study conducted by Cavalcante and collaborators (2017), zebrafish embryos
7 exposed to resveratrol, in a similar concentration range as used in our study, exhibited
8 toxicological effects at 46.9 and 100 mg L^{-1} , or 205 and 438 μM , respectively. These
9 effects included lethality, lack of eyes and body pigmentation, slowed heartbeat rate,
10 hatching delay, and morphological alterations such as pericardial and yolk sac edema, and
11 spine deformity. In contrast, our study showed that embryos exposed to polydatin did not
12 display developmental alterations as observed in the study mentioned earlier with
13 resveratrol (aglycone form). Cadena and collaborators (2020) explored the protective
14 effects of polydatin, quercetin, folic acid, and their mixtures in a zebrafish model of fetal
15 alcohol spectrum disorder. Exposure to polydatin as a single compound at 75 μM has been
16 shown not to affect larval mortality and development. However, despite this lack of
17 impact on mortality and development, embryonic exposure to polydatin was found to
18 influence the swimming activity of zebrafish larvae. Notably, the presence of a glucose
19 moiety in polyphenols or in bioactive compounds in general, may impact their
20 biological/physicochemical properties as well as their toxicity, as glycosylated forms
21 have been reported to be less toxic than their aglycones. This observation aligns with an
22 *in vitro* study where glycosylated derivatives of resveratrol demonstrated lower
23 cytotoxicity in HEK-293 cells compared to resveratrol (Falomir *et al.* 2016). Similar
24 trends were noted in studies involving glucosides derivatives of (-)-epigallocatechin
25 gallate (Nadim *et al.* 2014, Gonzalez-Alfonso *et al.* 2019, Méndez-Líte *et al.* 2022) and
26 quercetin (Nyska *et al.* 2016, Hobbs *et al.* 2018), where experiments conducted in both
27 *in vitro* and *in vivo* models confirmed the non-toxic profile of glycosylated forms.

28 Zebrafish plays a crucial role in toxicological assessment research of synthetic
29 or bioactive compounds, aiding in drug discovery and identifying potential harmful
30 effects (Modarresi Chahardehi *et al.* 2020, Abrão *et al.* 2022, Rivero-Wendt *et al.* 2023,
31 Rodrigues Esperandim *et al.* 2023). The zebrafish FET test is a valuable tool for rapidly
32 forecasting and tracking multiple toxicity endpoints, detecting phenotypic alterations
33 during organogenesis. To assess potential lethality and teratogen effects of natural

1 products like polydatin, various endpoints (i.e. coagulation, absence of heartbeat, tail and
2 notochord malformation, scoliosis, pericardial and yolk edema, and growth delays) are
3 examined. Additionally, adverse effects in different target tissues are analyzed, generating
4 results that can complement and be compared with mammalian models, and surpass those
5 observed in single *in vitro* methods (Lantz-McPeak *et al.* 2015, von Hellfeld *et al.* 2020).

6 Zebrafish FET is based on mortality and teratogenesis endpoints, as mentioned
7 before, providing valuable information on various toxicity endpoints during development.
8 On the other hand, the evaluation of behavior parameters could also act as a crucial
9 analysis in toxicological studies. By monitoring locomotor activity, responses to
10 environmental stimuli, social interactions, and other behavioral patterns, can reflect into
11 their sensory, motor, and cognitive functions condition (Goldsmith 2004, Irons *et al.*
12 2010). Additionally, behavior evaluation can help identify neurodevelopmental
13 abnormalities that may be subtle and not directly related to gross morphological
14 alterations in the zebrafish embryos (Padilla *et al.* 2011, Ogungbemi *et al.* 2019). In our
15 study, we did not observe morphological malformations or developmental delays caused
16 by polydatin exposure. To further investigate the effects of polydatin on zebrafish larvae,
17 we extended the experimental protocol to 120 hpf and employed the photolocomotor
18 response to assess locomotor activity. Zebrafish larvae possess a well-developed nervous
19 system, making them highly sensitive to changes in locomotor behavior in response to
20 various stimuli, including light and dark environments and the test used takes advantage
21 of the innate preference of zebrafish larvae to avoid brightly lit areas and seek darker
22 regions (MacPhail *et al.* 2009). For quantifying the behavior parameters, we adopted the
23 first minute after each light-dark transition to ensure a reliable stimuli response avoiding
24 the habituation interference as the test progressed (Wong *et al.* 2010). Our observations
25 revealed that zebrafish larvae exposed to polydatin at 120 hpf exhibited locomotor
26 activity similar to control larvae, indicating that polydatin did not disrupt the developing
27 brain environment. Although we noticed a transient effect in distance moved during the
28 first dark cycle in larvae exposed to 310 μ M of polydatin, this effect has not remained as
29 the cycles progressed and no significant differences were observed in mean velocity
30 parameter. This effect can be explained by the intrinsic data variability normally observed
31 in behavioral datasets (Gerhardt 2007), besides the experiments were conducted in
32 independent replicates, with embryos from different breedings, following the
33 recommendations of FET test. Thus, integrating behavior assessment with the zebrafish

FET test not only enhances the sensitivity and comprehensiveness of the toxicological evaluation but also offers an overall understanding of the harmful effects on neurodevelopment, providing valuable data to support regulatory decisions and ensure the safety of various substances (Basnet *et al.* 2019).

Polydatin, despite exhibiting higher hydrosolubility than resveratrol, still has limited solubility (Subramanian *et al.* 2010, Amri *et al.* 2012, Du *et al.* 2013). DMSO is a solvent commonly as vehicle in developmental toxicity testing (Castro *et al.* 1995, Cavaletti *et al.* 2000). Some studies have shown that concentrations up to 2% of DMSO are well-tolerated by zebrafish embryos (Maes *et al.* 2012, Hoyberghs *et al.* 2021), while others reported reduced larval movement with 0.05-1% DMSO exposure (Chen *et al.* 2011, Huang *et al.* 2018, Christou *et al.* 2020). In our study, exposure to 0.85% of DMSO was also not able to cause mortality or embryo development alterations, as evaluated through hatching rate and morphology. However, locomotor behavior analysis revealed hypolocomotion activity in larvae caused by DMSO, possibly attributable to its anxiolytic effects already reported (Sackerman *et al.* 2010). Although polydatin appears to have a low toxicity profile, the solvent DMSO can exert toxic effects even at concentrations considered low. Researchers should aim to minimize the use of DMSO or use alternative solvents to improve the bioavailability of polydatin without compromising safety.

5. Conclusion

Our study provides valuable information on the toxicity of polydatin through a comprehensive toxicological screening using the zebrafish model. The zebrafish model, coupled with the FET test, proved to be an effective and efficient tool for assessing the toxicological profile of polydatin during critical developmental stages. By analyzing different parameters, including survival, morphological observations, hatchability, cardiac function and behavioral assessments, we obtained a comprehensive understanding of polydatin's effects in this model organism. Finally, results shown here indicate a promising non-toxic profile for polydatin, suggesting its potential as a viable candidate for various human therapeutic applications and consumer products, and also encouraging researchers for further preclinical studies and clinicians in future exploration.

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3 **Disclosure statement**

4 The authors report there are no competing interests to declare.

5 **Data Availability Statement**

6 The data that support the findings of this study are available from the corresponding
7 author, [M.A.H], upon reasonable request.

References

- Abrão, L. da C., Costa-Silva, D.G., Santos, M.G. dos, Cerqueira, M.B.R., Badiale-Furlong, E., Muccillo-Baisch, A.L., and Hort, M.A., 2022. Toxicity evaluation of traditional and organic yerba mate (*Ilex paraguariensis* A. St.-Hil.) extracts. *Journal of Toxicology and Environmental Health, Part A*, 85 (11), 461–479.
- Aleström, P., D'Angelo, L., Midtlyng, P.J., Schorderet, D.F., Schulte-Merker, S., Sohm, F., and Warner, S., 2020. Zebrafish: Housing and husbandry recommendations. *Laboratory Animals*, 54 (3), 213–224.
- Amri, A., Chaumeil, J.C., Sfar, S., and Charrueau, C., 2012. Administration of resveratrol: What formulation solutions to bioavailability limitations? *Journal of Controlled Release*, 158 (2), 182–193.
- Avdesh, A., Chen, M., Martin-Iverson, M.T., Mondal, A., Ong, D., Rainey-Smith, S., Taddei, K., Lardelli, M., Groth, D.M., Verdile, G., and Martins, R.N., 2012. Regular Care and Maintenance of a Zebrafish (*Danio rerio*) Laboratory: An Introduction. *Journal of Visualized Experiments*, (69).
- Bai, L., Ma, Y., Wang, X., Feng, Q., Zhang, Z., Wang, S., Zhang, H., Lu, X., Xu, Y., Zhao, E., and Cui, H., 2021. Polydatin Inhibits Cell Viability, Migration, and Invasion Through Suppressing the c-Myc Expression in Human Cervical Cancer. *Frontiers in Cell and Developmental Biology*, 9 (587218).
- Basnet, R., Zizioli, D., Taweedet, S., Finazzi, D., and Memo, M., 2019. Zebrafish Larvae as a Behavioral Model in Neuropharmacology. *Biomedicines*, 7 (1), 23.
- Bauer, B., Mally, A., and Liedtke, D., 2021. Zebrafish Embryos and Larvae as Alternative Animal Models for Toxicity Testing. *International Journal of Molecular Sciences*, 22 (24), 13417.
- Brannen, K.C., Panzica-Kelly, J.M., Danberry, T.L., and Augustine-Rauch, K.A., 2010. Development of a zebrafish embryo teratogenicity assay and quantitative prediction model. *Birth Defects Research Part B - Developmental and Reproductive Toxicology*, 89 (1), 66–77.
- Cadena, P.G., Sales Cadena, M.R., Sarmah, S., and Marrs, J.A., 2020. Protective effects of quercetin, polydatin, and folic acid and their mixtures in a zebrafish (*Danio rerio*) fetal

- 1 alcohol spectrum disorder model. *Neurotoxicology and Teratology*, 82, 106928.
- 2 Castro, C.A., Hogan, J.B., Benson, K.A., Shehata, C.W., and Landauer, M.R., 1995.
- 3 Behavioral effects of vehicles: DMSO, ethanol, Tween-20, Tween-80, and emulphor-620.
- 4 *Pharmacology Biochemistry and Behavior*, 50 (4), 521–526.
- 5 Cavalcante, A.K., Lopes-Ferreira, M., Rogero, S.O., and Rogero, J.R., 2017. Evaluation
- 6 of resveratrol toxicity in the embryolarval stage of Danio rerio fish. *Ecotoxicology and*
- 7 *Environmental Contamination*, 12 (1), 133–139.
- 8 Cavaletti, G., Oggioni, N., Sala, F., Pezzoni, G., Cavalletti, E., Marmiroli, P., Petruccioli,
- 9 M., Frattola, L., and Tredici, G., 2000. Effect on the peripheral nervous system of
- 10 systemically administered dimethylsulfoxide in the rat: a neurophysiological and
- 11 pathological study. *Toxicology Letters*, 118 (1–2), 103–107.
- 12 Chen, G., Yang, Z., Wen, D., Guo, J., Xiong, Q., Li, P., Zhao, L., Wang, J., Wu, C., and
- 13 Dong, L., 2021. Polydatin has anti-inflammatory and antioxidant effects in LPS-induced
- 14 macrophages and improves DSS-induced mice colitis. *Immunity, Inflammation and*
- 15 *Disease*, 9 (3), 959–970.
- 16 Chen, T.-H., Wang, Y.-H., and Wu, Y.-H., 2011. Developmental exposures to ethanol or
- 17 dimethylsulfoxide at low concentrations alter locomotor activity in larval zebrafish:
- 18 Implications for behavioral toxicity bioassays. *Aquatic Toxicology*, 102 (3–4), 162–166.
- 19 Chen, Y., Zhang, D., Liao, Z., Wang, B., Gong, S., Wang, C., Zhang, M., Wang, G., Cai,
- 20 H., Liao, F.-F., and Xu, J., 2015. Anti-oxidant polydatin (piceid) protects against
- 21 substantia nigral motor degeneration in multiple rodent models of Parkinson's disease.
- 22 *Molecular Neurodegeneration*, 10 (1), 4.
- 23 Chiffre, A., Clérandeau, C., Dwoinikoff, C., Le Bihanic, F., Budzinski, H., Geret, F., and
- 24 Cachot, J., 2016. Psychotropic drugs in mixture alter swimming behaviour of Japanese
- 25 medaka (*Oryzias latipes*) larvae above environmental concentrations. *Environmental*
- 26 *Science and Pollution Research*, 23 (6), 4964–4977.
- 27 Christou, M., Kavaliauskis, A., Ropstad, E., and Fraser, T.W.K., 2020. DMSO effects
- 28 larval zebrafish (*Danio rerio*) behavior, with additive and interaction effects when
- 29 combined with positive controls. *Science of The Total Environment*, 709, 134490.
- 30 CONCEA, 2018. Diretriz Da Prática de Eutanásia Do CONCEA - Resolução Normativa

- 1 n°37. In: *Brazil: Conselho Nacional de Experimentação Animal*.
- 2 Du, Q.H., Peng, C., and Zhang, H., 2013. Polydatin: A review of pharmacology and
- 3 pharmacokinetics. *Pharmaceutical Biology*, 51 (11), 1347–1354.
- 4 Falcão, M.A.P., de Souza, L.S., Dolabella, S.S., Guimarães, A.G., and Walker, C.I.B.,
- 5 2018. Zebrafish as an alternative method for determining the embryo toxicity of plant
- 6 products: a systematic review. *Environmental Science and Pollution Research*, 25 (35),
- 7 35015–35026.
- 8 Falomir, E., Lucas, R., Peñalver, P., Martí-Centelles, R., Dupont, A., Zafra-Gómez, A.,
- 9 Carda, M., and Morales, J.C., 2016. Cytotoxic, Antiangiogenic and Antitelomerase
- 10 Activity of Glucosyl- and Acyl- Resveratrol Prodrugs and Resveratrol Sulfate
- 11 Metabolites. *ChemBioChem*, 17 (14), 1343–1348.
- 12 Gao, Y., Chen, T., Lei, X., Li, Y., Dai, X., Cao, Y., Ding, Q., Lei, X., Li, T., and Lin, X.,
- 13 2016. Neuroprotective effects of polydatin against mitochondrial-dependent apoptosis in
- 14 the rat cerebral cortex following ischemia/reperfusion injury. *Molecular Medicine*
- 15 *Reports*, 14 (6), 5481–5488.
- 16 Gerhardt, A., 2007. Aquatic Behavioral Ecotoxicology—Prospects and Limitations.
- 17 *Human and Ecological Risk Assessment: An International Journal*, 13 (3), 481–491.
- 18 Goldsmith, P., 2004. Zebrafish as a pharmacological tool: the how, why and when.
- 19 *Current Opinion in Pharmacology*, 4 (5), 504–512.
- 20 Gonzalez-Alfonso, J.L., Peñalver, P., Ballesteros, A.O., Morales, J.C., and Plou, F.J.,
- 21 2019. Effect of α -Glucosylation on the Stability, Antioxidant Properties, Toxicity, and
- 22 Neuroprotective Activity of (–)-Epigallocatechin Gallate. *Frontiers in Nutrition*, 6 (30).
- 23 von Hellfeld, R., Brotzmann, K., Baumann, L., Strecker, R., and Braunbeck, T., 2020.
- 24 Adverse effects in the fish embryo acute toxicity (FET) test: a catalogue of unspecific
- 25 morphological changes versus more specific effects in zebrafish (*Danio rerio*) embryos.
- 26 *Environmental Sciences Europe*, 32 (1), 122.
- 27 Hobbs, C.A., Koyanagi, M., Swartz, C., Davis, J., Kasamoto, S., Maronpot, R., Recio, L.,
- 28 and Hayashi, S., 2018. Comprehensive evaluation of the flavonol anti-oxidants, alpha-
- 29 glycosyl isoquercitrin and isoquercitrin, for genotoxic potential. *Food and Chemical*
- 30 *Toxicology*, 113, 218–227.

- 1 Hoyberghs, J., Bars, C., Ayuso, M., Van Ginneken, C., Foubert, K., and Van Cruchten, S.,
2 2021. DMSO Concentrations up to 1% are Safe to be Used in the Zebrafish Embryo
3 Developmental Toxicity Assay. *Frontiers in Toxicology*, 3.
- 4 Hu, W.-H., Wang, H.-Y., Kong, X.-P., Xiong, Q.-P., Poon, K.K.-M., Xu, L., Duan, R.,
5 Chan, G.K.-L., Dong, T.T.-X., and Tsim, K.W.-K., 2019. Polydatin suppresses VEGF-
6 induced angiogenesis through binding with VEGF and inhibiting its receptor signaling.
7 *The FASEB Journal*, 33 (1), 532–544.
- 8 Huang, Y., Cartlidge, R., Walpitagama, M., Kaslin, J., Campana, O., and Wlodkowic, D.,
9 2018. Unsuitable use of DMSO for assessing behavioral endpoints in aquatic model
10 species. *Science of The Total Environment*, 615, 107–114.
- 11 Irons, T.D., MacPhail, R.C., Hunter, D.L., and Padilla, S., 2010. Acute neuroactive drug
12 exposures alter locomotor activity in larval zebrafish. *Neurotoxicology and Teratology*,
13 32 (1), 84–90.
- 14 Jiang, C., Ma, L., Lv, Z., Feng, F., Chen, Z., and Liu, Z.-D., 2020. Polydatin induces
15 apoptosis and autophagy via STAT3 signaling in human osteosarcoma MG-63 cells.
16 *Journal of Natural Medicines*, 74 (3), 533–544.
- 17 Kalueff, A. V., Stewart, A.M., and Gerlai, R., 2014. Zebrafish as an emerging model for
18 studying complex brain disorders. *Trends in Pharmacological Sciences*, 35 (2), 63–75.
- 19 Karami, A., Fakhri, S., Kooshki, L., and Khan, H., 2022. Polydatin: Pharmacological
20 Mechanisms, Therapeutic Targets, Biological Activities, and Health Benefits. *Molecules*,
21 27 (19), 6474.
- 22 Kilkenney, C., Browne, W., Cuthill, I.C., Emerson, M., and Altman, D.G., 2010. Animal
23 research: Reporting in vivo experiments: The ARRIVE guidelines. *British Journal of*
24 *Pharmacology*, 160 (7), 1577–1579.
- 25 Kimmel, C.B., Ballard, W.W., Kimmel, S.R., Ullmann, B., and Schilling, T.F., 1995.
26 Stages of embryonic development of the zebrafish. *Developmental Dynamics*, 203 (3),
27 253–310.
- 28 Lantz-McPeak, S., Guo, X., Cuevas, E., Dumas, M., Newport, G.D., Ali, S.F., Paule,
29 M.G., and Kanungo, J., 2015. Developmental toxicity assay using high content screening
30 of zebrafish embryos. *Journal of Applied Toxicology*, 35 (3), 261–272.

- 1 Luo, J., Chen, S., Wang, L., Zhao, X., and Piao, C., 2022. Pharmacological effects of
2 polydatin in the treatment of metabolic diseases: A review. *Phytomedicine*, 102, 154161.
- 3 Ma, Y., Gong, X., Mo, Y., and Wu, S., 2016. Polydatin inhibits the oxidative stress-
4 induced proliferation of vascular smooth muscle cells by activating the eNOS/SIRT1
5 pathway. *International Journal of Molecular Medicine*, 37 (6), 1652–1660.
- 6 MacPhail, R.C., Brooks, J., Hunter, D.L., Padnos, B., Irons, T.D., and Padilla, S., 2009.
7 Locomotion in larval zebrafish: Influence of time of day, lighting and ethanol.
8 *NeuroToxicology*, 30 (1), 52–58.
- 9 Maeda, H., Fukushima, N., and Hasumi, A., 2021. Standardized Method for the
10 Assessment of Behavioral Responses of Zebrafish Larvae. *Biomedicines*, 9 (8), 884.
- 11 Maes, J., Verlooy, L., Buenafe, O.E., de Witte, P.A.M., Esguerra, C. V., and Crawford,
12 A.D., 2012. Evaluation of 14 Organic Solvents and Carriers for Screening Applications
13 in Zebrafish Embryos and Larvae. *PLoS ONE*, 7 (10), e43850.
- 14 Méndez-Líter, J.A., Pozo-Rodríguez, A., Madruga, E., Rubert, M., Santana, A.G., de
15 Eugenio, L.I., Sánchez, C., Martínez, A., Prieto, A., and Martínez, M.J., 2022.
16 Glycosylation of Epigallocatechin Gallate by Engineered Glycoside Hydrolases from
17 *Talaromyces amestolkiae*: Potential Antiproliferative and Neuroprotective Effect of
18 These Molecules. *Antioxidants*, 11 (7), 1325.
- 19 Meng, Q., Liu, H., and Wang, J., 2016. Polydatin ameliorates renal ischemia/reperfusion
20 injury by decreasing apoptosis and oxidative stress through activating sonic hedgehog
21 signaling pathway. *Food and Chemical Toxicology*, 96, 215–225.
- 22 Modarresi Chahardehi, A., Arsad, H., and Lim, V., 2020. Zebrafish as a Successful
23 Animal Model for Screening Toxicity of Medicinal Plants. *Plants*, 9 (10), 1345.
- 24 Nadim, M., Auriol, D., Lamerant-FayeL, N., Lefèvre, F., Dubanet, L., Redziniak, G.,
25 Kieda, C., and Grillon, C., 2014. Improvement of polyphenol properties upon
26 glucosylation in a UV-induced skin cell ageing model. *International Journal of Cosmetic
27 Science*, 36 (6), 579–587.
- 28 Nyska, A., Hayashi, S., Koyanagi, M., Davis, J.P., Jokinen, M.P., Ramot, Y., and
29 Maronpot, R.R., 2016. Ninety-day toxicity and single-dose toxicokinetics study of alpha-
30 glycosyl isoquercitrin in Sprague-Dawley rats. *Food and Chemical Toxicology*, 97, 354–

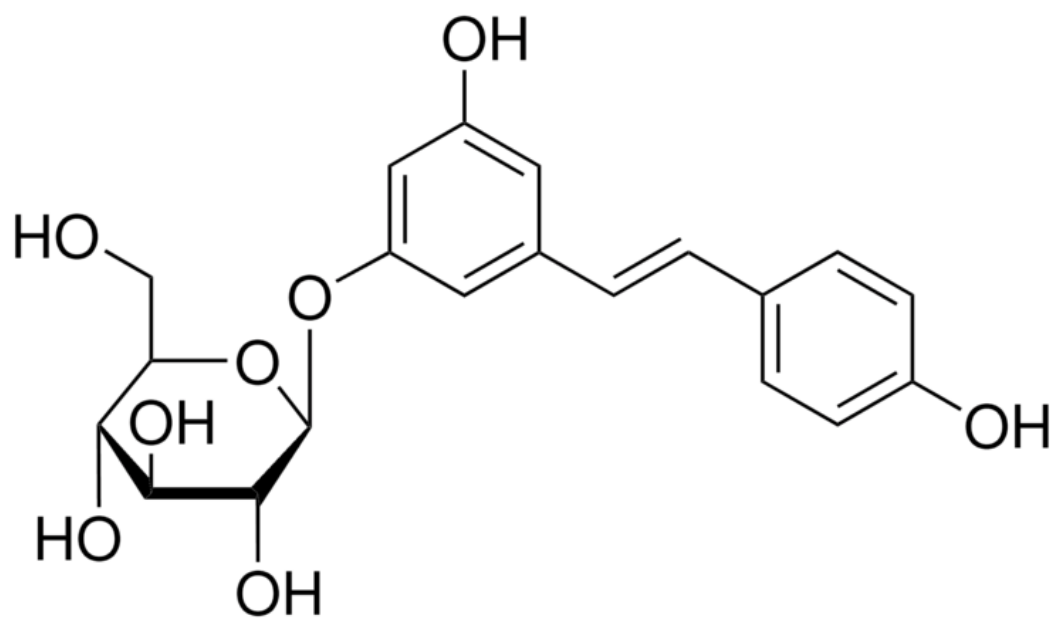
- 1 366.
- 2 OECD, 2006. *Current Approaches in the Statistical Analysis of Ecotoxicity Data*. OECD
- 3 Series on Testing and Assessment. Paris: OECD Publishing.
- 4 OECD, 2013. *Test No. 236: Fish Embryo Acute Toxicity (FET) Test, OECD Guidelines*
- 5 *for the Testing of Chemicals*. Paris: OECD Publishing.
- 6 Ogungbemi, A., Leuthold, D., Scholz, S., and Küster, E., 2019. Hypo- or hyperactivity of
- 7 zebrafish embryos provoked by neuroactive substances: a review on how experimental
- 8 parameters impact the predictability of behavior changes. *Environmental Sciences*
- 9 *Europe*, 31 (1), 88.
- 10 Padilla, S., Hunter, D.L., Padnos, B., Frady, S., and MacPhail, R.C., 2011. Assessing
- 11 locomotor activity in larval zebrafish: Influence of extrinsic and intrinsic variables.
- 12 *Neurotoxicology and Teratology*, 33 (6), 624–630.
- 13 Peritore, A.F., D’Amico, R., Cordaro, M., Siracusa, R., Fusco, R., Gugliandolo, E.,
- 14 Genovese, T., Crupi, R., Di Paola, R., Cuzzocrea, S., and Impellizzeri, D., 2021.
- 15 PEA/Polydatin: Anti-Inflammatory and Antioxidant Approach to Counteract DNBS-
- 16 Induced Colitis. *Antioxidants*, 10 (3), 464.
- 17 Pype, C., Verbueken, E., Saad, M.A., Casteleyn, C.R., Van Ginneken, C.J., Knapen, D.,
- 18 and Van Cruchten, S.J., 2015. Incubation at 32.5°C and above causes malformations in
- 19 the zebrafish embryo. *Reproductive Toxicology*, 56, 56–63.
- 20 Rivero-Wendt, C.L.G., Fernandes, L.G., dos Santos, A.N., Brito, I.L., dos Santos Jaques,
- 21 J.A., dos Santos dos Anjos, E., and Fernandes, C.E., 2023. Effects of Chloramine T on
- 22 zebrafish embryos malformations associated with cardiotoxicity and neurotoxicity.
- 23 *Journal of Toxicology and Environmental Health, Part A*, 86 (11), 372–381.
- 24 Rodrigues Esperandim, T., Barcelos Ribeiro, A., Silva Squarisi, I., Teixeira Marcos de
- 25 Souza, L., Olimpio de Souza, T., Oliveira Acésio, N., Ferreira Conceição Santos, M.,
- 26 Kenupp Bastos, J., Ricardo Ambrósio, S., and Crispim Tavares, D., 2023. Toxicological
- 27 and chemoprevention studies of Brazilian brown propolis from Araucaria sp. *Journal of*
- 28 *Toxicology and Environmental Health, Part A*, 1–12.
- 29 Rosa, J.G.S., Lima, C., and Lopes-Ferreira, M., 2022. Zebrafish Larvae Behavior Models
- 30 as a Tool for Drug Screenings and Pre-Clinical Trials: A Review. *International Journal of*

- 1 *Molecular Sciences*, 23 (12), 6647.
- 2 Sackerman, J., Donegan, J.J., Cunningham, C.S., Nguyen, N.N., Lawless, K., Long, A.,
3 Benno, R.H., and Gould, G.G., 2010. Zebrafish Behavior in Novel Environments: Effects
4 of Acute Exposure to Anxiolytic Compounds and Choice of *Danio rerio* Line.
5 *International journal of comparative psychology*, 23 (1), 43–61.
- 6 Schimith, L.E., dos Santos, M.G., Arbo, B.D., André-Miral, C., Muccillo-Baisch, A.L.,
7 and Hort, M.A., 2022. Polydatin as a therapeutic alternative for central nervous system
8 disorders: A systematic review of animal studies. *Phytotherapy Research*, 36 (7), 2852–
9 2877.
- 10 Shah, M.A., Hamid, A., Faheem, H.I., Rasul, A., Baokbah, T.A.S., Haris, M., Yousaf, R.,
11 Saleem, U., Iqbal, S., Alves, M.S., Khan, Z., Hussain, G., Alsharfi, I., Khan, H., and
12 Jeandet, P., 2022. Uncovering the Anticancer Potential of Polydatin: A Mechanistic
13 Insight. *Molecules*, 27 (21), 7175.
- 14 Stewart, A.M., Braubach, O., Spitsbergen, J., Gerlai, R., and Kalueff, A. V., 2014.
15 Zebrafish models for translational neuroscience research: from tank to bedside. *Trends in*
16 *Neurosciences*, 37 (5), 264–278.
- 17 Subramanian, L., Youssef, S., Bhattacharya, S., Kenealey, J., Polans, A.S., and van
18 Ginkel, P.R., 2010. Resveratrol: Challenges in Translation to the Clinic — A Critical
19 Discussion. *Clinical Cancer Research*, 16 (24), 5942–5948.
- 20 Tan, J.X.M., Ang, R.J.W., and Wee, C.L., 2022. Larval Zebrafish as a Model for
21 Mechanistic Discovery in Mental Health. *Frontiers in Molecular Neuroscience*, 15.
- 22 Tan, Y.-Y., Chen, L.-X., Fang, L., and Zhang, Q., 2020. Cardioprotective effects of
23 polydatin against myocardial injury in diabetic rats via inhibition of NADPH oxidase and
24 NF- κ B activities. *BMC Complementary Medicine and Therapies*, 20 (1), 378.
- 25 Tang, D., Zhang, Q., Duan, H., Ye, X., Liu, J., Peng, W., and Wu, C., 2022. Polydatin: A
26 Critical Promising Natural Agent for Liver Protection via Antioxidative Stress. *Oxidative*
27 *Medicine and Cellular Longevity*, 2022, 1–14.
- 28 Westerfield, M., 2000. *The zebrafish book. A guide for the laboratory use of zebrafish*
29 (*Danio rerio*). 4th Editio. Eugene, USA: University of Oregon Press.
- 30 Wong, K., Elegante, M., Bartels, B., Elkhayat, S., Tien, D., Roy, S., Goodspeed, J., Suci, S.,

- 1 C., Tan, J., Grimes, C., Chung, A., Rosenberg, M., Gaikwad, S., Denmark, A., Jackson,
2 A., Kadri, F., Chung, K.M., Stewart, A., Gilder, T., Beeson, E., Zapolsky, I., Wu, N.,
3 Cachat, J., and Kalueff, A. V., 2010. Analyzing habituation responses to novelty in
4 zebrafish (*Danio rerio*). *Behavioural Brain Research*, 208 (2), 450–457.
- 5 Wu, M., Li, X., Wang, S., Yang, S., Zhao, R., Xing, Y., and Liu, L., 2020. Polydatin for
6 treating atherosclerotic diseases: A functional and mechanistic overview. *Biomedicine &*
7 *Pharmacotherapy*, 128 (May), 110308.
- 8 Wu, Y., Xue, L., Du, W., Huang, B., Tang, C., Liu, C., Qiu, H., and Jiang, Q., 2015.
9 Polydatin Restores Endothelium-Dependent Relaxation in Rat Aorta Rings Impaired by
10 High Glucose: A Novel Insight into the PPAR β -NO Signaling Pathway. *PLOS ONE*, 10
11 (5), e0126249.
- 12 Zhang, Q., Tan, Y., Zhang, N., and Yao, F., 2015. Polydatin prevents angiotensin II-
13 induced cardiac hypertrophy and myocardial superoxide generation. *Experimental*
14 *Biology and Medicine*, 240 (10), 1352–1361.
- 15 Zou, J., Yang, Y., Yang, Y., and Liu, X., 2018. Polydatin suppresses proliferation and
16 metastasis of non-small cell lung cancer cells by inhibiting NLRP3 inflammasome
17 activation via NF- κ B pathway. *Biomedicine & Pharmacotherapy*, 108, 130–136.

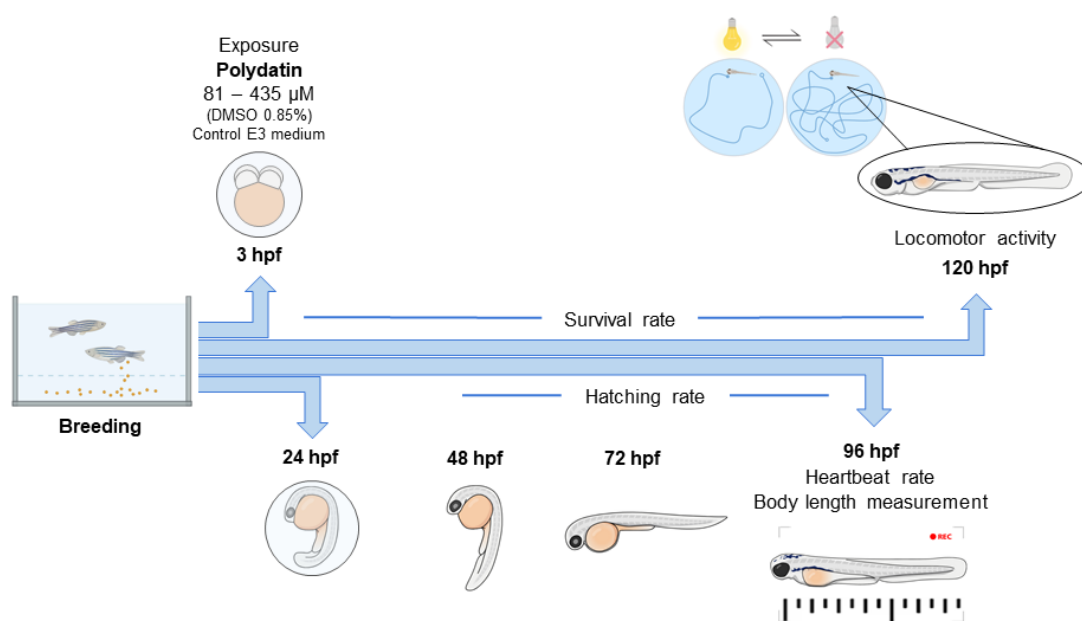
1 **Figures**

2 **Figure 1.**



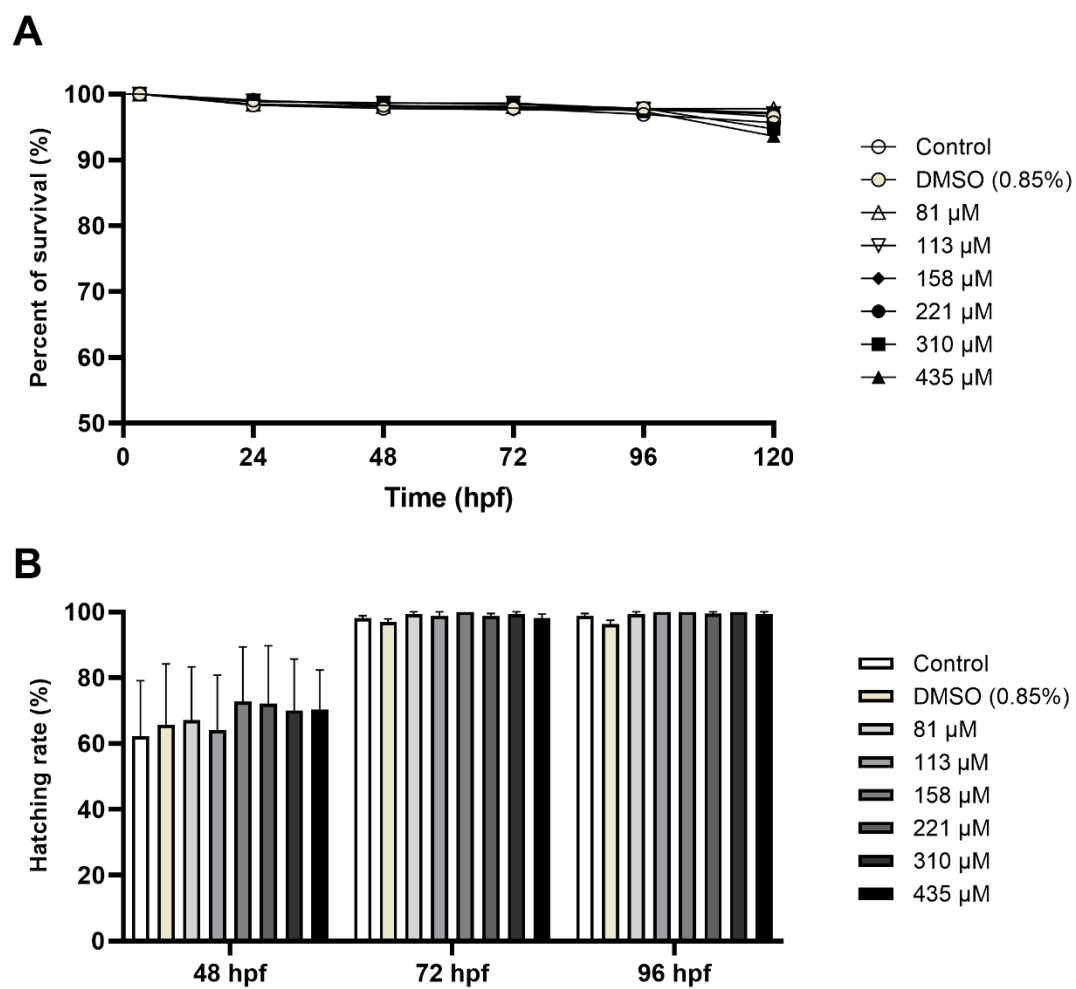
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1 **Figure 2.**

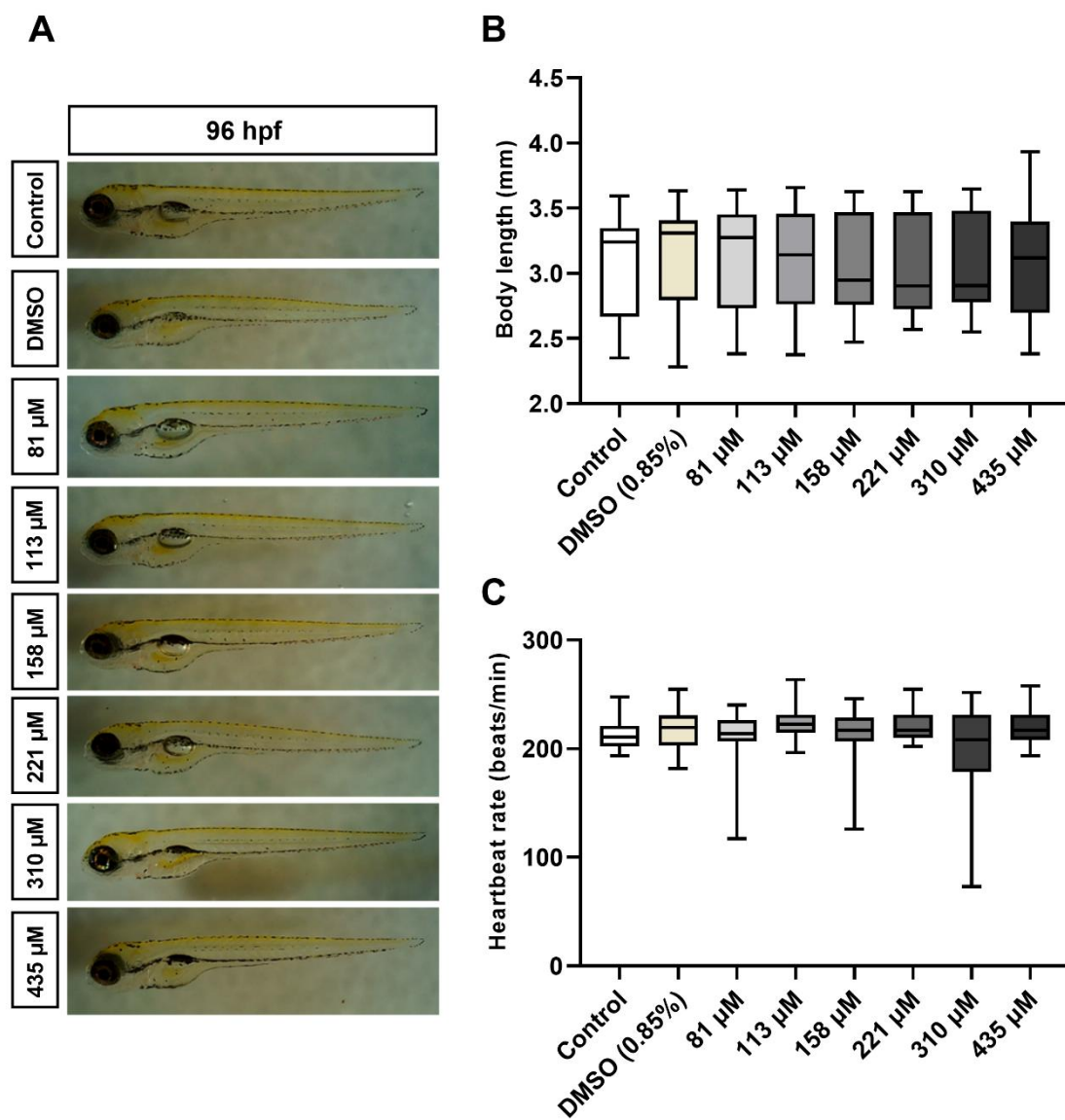
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1 **Figure 3.**

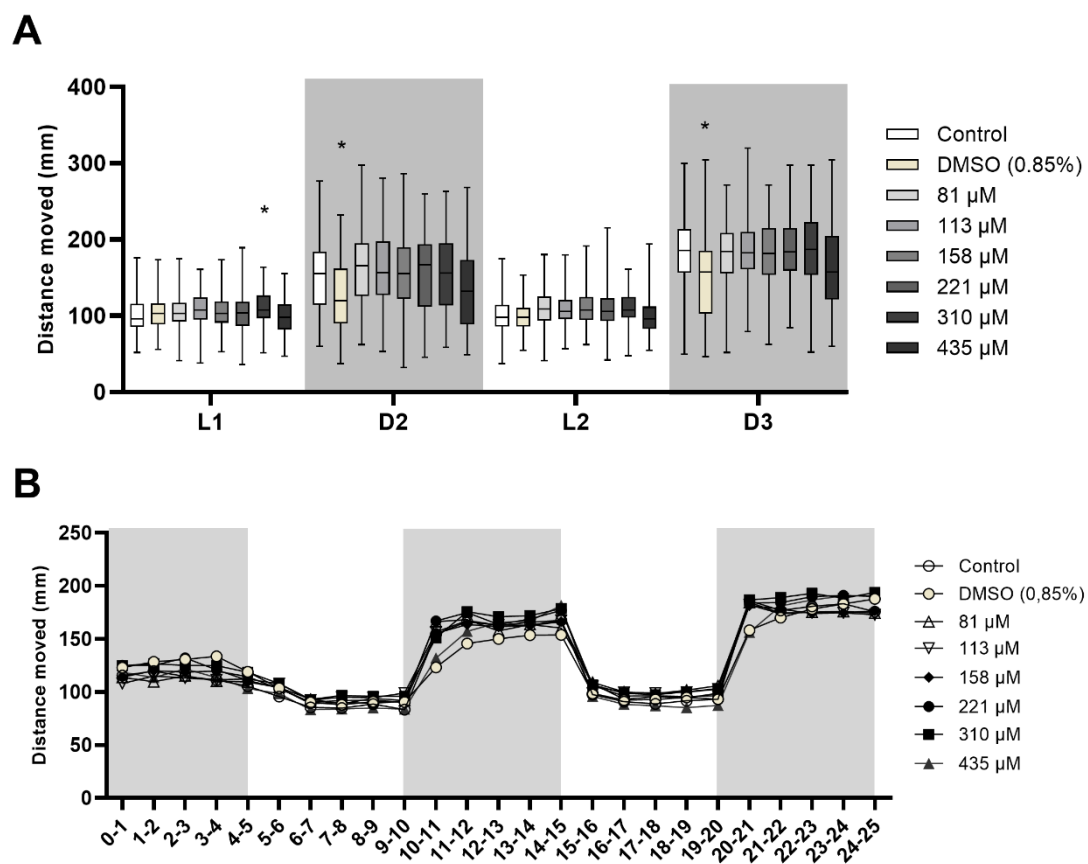
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1 **Figure 4.**

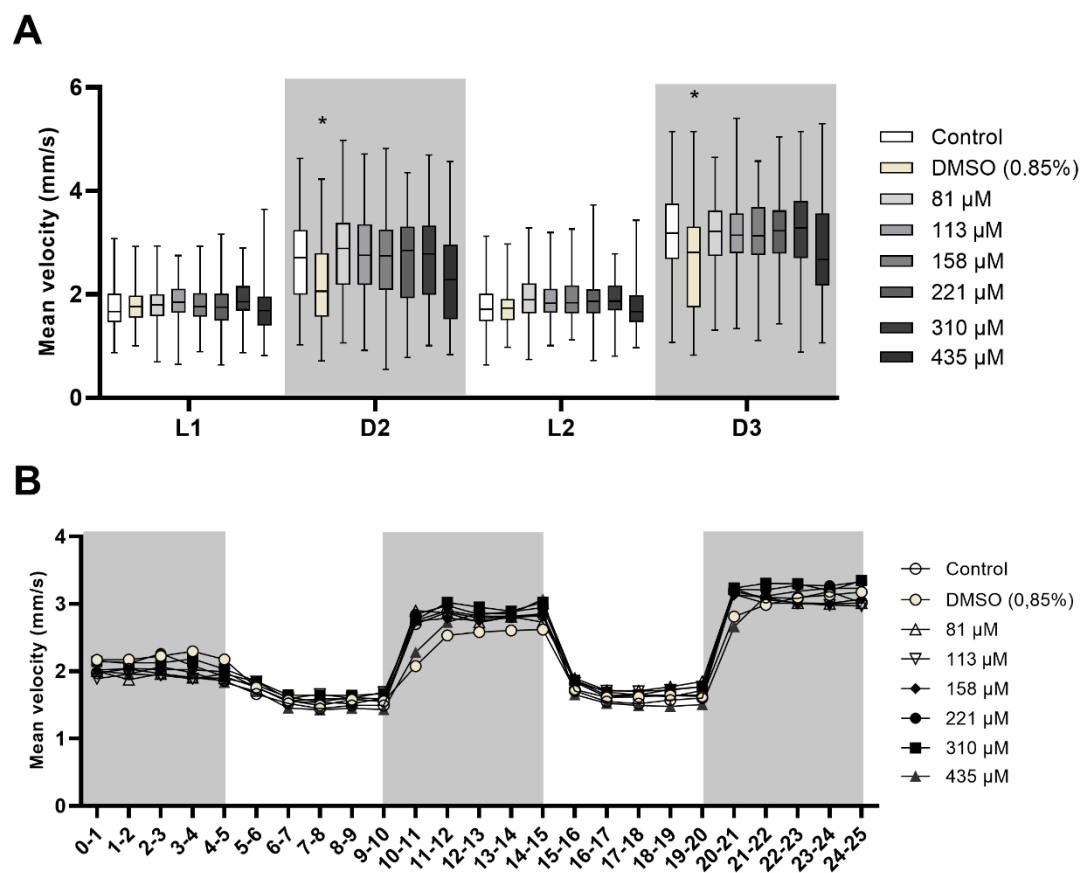
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1 **Figure 5.**

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1 **Figure 6.**

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Figure Captions

Figure 1. Chemical structure of polydatin (3,4',5-trihydroxystilbene-3- β -D-glucoside).

Figure 2. Experimental design. Based on OECD guideline 236 (FET test – Fish embryo toxicity test).

Figure 3. Polydatin exhibits a wide range of non-lethal concentrations from 81-435 μ M. 3 hours post-fertilization zebrafish embryos (hpf) were static exposed to 0 (E3 medium, control group), 81, 113, 158, 221, 310, and 435 μ M of polydatin, and DMSO (0.85%, vehicle group) up to 120 hpf. (A) Animals were monitored daily for survival rate and data were analyzed using Mantel-Cox test and expressed as percent of survival. (B) Hatching rate was evaluated at 48, 72, and 96 hpf; data analyzed through one-way ANOVA followed by Tukey's post-hoc test and expressed as mean $\pm$ SEM. *Represents significant at $p < 0.05$ in relation to control group (n=156).

Figure 4. (A) Representative images, (B) body length (mm), and (C) heartbeat rate (bpm) of 96 hpf zebrafish larvae exposed to 0 (E3 medium, control group), 81, 113, 158, 221, 310, and 435 μ M of polydatin, and DMSO (0.85%, vehicle group). Animals were anesthetized by immersion in MS-222 (0.02%) and immobilized in methylcellulose (3%) for images acquisition in a G1200 digital microscope. Data analyzed through Kruskal-Wallis test followed by Dunn's post-hoc test and expressed as median $\pm$ interquartile range. *Represents significant at $p < 0.05$ in relation to control group (n=21-38 animals/group).

Figure 5. Distance moved (mm) in locomotor activity of zebrafish larvae exposed to exposed to 0 (E3 medium, control group), 81, 113, 158, 221, 310, and 435 μ M of polydatin, and DMSO (0.85%, vehicle group). 120 hpf animals were submitted to photolocomotor response assay alternating light and dark conditions (25 min: 5 min cycles). (A) Distance moved during the first minute of each light-dark transition analyzed through Kruskal-Wallis test followed by Dunn's post-hoc test and expressed as median $\pm$ interquartile range. *Represents significant at $p < 0.05$ in relation to control group (n=32-38). (B) Representative diagram of distance moved (mm) throughout the photolocomotor response assay.

Figure 6. Mean velocity (mm/s) in locomotor activity of zebrafish larvae exposed to exposed to 0 (E3 medium, control group), 81, 113, 158, 221, 310, and 435 μ M of

1 polydatin, and DMSO (0.85%, vehicle group). 120 hpf animals were submitted to
2 photolocomotor response assay alternating light and dark conditions (25 min: 5 min
3 cycles). (A) Mean velocity during the first minute of each light-dark transition analyzed
4 through Kruskal-Wallis test followed by Dunn's post-hoc test and expressed as median $\pm$
5 interquartile range. *Represents significant at $p < 0.05$ in relation to control group (n=32-
6 38). (B) Representative chronological diagram of mean velocity (mm/s) throughout the
7 photolocomotor response assay.

CAPÍTULO 3 – MANUSCRITO 3**“Resveratrol glycosylated derivatives protect MPTP-induced locomotor deficit in zebrafish model of parkinsonism”**

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Abstract

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by the loss of dopaminergic neurons in the substantia nigra, leading to motor impairments such as bradykinesia, rigidity, and tremors. Resveratrol (3,4',5-*trans*-trihydroxystilbene), a polyphenol with neuroprotective properties, has been extensively studied for its antioxidant and anti-inflammatory effects. However, its clinical application is limited due to low bioavailability and rapid metabolism. Structural modifications, such as glycosylation, have emerged as a promising strategy to improve its solubility, stability, and bioavailability. In this study, we evaluated the neuroprotective effects of polydatin (resveratrol-3-*O*- β -D-glucoside, piceid) and resveratrol-3- α -glucoside (resveratrol-3-*O*- α -D-glucoside) in a zebrafish model of MPTP-induced parkinsonism. Larvae at 96 hours post-fertilization (hpf) were submitted to a pretreatment with polydatin or resveratrol-3- α -glucoside at 100, 200, and 400 μ M. At 120 hpf, larvae were exposed to MPTP (50 μ M) to induce dopaminergic neurotoxicity, and locomotor activity was assessed under basal conditions and light-dark transition at 168 hpf. Our results demonstrated that MPTP exposure significantly reduced distance moved and mean velocity, indicating severe locomotor impairment. Pretreatment with polydatin and resveratrol-3- α -glucoside (100 μ M) effectively mitigated these deficits, preserving basal locomotion. However, resveratrol-3- α -glucoside exhibited a transient enhancement in locomotor response to light-dark transitions, while polydatin did not alter stimulus-induced locomotion. The neuroprotective effects observed may be related to antioxidant and anti-inflammatory properties. These findings suggest that polydatin and resveratrol-3- α -glucoside have potential as therapeutic alternatives for PD, offering neuroprotective benefits on locomotor deficit.

Keywords: Parkinson's disease, neuroprotection, glycosylations, polydatin, piceid

1. Introduction

Parkinson's Disease (PD) is one of the most prevalent neurodegenerative disorders, affecting millions of people worldwide and significantly impairing their quality of life (Dorsey et al., 2018; Pringsheim et al., 2014). It is primarily characterized by the progressive loss of dopaminergic neurons in the substantia nigra, a brain region responsible for movement coordination. This neuronal loss leads to a decline in dopamine levels, a crucial neurotransmitter for motor control regulation (Kalia and Lang, 2015; Lees et al., 2009). The resulting dopaminergic deficiency is directly related to the development of the hallmark motor symptoms of PD, including resting tremors, muscle rigidity, bradykinesia, and postural instability (Rodriguez-Oroz et al., 2009; Váradi, 2020). In addition to motor symptoms, PD also manifests non-motor complications such as sleep disturbances and cognitive dysfunction (Chaudhuri and Schapira, 2009). Currently available therapeutic options primarily focus on symptom management, without offering a cure or reversing PD progression (Connolly and Lang, 2014; Murakami et al., 2023; Zahoor et al., 2018). This highlights the urgent need for novel therapeutic strategies capable of not only alleviating symptoms but also modifying the course of the disease.

In this context, studies have shown that resveratrol (3,4',5-*trans*-trihydroxystilbene), a polyphenol found in various plant sources, exhibits neuroprotective properties, related to its antioxidant and anti-inflammatory activities, that may be beneficial in the treatment of neurodegenerative diseases, including PD (Anandhan et al., 2010; Arbo et al., 2020; dos Santos et al., 2022; Jin et al., 2008; Khan et al., 2010; Lofrumento et al., 2014; Zeng et al., 2017; Zhang et al., 2018). However, its therapeutic potential use is significantly compromised by several limitations. One of the main issues is its low bioavailability, which refers to the fraction of the compound that reaches systemic circulation and is available to exert biological effects (Walle, 2011). After ingestion, resveratrol is rapidly metabolized in the liver and intestines, resulting in a quick elimination from the body, consequently reducing its concentration in plasma and limiting its ability to reach target tissues such as the central nervous system (Walle et al., 2004).

The glycosylation of resveratrol has been proposed as a strategy to increase its bioavailability, potentially improving its neuroprotective properties. This approach is based on the conjugation of the molecule of interest with a sugar moiety, increasing its polarity and, consequently, its solubility (Hu et al., 2024; Moradi et al., 2016; Regev-

Shoshani et al., 2003; Xiao et al., 2014). One of the glycosylated derivatives of resveratrol that has been studied is polydatin (resveratrol-3-*O*- β -D-glucoside) (Fig. 1A), a glycosylated precursor in the biosynthesis of resveratrol in plants. Polydatin exhibits a β -type linkage between glucose and the resveratrol backbone, a bond type known to occur naturally during the synthesis of these phytoalexins in plants (Du et al., 2013; Schimith et al., 2022). Adding a glycosidic moiety can influence key pharmacokinetic processes, including absorption, distribution, metabolism, and excretion of the aglycone. This modification serves as a strategy to enhance the solubility of natural compounds, thereby improving their bioavailability and biological activity (Klingel et al., 2019; Slámová et al., 2018). Studies have demonstrated that different glycosylated derivatives of resveratrol exhibit antioxidant, anti-inflammatory, and neuroprotective properties, often surpassing those of its aglycone form (Larrosa et al., 2010; Peñalver et al., 2018; Wang et al., 2015). Glycosylation can also be performed synthetically through enzymes capable of catalyzing transglycosylation reactions (Shaikh and Withers, 2008). Kraus and colleagues (2017) investigated the enzyme sucrose phosphorylase from *Bifidobacterium adolescentis*, a glycosidase that catalyzes the reversible phosphorolysis reaction of sucrose into α -D-glucose, allowing the transfer of the glucose unit to different acceptors, including resveratrol. Using the Q345F variant of sucrose phosphorylase, they synthesized resveratrol-3- α -glucoside (resveratrol-3-*O*- α -D-glucoside) (Fig. 1B).

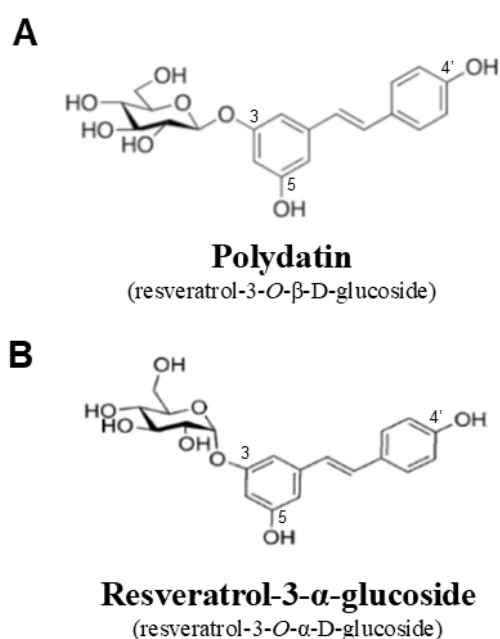


Figure 1. Chemical structure of (A) polydatin and (B) resveratrol-3- α -glucoside.

Experimental models, such as zebrafish larvae, have proven effective for evaluating new therapeutic strategies under neurotoxic conditions, such as parkinsonism induced by the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (Bríñez-Gallego et al., 2023; Buhidma et al., 2020). Despite the promising evidence regarding the neuroprotective effects of resveratrol, literature lacks studies that assess the effects of glycosylation of this compound in models of induced parkinsonism, particularly of resveratrol-3- α -glucoside. This study aims to assess the neuroprotective effects of polydatin and resveratrol-3- α -glucoside in a locomotor deficit model of parkinsonism induced by MPTP in zebrafish larvae, contributing to the understanding of the therapeutic potential of glycosylated resveratrol derivatives. Additionally, we investigate how the type of glycosidic link, whether α or β , influences the biological activity and neuroprotective properties of these compounds.

2. Material and methods

2.1. Chemicals and solutions

Polydatin (purity $\geq 95\%$, CAS number 65914-17-2) and MPTP (CAS number 23007-85-4) were purchased from Sigma-Aldrich (St. Louis, USA). Resveratrol-3- α -glucoside was synthesized from resveratrol (CAS number 501-36-0) using the Q345F mutant of sucrose phosphorylase from *Bifidobacterium adolescentis*, following the protocols of Kraus et al., (2017) and Demonceaux et al., (2023). Stock solutions of polydatin and resveratrol-3- α -glucoside were prepared in dimethyl sulfoxide (DMSO, CAS number: 67-68-5), while MPTP stock solution was prepared by dissolving the powder in ultrapure water. All stock solutions were stored at $-20\text{ }^{\circ}\text{C}$. Test solutions of polydatin, resveratrol-3- α -glucoside, and MPTP were prepared by diluting the required volume of their respective stock solution in E3 medium (5 mM NaCl, 0.17 mM KCl, 0.33 mM CaCl_2 , 0.33 mM MgSO_4 , pH 7.3, and dissolved oxygen (8 mg/L)).

2.2. Animals

Adult wild-type zebrafish (*Danio rerio*) were purchased from Delphis Aquários e Pet Shop (Porto Alegre, RS, Brazil) and maintained in a recirculating aquatic system (Altamar, Brazil) in 10 L tanks in a fish density of 1.5 fish/L, under standard conditions of pH 7.2 ± 0.5 , temperature $27.5 \pm 1\text{ }^{\circ}\text{C}$, conductivity $500 \pm 50\text{ }\mu\text{S/cm}$, water hardness 75–200 mg/L CaCO_3 , and 12/12h light/dark cycle. Adult fish were fed 2–3 times daily with brine shrimp (*Artemia salina*) and commercial dry flakes (Aleström et al., 2020;

Avdesh et al., 2012; Westerfield, 2000). For breeding, females and males were maintained at a ratio of 1:1 overnight, and to prevent egg predation, the bottom of the tanks was equipped with a net, allowing only the eggs to pass through. The following morning, fertilized eggs were collected, and embryos were then sanitized and maintained in E3 medium in a biochemical oxygen demand (B.O.D) incubator, under constant temperature (27.5 ± 0.5 °C) and light/dark cycle of 12/12h until the beginning of the experiments. During this period, the E3 medium was renewed daily.

2.3. Experimental design

Larvae at 96 hours post-fertilization (hpf) were arbitrarily distributed into 96-well plate, following the arrangement of one animal per well. Polydatin and resveratrol-3- α -glucoside concentrations used were selected based on a previous study conducted by our research group, which determined the non-toxic concentrations of polydatin (Schimith et al., 2024). MPTP concentration used to induce parkinsonism (50 μ M) was based on literature (Mo et al., 2023; Ren et al., 2022; Zhang et al., 2020). Animals in the vehicle group were exposed to DMSO (0.78%), corresponding to the highest DMSO concentration present in the polydatin and resveratrol-3- α -glucoside treatments, diluted in E3 medium. To check the influence of DMSO on the neurotoxicity induced by MPTP, a group of larvae was exposed to MPTP 50 μ M+DMSO 0.78%, and another one only to MPTP 50 μ M. Control group was exposed exclusively to E3 medium.

Larvae at 96 hpf were exposed by immersion to a pretreatment of polydatin and resveratrol-3- α -glucoside at concentrations of 100, 200, and 400 μ M. The 96-well plates were maintained in a B.O.D incubator, under a constant temperature of 27.5 ± 0.5 °C and 12/12h light/dark cycle (~300 lux) during the experiment period. At 120 hpf, the pretreatment solutions were renewed and, immediately after, MPTP (50 μ M) was added to the already existing exposure solution. After 24 hours, animals were monitored for possible mortality. At 168 hpf, larvae without detectable morphological alterations were submitted to locomotor activity assessment (Fig. 2).

All procedures followed the guidelines of the National Council for the Control of Animal Experimentation (CONCEA). At the end of the experiments, larvae were euthanized according to the Brazilian Guide to Euthanasia Practices of the CONCEA by immersion in cooled and freezing water (0–4 °C) for 20 minutes until complete cessation of movement, ensuring death by hypoxia (CONCEA, 2018). Experimental procedures

were approved by the Animal Use Ethics Committee of the Federal University of Rio Grande (process number 23116.006597/2023-07). The ARRIVE guideline was used for reporting *in vivo* experiments (Kilkenny et al., 2010).

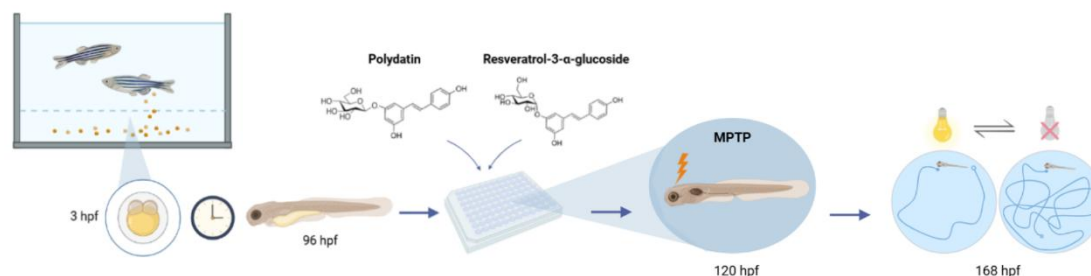


Figure 2. Experimental design.

2.4. Locomotor activity

Larval locomotor activity was assessed using two different open-field behavioral methods, under darkness and under the influence of light and dark transitions. Tests were performed between 10:00 a.m. and 3:30 p.m. to minimize variations in the metabolic activity, using DanioVision acquisition system (Noldus Information Technology®, Netherlands). At 168 hpf, the experiment 96-well plate was placed inside the DanioVision chamber and acclimated at 27.5 ± 0.5 °C for 10 minutes in darkness. Larval locomotion was first tracked under constant dark conditions (infrared light) for 25 minutes. Immediately afterward, a light-dark transition stimulus was applied, consisting of 25 minutes of alternating light (~900 lux) and dark conditions in five 5 transition cycles of 5 minutes each (Maeda et al., 2021). The parameters analyzed included distance moved (mm), mean velocity (mm/s), and maximum acceleration (mm/s^2) using Ethovision XT version 11.5 (Noldus Information Technology®, Netherlands).

2.5. Statistical analysis

Statistical analyses were performed using GraphPad Prism software, version 9 for Windows (GraphPad Software, USA). Outliers were identified using interquartile range extreme method ($\text{IQR} \times 3$), and data normal distribution was checked using Kolmogorov-Smirnov. As data were determined to be non-parametric, the Kruskal-Wallis non-parametric test was applied, followed by Dunn's post-hoc test. Results are presented as median $\pm$ interquartile range values and the level of significance was set at $p \leq 0.05$.

3. Results and discussion

1 Zebrafish central nervous and catecholaminergic systems exhibit notably
2 evolutionary conservation compared to other vertebrates, including mammals and
3 humans. Their neuroanatomical organization, homologous regions shared, such as
4 dopaminergic midbrain, and neurotransmitters regulation, including dopamine and
5 noradrenaline, are highly similar (Panula et al., 2010). All these characteristics make
6 zebrafish a robust model for studying neurodegenerative diseases such as PD. The clinical
7 motor symptoms of PD reflect movement dysfunction resulting from the loss of
8 dopaminergic neurons. By analyzing locomotor phenotypes in experimental models,
9 such as zebrafish larvae, it is possible to observe locomotor alterations that mimic these
10 symptoms, allowing the investigation of the neuroprotective potential of new treatment
11 strategies (Meredith and Rademacher, 2011; Raza et al., 2019).

12 Zebrafish locomotor network develops in distinct phases, beginning with
13 spontaneous tail coiling movements around 17 hpf, followed by an escape response to
14 mechanical stimuli at 2-3 days post-fertilization (dpf) and showing a mature swimming
15 pattern by 5 dpf (Brustein et al. 2003). The assessment of locomotor activity in
16 parkinsonism models provides valuable insights into motor behavior and neurological
17 function (Sheta et al., 2024). Basal locomotor response reflects the spontaneous motor
18 activity of the animal in a controlled environment, allowing the identification of motor
19 deficits associated with neurotoxicity, such as that induced by MPTP (Seibenhener and
20 Wooten, 2015). On the other hand, the light-dark transition test evaluated not only
21 locomotor deficits but also alterations in response to visual stimuli. Typically, a transition
22 from light to dark conditions induces an increase in swimming activity, while a transition
23 from dark to light causes an immediate decrease in activity (Irons et al., 2010; MacPhail
24 et al., 2009). Evaluation under light conditions can reveal alterations in motor
25 coordination and locomotor activity that may not be evident under constant darkness
26 (Burgess and Granato, 2007). This approach is particularly relevant for understanding
27 how sensory perception influences locomotor behavior in parkinsonism models,
28 providing a more comprehensive understanding of the underlying mechanisms of motor
29 dysfunction and their implications for PD.

30 Previous studies have demonstrated locomotor impairment in the MPTP-induced
31 zebrafish parkinsonism model. MPTP neurotoxicity occurs through its metabolism
32 into 1-methyl-4-phenylpyridinium ion (MPP⁺) by the enzyme monoamine oxidase B,
33 present in glial cells. MPP⁺ is the toxic metabolite that selectively targets dopaminergic

neurons, leading to their death (Przedborski et al., 2004). Our data indicate that the DMSO (0.78%) vehicle did not induce significant changes in the distance moved compared to the control group (Fig. S1A), it also did not alter the effects of MPTP when co-administered. Therefore, we used the DMSO (0.78%) group as vehicle control and the DMSO (0.78%) + MPTP (50 μ M) group as the parkinsonism model in subsequent experiments (Fig. S1B).

In our study, exposure to MPTP (50 μ M) resulted in a significant reduction in larval locomotor activity compared to the vehicle group ($p < 0.05$) in both behavioral tests, under dark conditions (Fig. 3) and light-dark transition stimuli (Fig. 4). Distance moved and mean velocity were significantly decreased following MPTP exposure, indicating pronounced motor impairment consistent with dopaminergic neurotoxicity. These alterations in locomotor parameters might suggest a diminished capacity for spontaneous movement and a disruption in motor coordination.

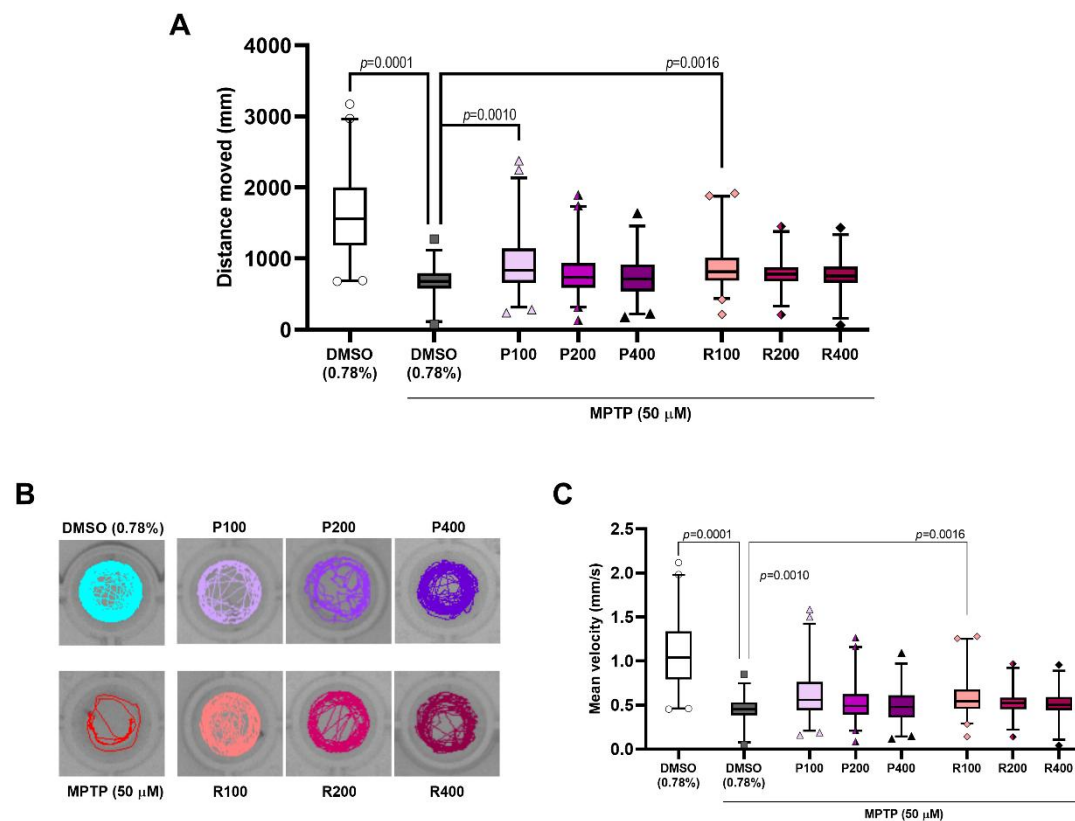


Figure 3. Effect of polydatin and resveratrol-3- α -glucoside on MPTP-induced locomotor impairment under constant dark conditions. (A) Distance moved (mm). (B) Representative track map. (C) Mean velocity (mm/s). Data were analyzed through Kruskal-Wallis test followed by Dunn's post-hoc test and expressed as median $\pm$ interquartile range (DMSO (0.78%) $n=93$; DMSO (0.78%) + MPTP (50 μ M) $n=74$; P100 $n=91$; P200 $n=88$; P400 $n=83$; R100 $n=84$; R200 $n=77$; R400 $n=63$).

Polydatin and resveratrol-3- α -glucoside pretreatment at 100 μ M effectively minimized the locomotor deficits caused by MPTP-induced parkinsonism. Larvae exhibited an increase in distance moved and mean velocity compared to the MPTP-treated group ($p=0.0010$ and $p=0.0016$, respectively), indicating a consistent preservation of basal locomotion (Fig. 3). In contrast, resveratrol-3- α -glucoside exhibited a transient effect on locomotor activity in response to light-dark transitions, specifically in the minute immediately following the transitions (Fig. 4 and 5). Meanwhile, polydatin showed no neuroprotective effect when locomotor response to stimuli was evaluated. During first dark-light transition, larvae treated with resveratrol-3- α -glucoside 400 at μ M showed a significant increase in distance moved (Fig. 4B) and mean velocity (Fig. 5A) compared to MPTP-treated group ($p=0.001$). At 10-11 min, when the light was switched off again, resveratrol-3- α -glucoside at 100 μ M increased the locomotor response by increasing distance moved ($p=0.0065$) (Fig. 4B), mean velocity ($p=0.0077$) (Fig. 5A), and maximum acceleration ($p=0.0094$) (Fig. 4B) compared to the MPTP-treated group. However, in the final two transitions (15-16 min and 20-21 min) no differences were observed between resveratrol-3- α -glucoside-treated groups and MPTP-treated larvae (Fig. 4 and 5). The lack of effect in the final light-dark transitions might be attributed to behavioral habituation over the analyzed period. Habituation, or reduced response to repeated stimuli, is a common form of simple non-associative learning. It has been observed in sensorimotor behavior alterations triggered by repeated abrupt transitions between adverse conditions, leading to a progressive decrease in behavioral startle responses (Rankin et al., 2009; Thompson and Spencer, 1966).

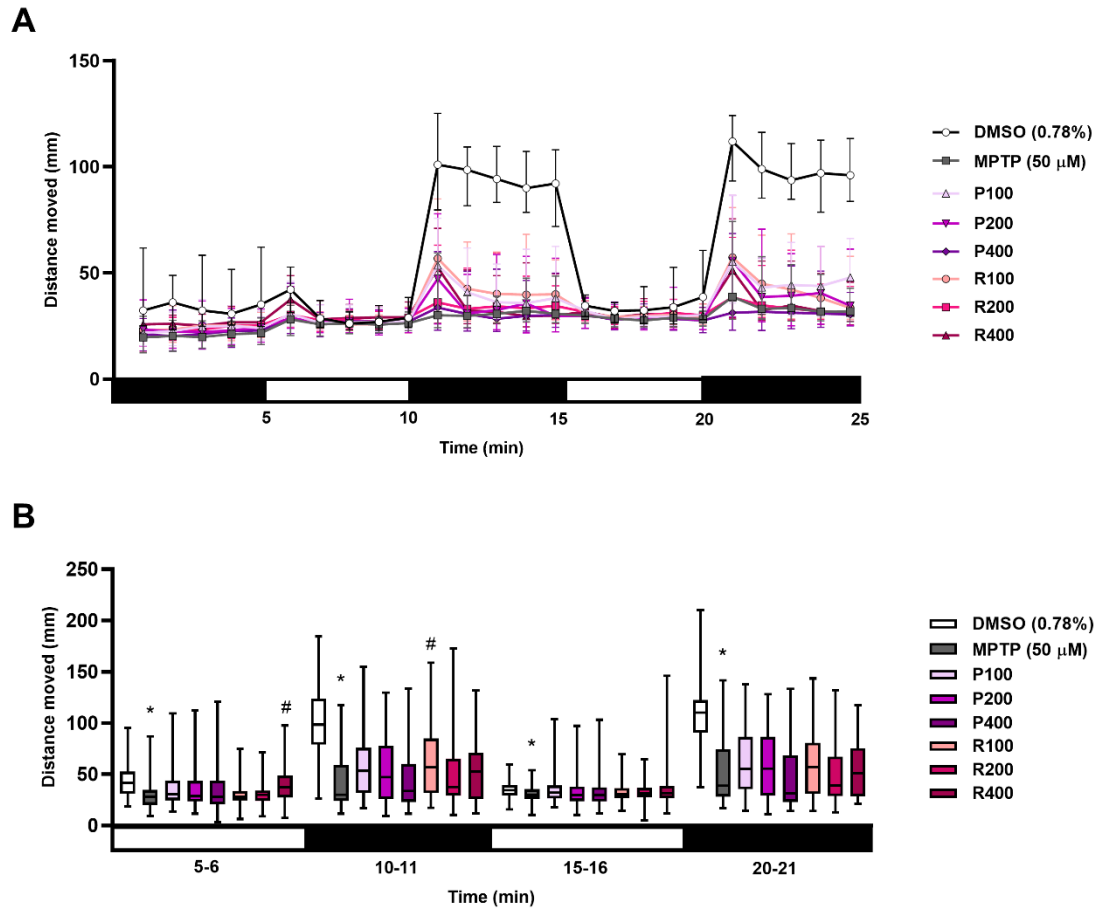


Figure 4. Distance moved (mm) in locomotor activity of zebrafish larvae under light-dark transitions (A) Representative diagram of distance moved (mm) throughout light-dark transitions. (B) Distance moved during the first minute of each light-dark transition analyzed through Kruskal-Wallis test followed by Dunn's post-hoc test and expressed as median $\pm$ interquartile range. *represents significant difference at $p < 0.05$ in relation to control group and # in relation to MPTP-treated group (DMSO (0.78%) $n=93$; DMSO (0.78%) + MPTP (50 μ M) $n=74$; P100 $n=91$; P200 $n=88$; P400 $n=83$; R100 $n=84$; R200 $n=77$; R400 $n=63$).

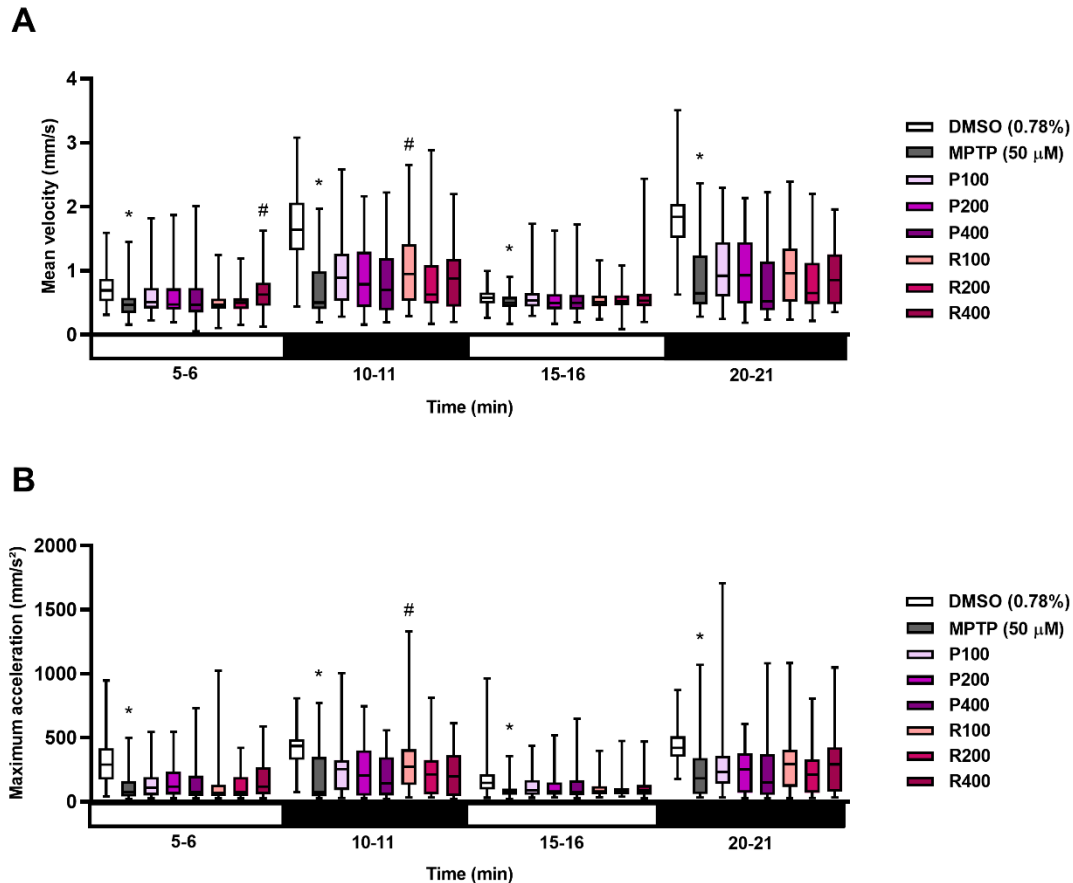


Figure 5. (A) Mean velocity and (B) maximum acceleration in locomotor activity of zebrafish larvae under light-dark transitions. Parameters were analyzed during the first minute of each light-dark transition using Kruskal-Wallis test followed by Dunn's post-hoc test and expressed as median $\pm$ interquartile range. *represents significant difference at $p < 0.05$ in relation to control group and # in relation to MPTP-treated group (DMSO (0.78%) $n=93$; DMSO (0.78%) + MPTP (50 μ M) $n=74$; P100 $n=91$; P200 $n=88$; P400 $n=83$; R100 $n=84$; R200 $n=77$; R400 $n=63$).

Polydatin and resveratrol-3- α -glucoside likely exert their neuroprotective effects through multiple mechanisms, including antioxidant and anti-inflammatory. Resveratrol and its derivatives, such as polydatin, are well-documented for their ability to mitigate oxidative stress by scavenging reactive oxygen species (ROS) and enhancing endogenous antioxidant defenses mainly via Nrf-2 activation (Anandhan et al., 2010; Chen et al., 2015; Huang et al., 2018). Additionally, resveratrol and polydatin are known for modulating inflammatory responses by the inhibition of NF- κ B activation and reduction of pro-inflammatory cytokine levels, which are implicated in PD progression (Ji et al., 2012; Lanzilli et al., 2012; Xu et al., 2018; Ye et al., 2017). Taking into account that MPTP-induced neurotoxicity is largely related to mitochondrial dysfunction, polydatin and resveratrol-3- α -glucoside may also protect against dopaminergic neuron loss by preserving mitochondrial membrane potential and preventing excessive apoptosis through pathways involving SIRT1 and PGC-1 α activation (Bheerreddy et al., 2021; Li et

al., 2019, 2015; Yang et al., 2017; Zhu et al., 2025). Furthermore, the addition of a glycosidic moiety appears to be associated with reduced toxicity, as polydatin has been shown to be less toxic than resveratrol. Zebrafish embryos exposed to resveratrol at a concentration range similar to those used in this study exhibited a high mortality rate and significant morphological abnormalities, including yolk sac and pericardial edema, absence of eyes and pigmentation, and spinal deformities (Cavalcante et al., 2017). In contrast, polydatin, even at concentrations up to 435 μ M, did not induce any developmental alterations (Schimith et al., 2024). Taken together, these findings indicate that the neuroprotection conferred by resveratrol glycosylated derivatives is likely mediated by a combination of antioxidative, anti-inflammatory, and mitochondrial-protective effects, with possible neuroprotection of dopaminergic pathways, making them promising candidates for further investigation in PD models.

4. Conclusion

Our findings suggest that polydatin and resveratrol-3- α -glucoside showed neuroprotective activity in locomotor deficit caused by MPTP-induced parkinsonism. These effects may be related to the beneficial properties of resveratrol glycosylated derivatives, including its antioxidant and anti-inflammatory activities. Further studies are needed to investigate the mechanism of polydatin and resveratrol-3- α -glucoside neuroprotection.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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References

- Aleström, P., D'Angelo, L., Midtlyng, P.J., Schorderet, D.F., Schulte-Merker, S., Sohm, F., Warner, S., 2020. Zebrafish: Housing and husbandry recommendations. *Lab Anim* 54, 213–224. <https://doi.org/10.1177/0023677219869037>
- Anandhan, A., Tamilselvam, K., Vijayaraja, D., Kumar, N.A., Rajasankar, S., Manivasagam, T., 2010. Resveratrol attenuates oxidative stress and improves behaviour in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) challenged parkinsonic mice. *Ann Neurosci* 17. <https://doi.org/10.5214/ans.0972-7531.1017304>
- Arbo, B.D., André-Miral, C., Nasre-Nasser, R.G., Schimith, L.E., Santos, M.G., Costa-Silva, D., Muccillo-Baisch, A.L., Hort, M.A., 2020. Resveratrol derivatives as potential treatments for Alzheimer's and Parkinson's disease. *Front Aging Neurosci* 12. <https://doi.org/10.3389/fnagi.2020.00103>
- Avdesh, A., Chen, M., Martin-Iverson, M.T., Mondal, A., Ong, D., Rainey-Smith, S., Taddei, K., Lardelli, M., Groth, D.M., Verdile, G., Martins, R.N., 2012. Regular Care and Maintenance of a Zebrafish (*Danio rerio*) Laboratory: An Introduction. *Journal of Visualized Experiments*. <https://doi.org/10.3791/4196>
- Bheerreddy, P., Yerra, V.G., Kalvala, A.K., Sherkhane, B., Kumar, A., 2021. SIRT1 activation by polydatin alleviates oxidative damage and elevates mitochondrial biogenesis in experimental diabetic neuropathy. *Cell Mol Neurobiol* 41, 1563–1577. <https://doi.org/10.1007/s10571-020-00923-1>
- Bríñez-Gallego, P., da Costa Silva, D.G., Cordeiro, M.F., Horn, A.P., Hort, M.A., 2023. Experimental models of chemically induced Parkinson's disease in zebrafish at the embryonic larval stage: a systematic review. *Journal of Toxicology and Environmental Health, Part B* 26, 201–237. <https://doi.org/10.1080/10937404.2023.2182390>
- Buhidma, Y., Rukavina, K., Chaudhuri, K.R., Duty, S., 2020. Potential of animal models for advancing the understanding and treatment of pain in Parkinson's disease. *NPJ Parkinsons Dis* 6, 1. <https://doi.org/10.1038/s41531-019-0104-6>
- Burgess, H.A., Granato, M., 2007. Modulation of locomotor activity in larval zebrafish during light adaptation. *Journal of Experimental Biology* 210, 2526–2539. <https://doi.org/10.1242/jeb.003939>
- Cavalcante, A.K., Lopes-Ferreira, M., Rogero, S.O., Rogero, J.R., 2017. Evaluation of resveratrol toxicity in the embryolarval stage of *Danio rerio* fish. *Ecotoxicology and Environmental Contamination* 12, 133–139. <https://doi.org/10.5132/eec.2017.01.15>
- Chaudhuri, K.R., Schapira, A.H., 2009. Non-motor symptoms of Parkinson's disease: dopaminergic pathophysiology and treatment. *Lancet Neurol* 8, 464–474. [https://doi.org/10.1016/S1474-4422\(09\)70068-7](https://doi.org/10.1016/S1474-4422(09)70068-7)
- Chen, Y., Zhang, D.Q., Liao, Z., Wang, B., Gong, S., Wang, C., Zhang, M.Z., Wang, G.H., Cai, H., Liao, F.F., Xu, J.P., 2015. Anti-oxidant polydatin (piceid) protects against

- 1 substantia nigral motor degeneration in multiple rodent models of Parkinson's disease.
- 2 Mol Neurodegener 10, 1–14. <https://doi.org/10.1186/1750-1326-10-4>
- 3 CONCEA, 2018. Diretriz Da Prática de Eutanásia Do CONCEA - Resolução Normativa
- 4 nº37, in: Brazil: Conselho Nacional de Experimentação Animal.
- 5 Connolly, B.S., Lang, A.E., 2014. Pharmacological treatment of Parkinson disease: A
- 6 review. JAMA - Journal of the American Medical Association 311, 1670–1683.
- 7 <https://doi.org/10.1001/jama.2014.3654>
- 8 Demonceaux, M., Goux, M., Emanuelli Schimith, L., Goulart Dos Santos, M.,
- 9 Hendrickx, J., Offmann, B., André-Miral, C., 2023. Enzymatic synthesis,
- 10 characterization and molecular docking of a new functionalized polyphenol:
- 11 Resveratrol-3,4'- α -diglucoside. Results Chem 5, 100956.
- 12 <https://doi.org/10.1016/j.rechem.2023.100956>
- 13 Dorsey, E.R., Elbaz, A., Nichols, E., Abd-Allah, F., Abdelalim, A., Adsuar, J.C., Ansha,
- 14 M.G., Brayne, C., Choi, J.-Y.J., Collado-Mateo, D., Dahodwala, N., Do, H.P., Edessa,
- 15 D., Endres, M., Fereshtehnejad, S.-M., Foreman, K.J., Gankpe, F.G., Gupta, R., Hankey,
- 16 G.J., Hay, S.I., Hegazy, M.I., Hibstu, D.T., Kasaeian, A., Khader, Y., Khalil, I., Khang,
- 17 Y.-H., Kim, Y.J., Kokubo, Y., Logroscino, G., Massano, J., Mohamed Ibrahim, N.,
- 18 Mohammed, M.A., Mohammadi, A., Moradi-Lakeh, M., Naghavi, M., Nguyen, B.T.,
- 19 Nirayo, Y.L., Ogbo, F.A., Owolabi, M.O., Pereira, D.M., Postma, M.J., Qorbani, M.,
- 20 Rahman, M.A., Roba, K.T., Safari, H., Safiri, S., Satpathy, M., Sawhney, M.,
- 21 Shafieesabet, A., Shiferaw, M.S., Smith, M., Szoeki, C.E.I., Tabarés-Seisdedos, R.,
- 22 Truong, N.T., Ukwaja, K.N., Venketasubramanian, N., Villafaina, S., Weldegwergs, K.
- 23 gidey, Westerman, R., Wijeratne, T., Winkler, A.S., Xuan, B.T., Yonemoto, N., Feigin,
- 24 V.L., Vos, T., Murray, C.J.L., 2018. Global, regional, and national burden of Parkinson's
- 25 disease, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016.
- 26 Lancet Neurol 17, 939–953. [https://doi.org/10.1016/S1474-4422\(18\)30295-3](https://doi.org/10.1016/S1474-4422(18)30295-3)
- 27 dos Santos, M.G., Schimith, L.E., André-Miral, C., Muccillo-Baisch, A.L., Arbo, B.D.,
- 28 Hort, M.A., 2022. Neuroprotective Effects of Resveratrol in In vivo and In vitro
- 29 Experimental Models of Parkinson's Disease: a Systematic Review. Neurotox Res 40,
- 30 319–345. <https://doi.org/10.1007/s12640-021-00450-x>
- 31 Du, Q.H., Peng, C., Zhang, H., 2013. Polydatin: A review of pharmacology and
- 32 pharmacokinetics. Pharm Biol 51, 1347–1354.
- 33 <https://doi.org/10.3109/13880209.2013.792849>
- 34 Hu, S., Wang, B., Pei, L., Wang, J., Gan, Y., Jiang, L., Liu, B., Cheng, J., Li, W., 2024.
- 35 Advances and Challenges in Biomanufacturing of Glycosylation of Natural Products.
- 36 Fermentation 10, 349. <https://doi.org/10.3390/fermentation10070349>
- 37 Huang, B., Liu, J., Meng, T., Li, Y., He, D., Ran, X., Chen, G., Guo, W., Kan, X., Fu, S.,
- 38 Wang, W., Liu, D., 2018. Polydatin prevents lipopolysaccharide (LPS)-induced

- 1 Parkinson's disease via regulation of the AKT/GSK3 β -Nrf2/NF- κ B signaling axis. *Front*
2 *Immunol* 9. <https://doi.org/10.3389/fimmu.2018.02527>
- 3 Irons, T.D., MacPhail, R.C., Hunter, D.L., Padilla, S., 2010. Acute neuroactive drug
4 exposures alter locomotor activity in larval zebrafish. *Neurotoxicol Teratol* 32, 84–90.
5 <https://doi.org/10.1016/j.ntt.2009.04.066>
- 6 Ji, H., Zhang, X., Du, Y., Liu, H., Li, S., Li, L., 2012. Polydatin modulates inflammation
7 by decreasing NF- κ B activation and oxidative stress by increasing Gli1, Ptch1, SOD1
8 expression and ameliorates blood-brain barrier permeability for its neuroprotective
9 effect in pMCAO rat brain. *Brain Res Bull* 87, 50–59.
10 <https://doi.org/10.1016/j.brainresbull.2011.09.021>
- 11 Jin, F., Wu, Q., Lu, Y.-F., Gong, Q.-H., Shi, J.-S., 2008. Neuroprotective effect of
12 resveratrol on 6-OHDA-induced Parkinson's disease in rats. *Eur J Pharmacol* 600, 78–
13 82. <https://doi.org/10.1016/j.ejphar.2008.10.005>
- 14 Kalia, L. V., Lang, A.E., 2015. Parkinson's disease. *The Lancet* 386, 896–912.
15 [https://doi.org/10.1016/S0140-6736\(14\)61393-3](https://doi.org/10.1016/S0140-6736(14)61393-3)
- 16 Khan, Mohd.M., Ahmad, A., Ishrat, T., Khan, M.B., Hoda, Md.N., Khuwaja, G., Raza,
17 S.S., Khan, A., Javed, H., Vaibhav, K., Islam, F., 2010. Resveratrol attenuates 6-
18 hydroxydopamine-induced oxidative damage and dopamine depletion in rat model of
19 Parkinson's disease. *Brain Res* 1328, 139–151.
20 <https://doi.org/10.1016/j.brainres.2010.02.031>
- 21 Kilkenney, C., Browne, W., Cuthill, I.C., Emerson, M., Altman, D.G., 2010. Animal
22 research: Reporting in vivo experiments: The ARRIVE guidelines. *Br J Pharmacol* 160,
23 1577–1579. <https://doi.org/10.1111/j.1476-5381.2010.00872.x>
- 24 Klingel, T., Hadamjetz, M., Fischer, A., Wefers, D., 2019. Glucosylation of flavonoids
25 and flavonoid glycosides by mutant dextransucrase from *Lactobacillus reuteri* TMW
26 1.106. *Carbohydr Res* 483, 107741. <https://doi.org/10.1016/j.carres.2019.107741>
- 27 Kraus, M., Grimm, C., Seibel, J., 2017. Switching enzyme specificity from phosphate to
28 resveratrol glucosylation. *Chemical Communications* 53, 12181–12184.
29 <https://doi.org/10.1039/C7CC05993K>
- 30 Lanzilli, G., Cottarelli, A., Nicotera, G., Guida, S., Ravagnan, G., Fuggetta, M.P., 2012.
31 Anti-inflammatory Effect of Resveratrol and Polydatin by In Vitro IL-17 Modulation.
32 *Inflammation* 35, 240–248. <https://doi.org/10.1007/s10753-011-9310-z>
- 33 Larrosa, M., Tomé-Carneiro, J., Yáñez-Gascón, M.J., Alcántara, D., Selma, M. V.,
34 Beltrán, D., García-Conesa, M.T., Urbán, C., Lucas, R., Tomás-Barberán, F., Morales,
35 J.C., Espín, J.C., 2010. Preventive Oral Treatment with Resveratrol Pro-prodrugs
36 Drastically Reduce Colon Inflammation in Rodents. *J Med Chem* 53, 7365–7376.
37 <https://doi.org/10.1021/jm1007006>

- 1 Lees, A.J., Hardy, J., Revesz, T., 2009. Parkinson's disease. *The Lancet* 373, 2055–
2 2066. [https://doi.org/10.1016/S0140-6736\(09\)60492-X](https://doi.org/10.1016/S0140-6736(09)60492-X)
- 3 Li, L., Tan, H.P., Liu, C.Y., Yu, L.T., Wei, D.N., Zhang, Z.C., Lu, K., Zhao, K. Sen,
4 Maeglele, M., Cai, D.Z., Gu, Z.T., 2019. Polydatin prevents the induction of secondary
5 brain injury after traumatic brain injury by protecting neuronal mitochondria. *Neural*
6 *Regen Res* 14, 1573–1582. <https://doi.org/10.4103/1673-5374.255972>
- 7 Li, P., Wang, X., Zhao, M., Song, R., Zhao, K., 2015. Polydatin protects hepatocytes
8 against mitochondrial injury in acute severe hemorrhagic shock via SIRT1-SOD 2
9 pathway. *Expert Opin Ther Targets* 19, 997–1010.
10 <https://doi.org/10.1517/14728222.2015.1054806>
- 11 Lofrumento, D.D., Nicolardi, G., Cianciulli, A., Nuccio, F. De, Pesa, V. La, Carofiglio,
12 V., Dragone, T., Calvello, R., Panaro, M.A., 2014. Neuroprotective effects of resveratrol
13 in an MPTP mouse model of Parkinson's-like disease: Possible role of SOCS-1 in
14 reducing pro-inflammatory responses. *Innate Immun* 20, 249–260.
15 <https://doi.org/10.1177/1753425913488429>
- 16 MacPhail, R.C., Brooks, J., Hunter, D.L., Padnos, B., Irons, T.D., Padilla, S., 2009.
17 Locomotion in larval zebrafish: Influence of time of day, lighting and ethanol.
18 *Neurotoxicology* 30, 52–58. <https://doi.org/10.1016/j.neuro.2008.09.011>
- 19 Meredith, G.E., Rademacher, D.J., 2011. MPTP Mouse Models of Parkinson's Disease:
20 An Update. *J Parkinsons Dis* 1, 19–33. <https://doi.org/10.3233/JPD-2011-11023>
- 21 Mo, C., Lin, S., Li, Z., Liu, X., Ye, S., Liang, H., Ma, H., Shen, C., Fan, W., Wang, Y.,
22 Liu, K., He, Q., 2023. Qilong Capsule Alleviated MPTP-Induced Neuronal Defects by
23 Inhibiting Apoptosis, Regulating Autophagy in Zebrafish Embryo Model. *Chem*
24 *Biodivers* 20. <https://doi.org/10.1002/cbdv.202200926>
- 25 Moradi, S.V., Hussein, W.M., Varamini, P., Simerska, P., Toth, I., 2016. Glycosylation,
26 an effective synthetic strategy to improve the bioavailability of therapeutic peptides.
27 *Chem Sci* 7, 2492–2500. <https://doi.org/10.1039/C5SC04392A>
- 28 Murakami, H., Shiraishi, T., Umehara, T., Omoto, S., Iguchi, Y., 2023. Recent Advances
29 in Drug Therapy for Parkinson's Disease. *Internal Medicine* 62, 8940–21.
30 <https://doi.org/10.2169/internalmedicine.8940-21>
- 31 Panula, P., Chen, Y.-C., Priyadarshini, M., Kudo, H., Semenova, S., Sundvik, M.,
32 Sallinen, V., 2010. The comparative neuroanatomy and neurochemistry of zebrafish
33 CNS systems of relevance to human neuropsychiatric diseases. *Neurobiol Dis* 40, 46–
34 57. <https://doi.org/10.1016/j.nbd.2010.05.010>
- 35 Peñalver, P., Belmonte-Reche, E., Adán, N., Caro, M., Mateos-Martín, M.L., Delgado,
36 M., González-Rey, E., Morales, J.C., 2018. Alkylated resveratrol prodrugs and
37 metabolites as potential therapeutics for neurodegenerative diseases. *Eur J Med Chem*
38 146, 123–138. <https://doi.org/10.1016/j.ejmech.2018.01.037>

- 1 Pringsheim, T., Jette, N., Frolkis, A., Steeves, T.D.L., 2014. The prevalence of
2 Parkinson's disease: A systematic review and meta-analysis. *Movement Disorders* 29,
3 1583–1590. <https://doi.org/10.1002/mds.25945>
- 4 Przedborski, S., Tieu, K., Perier, C., Vila, M., 2004. MPTP as a Mitochondrial
5 Neurotoxic Model of Parkinson's Disease. *J Bioenerg Biomembr* 36, 375–379.
6 <https://doi.org/10.1023/B:JOB.0000041771.66775.d5>
- 7 Rankin, C.H., Abrams, T., Barry, R.J., Bhatnagar, S., Clayton, D.F., Colombo, J.,
8 Coppola, G., Geyer, M.A., Glanzman, D.L., Marsland, S., McSweeney, F.K., Wilson,
9 D.A., Wu, C.-F., Thompson, R.F., 2009. Habituation revisited: An updated and revised
10 description of the behavioral characteristics of habituation. *Neurobiol Learn Mem* 92,
11 135–138. <https://doi.org/10.1016/j.nlm.2008.09.012>
- 12 Raza, C., Anjum, R., Shakeel, N. ul A., 2019. Parkinson's disease: Mechanisms,
13 translational models and management strategies. *Life Sci* 226, 77–90.
14 <https://doi.org/10.1016/j.lfs.2019.03.057>
- 15 Regev-Shoshani, G., Shoseyov, O., Bilkis, I., Kerem, Z., 2003. Glycosylation of
16 resveratrol protects it from enzymic oxidation. *Biochemical Journal* 374, 157–163.
17 <https://doi.org/10.1042/bj20030141>
- 18 Ren, Q., Jiang, X., Paudel, Y.N., Gao, X., Gao, D., Zhang, P., Sheng, W., Shang, X., Liu,
19 K., Zhang, X., Jin, M., 2022. Co-treatment with natural HMGB1 inhibitor Glycyrrhizin
20 exerts neuroprotection and reverses Parkinson's disease like pathology in Zebrafish. *J*
21 *Ethnopharmacol* 292, 115234. <https://doi.org/10.1016/j.jep.2022.115234>
- 22 Rodriguez-Oroz, M.C., Jahanshahi, M., Krack, P., Litvan, I., Macias, R., Bezard, E.,
23 Obeso, J.A., 2009. Initial clinical manifestations of Parkinson's disease: features and
24 pathophysiological mechanisms. *Lancet Neurol* 8, 1128–1139.
25 [https://doi.org/10.1016/S1474-4422\(09\)70293-5](https://doi.org/10.1016/S1474-4422(09)70293-5)
- 26 Schimith, L.E., dos Santos, M.G., Arbo, B.D., André-Miral, C., Muccillo-Baisch, A.L.,
27 Hort, M.A., 2022. Polydatin as a therapeutic alternative for central nervous system
28 disorders: A systematic review of animal studies. *Phytotherapy Research* 36, 2852–
29 2877. <https://doi.org/10.1002/ptr.7497>
- 30 Schimith, L.E., Machado da Silva, V., Costa-Silva, D.G. da, Seregni Monteiro, L.K.,
31 Muccillo-Baisch, A.L., André-Miral, C., Hort, M.A., 2024. Preclinical toxicological
32 assessment of polydatin in zebrafish model. *Drug Chem Toxicol* 47, 923–932.
33 <https://doi.org/10.1080/01480545.2024.2311287>
- 34 Seibenhener, M.L., Wooten, M.C., 2015. Use of the Open Field Maze to Measure
35 Locomotor and Anxiety-like Behavior in Mice. *Journal of Visualized Experiments*.
36 <https://doi.org/10.3791/52434-v>

- 1 Shaikh, F.A., Withers, S.G., 2008. Teaching old enzymes new tricks: engineering and
2 evolution of glycosidases and glycosyl transferases for improved glycoside synthesis.
3 *Biochemistry and Cell Biology* 86, 169–177. <https://doi.org/10.1139/O07-149>
- 4 Sheta, R., Bérard, M., Musiol, D., Martínez-Drudis, L., Oueslati, A., 2024. Behavioral
5 analysis of motor and non-motor impairment in rodent models of Parkinson’s disease.
6 *Front Aging Neurosci* 16. <https://doi.org/10.3389/fnagi.2024.1464706>
- 7 Slámová, K., Kapešová, J., Valentová, K., 2018. “Sweet Flavonoids”: Glycosidase-
8 Catalyzed Modifications. *Int J Mol Sci* 19, 2126. <https://doi.org/10.3390/ijms19072126>
- 9 Thompson, R.F., Spencer, W.A., 1966. Habituation: A model phenomenon for the study
10 of neuronal substrates of behavior. *Psychol Rev* 73, 16–43.
11 <https://doi.org/10.1037/h0022681>
- 12 Váradi, C., 2020. Clinical Features of Parkinson’s Disease: The Evolution of Critical
13 Symptoms. *Biology (Basel)* 9, 103. <https://doi.org/10.3390/biology9050103>
- 14 Walle, T., 2011. Bioavailability of resveratrol. *Ann N Y Acad Sci* 1215, 9–15.
15 <https://doi.org/10.1111/j.1749-6632.2010.05842.x>
- 16 Walle, T., Hsieh, F., DeLegge, M.H., Oatis, J.E., Walle, U.K., 2004. High absorption but
17 very low bioavailability of oral resveratrol in humans. *Drug Metabolism and*
18 *Disposition* 32, 1377–1382. <https://doi.org/10.1124/dmd.104.000885>
- 19 Wang, H.-L., Gao, J.-P., Han, Y.-L., Xu, X., Wu, R., Gao, Y., Cui, X.-H., 2015.
20 Comparative studies of polydatin and resveratrol on mutual transformation and
21 antioxidative effect in vivo. *Phytomedicine* 22, 553–559.
22 <https://doi.org/10.1016/j.phymed.2015.03.014>
- 23 Westerfield, M., 2000. The zebrafish book. A guide for the laboratory use of zebrafish
24 (*Danio rerio*)., 4th Editio. ed. University of Oregon Press, Eugene, USA.
- 25 Xiao, J., Muzashvili, T.S., Georgiev, M.I., 2014. Advances in the biotechnological
26 glycosylation of valuable flavonoids. *Biotechnol Adv* 32, 1145–1156.
27 <https://doi.org/10.1016/j.biotechadv.2014.04.006>
- 28 Xu, L., Botchway, B.O.A., Zhang, S., Zhou, J., Liu, X., 2018. Inhibition of NF-κB
29 Signaling Pathway by Resveratrol Improves Spinal Cord Injury. *Front Neurosci* 12.
30 <https://doi.org/10.3389/fnins.2018.00690>
- 31 Yang, X., Xu, S., Qian, Y., Xiao, Q., 2017. Resveratrol regulates microglia M1/M2
32 polarization via PGC-1α in conditions of neuroinflammatory injury. *Brain Behav*
33 *Immun* 64, 162–172. <https://doi.org/10.1016/j.bbi.2017.03.003>
- 34 Ye, J., Piao, H., Jiang, J., Jin, G., Zheng, M., Yang, J., Jin, X., Sun, T., Choi, Y.H., Li, L.,
35 Yan, G., 2017. Polydatin inhibits mast cell-mediated allergic inflammation by targeting
36 PI3K/Akt, MAPK, NF-κB and Nrf2/HO-1 pathways. *Sci Rep* 7, 11895.
37 <https://doi.org/10.1038/s41598-017-12252-3>

- 1 Zahoor, I., Shafi, A., Haq, E., 2018. Pharmacological Treatment of Parkinson's Disease,
2 in: Parkinson's Disease: Pathogenesis and Clinical Aspects. Codon Publications, pp.
3 129–144. <https://doi.org/10.15586/codonpublications.parkinsonsdisease.2018.ch7>
- 4 Zeng, W., Zhang, W., Lu, F., Gao, L., Gao, G., 2017. Resveratrol attenuates MPP+
5 induced mitochondrial dysfunction and cell apoptosis via AKT/GSK-3 β pathway in
6 SN4741 cells. *Neurosci Lett* 637, 50–56. <https://doi.org/10.1016/j.neulet.2016.11.054>
- 7 Zhang, L., Yu, X., Ji, M., Liu, S., Wu, X., Wang, Y., Liu, R., 2018. Resveratrol alleviates
8 motor and cognitive deficits and neuropathology in the A53T α -synuclein mouse model
9 of Parkinson's disease. *Food Funct* 9, 6414–6426. <https://doi.org/10.1039/C8FO00964C>
- 10 Zhang, S., Yu, Z., Xia, J., Zhang, X., Liu, K., Sik, A., Jin, M., 2020. Anti-Parkinson's
11 disease activity of phenolic acids from *Eucommia ulmoides* Oliver leaf extracts and
12 their autophagy activation mechanism. *Food Funct* 11, 1425–1440.
13 <https://doi.org/10.1039/C9FO02288K>
- 14 Zhu, L., Yang, M., Fan, L., Yan, Q., Zhang, L., Mu, P., Lu, F., 2025. Interaction between
15 resveratrol and SIRT1: role in neurodegenerative diseases. *Naunyn Schmiedebergs Arch*
16 *Pharmacol* 398, 89–101. <https://doi.org/10.1007/s00210-024-03319-w>

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1 Supplementary material

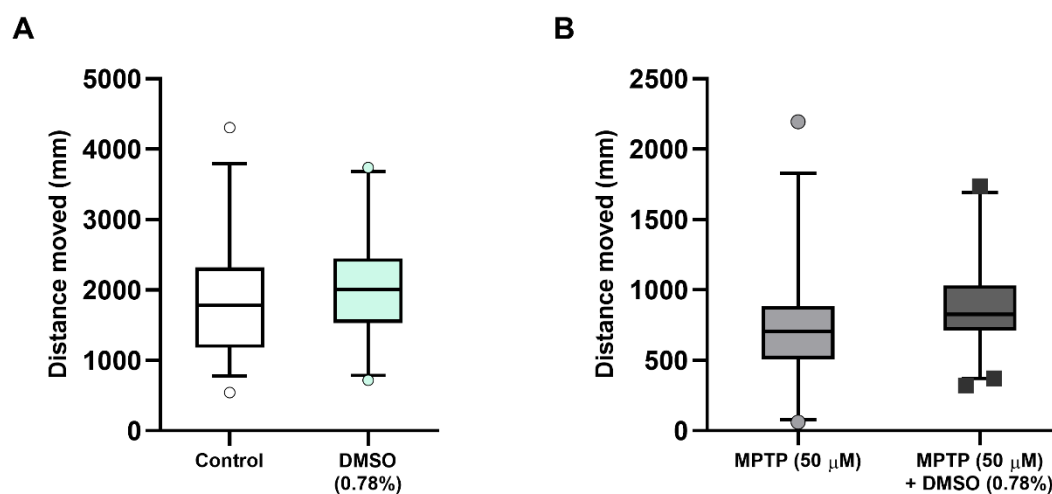


Figure S1. DMSO 0.78% does not influence in locomotor basal locomotion when compared to control group (A), where larvae were exposed exclusively to E3 medium, and in association with MPTP 50 μM (B). Data were analyzed through Kruskal-Wallis test followed by Dunn's post-hoc test and expressed as median ± interquartile range (n=72/group).

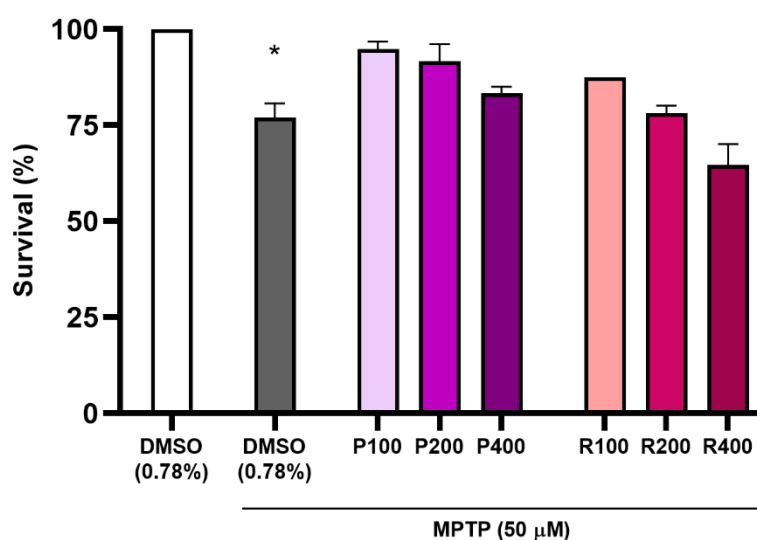


Figure S2. Survival rate. Animals were monitored daily for survival rate and data were analyzed using one-way ANOVA followed by Tukey's post-hoc test and expressed as mean ± SEM. *represents significant difference at $p < 0.05$ in relation to control group (n=72/group).

5. CONCLUSÕES

Neste estudo, investigamos os efeitos neuroprotetores dos derivados glicosilados do RV, polidatina e resveratrol-3- α -glicosídeo, utilizando abordagens *in silico* e *in vivo*. A glicosilação demonstrou-se uma estratégia promissora para otimizar as propriedades farmacocinéticas e a interação molecular desses compostos com alvos envolvidos na patogênese da DP. Nossos resultados sugerem que a modificação estrutural via glicosilação pode desempenhar um papel fundamental na modulação da afinidade e melhora na estabilidade das interações dos derivados glicosilados do RV com proteínas envolvidas na patogênese da DP, especialmente nos alvos TNF- α , PPARG e ERBB2, possivelmente potencializando seus mecanismos neuroprotetores. Além da presença da molécula de glicose, fatores estruturais como o tipo de ligação glicosídica (alfa e beta) e seus efeitos estéricos, parecem influenciar a orientação do ligante e sua acessibilidade aos sítios ativos, potencialmente influenciando a afinidade pelas proteínas avaliadas. Esses resultados contribuem para a compreensão dos determinantes moleculares subjacentes aos mecanismos que podem estar envolvidos nas atividades biológicas do resveratrol e seus derivados glicosilados.

O modelo do peixe-zebra, juntamente com o teste FET, provou ser uma ferramenta eficaz e eficiente para avaliar o perfil toxicológico da polidatina durante os estágios críticos de desenvolvimento. A avaliação *in vivo* da toxicidade aguda da polidatina demonstrou que essa apresenta um perfil de baixa toxicidade na faixa de concentrações avaliada. Parâmetros como sobrevivência, morfologia, taxa de eclosão, função cardíaca e atividade locomotora não foram alterados, reforçando seu perfil não tóxico para investigações futuras. Tais resultados nos guiaram no desenvolvimento do último manuscrito a compor essa tese, onde avaliamos os efeitos neuroprotetores da glicosilação do resveratrol no modelo *in vivo* de parkinsonismo induzido por MPTP em larvas de peixe-zebra. Ambos polidatina e resveratrol-3- α -glicosídeo foram eficazes na proteção contra o déficit locomotor induzido por MPTP, sendo o resveratrol-3- α -glicosídeo particularmente eficiente na resposta a estímulos sensoriais. Como perspectivas futuras, serão realizadas análises adicionais, incluindo a avaliação da expressão gênica de marcadores de neurônios dopaminérgicos, bem como de genes relacionados às respostas anti-inflamatória e antioxidante, a fim de aprofundar a compreensão dos mecanismos envolvidos na neuroproteção observada.

1 Nossos resultados, ainda que preliminares, apresentam achados promissores e
2 instigantes, sugerindo que a glicosilação do RV pode influenciar positivamente sua
3 interação com alvos moleculares da DP e potencializar seus efeitos neuroprotetores *in*
4 *vivo*. No entanto, nosso estudo apresenta limitações. Por se tratar de um *screening* inicial,
5 estudos complementares para aprofundar a compreensão dos mecanismos moleculares
6 envolvidos na neuroproteção apresentada pela polidatina e resveratrol-3- α -glicosídeo são
7 necessários, bem como a validação experimental das interações preditas para elucidar seu
8 potencial farmacológico na DP. Além disso, investigações adicionais são essenciais para
9 avaliar a farmacocinética, biodisponibilidade e os efeitos a longo prazo dos derivados
10 glicosilados do RV em modelos biológicos pré-clínicos mais complexos.

11 Por fim, nossos achados reforçam a viabilidade da glicosilação como estratégia
12 para melhorar os efeitos do resveratrol no contexto da neuroproteção, contribuindo para
13 o desenvolvimento de novas abordagens terapêuticas para DP. Estudos futuros devem
14 explorar e detalhar os mecanismos moleculares envolvidos na atividade neuroprotetora
15 da polidatina e resveratrol-3- α -glicosídeo, além de validar o potencial terapêutico desses
16 compostos em modelos pré-clínicos biológicos mais complexos, visando sua possível
17 aplicação no tratamento da doença.

ANEXOS

CERTIFICADO Nº P022/2022

Certificamos que o projeto intitulado “Avaliação da toxicidade do resveratrol e suas formas glicosiladas em um modelo experimental utilizando zebrafish no seu estágio embrio-larval”, protocolo nº 23116.000500/2022-63, sob a responsabilidade de Mariana Appel Hort - que envolve a produção, manutenção e/ou utilização de animais pertencentes ao Filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa – encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi APROVADO pela COMISSÃO DE ÉTICA EM USO ANIMAL DA UNIVERSIDADE FEDERAL DO RIO GRANDE (CEUA-FURG), em reunião de 27 de abril de 2022 (Ata 004/2022).

A CEUA lembra aos pesquisadores que qualquer alteração no protocolo experimental ou na equipe deve ser encaminhada à comissão para avaliação e aprovação. Um relatório final deve ser enviado à CEUA no término da vigência do seu projeto.

CEUA Nº	Pq004/2022
COLABORADORES AUTORIZADOS A MANIPULAR OS ANIMAIS	Dennis Guilherme da Costa Silva; Lucia Emanueli Schimith
VIGÊNCIA DO PROJETO	31/12/2024
ESPÉCIE/ LINHAGEM / RAÇA	<i>Danio rerio</i> (zebrafish)
NÚMERO DE ANIMAIS	15 adultos (10 machos e 5 fêmeas) 606 embriões
PESO/ IDADE	Adultos – 400 mg Embriões
SEXO	10 Machos e 5 Fêmeas. Demais indeterminado
ORIGEM	Centro de Modelos Biológicos Experimentais (CeMBE), Pontifícia Universidade Católica do Rio Grande do Sul (PUC-RS), Porto Alegre, RS
ENVIO RELATÓRIO PARCIAL	Janeiro de 2023; Janeiro de 2024
ENVIO DO RELATÓRIO FINAL	Janeiro de 2025

Rio Grande, 04 de maio de 2022.

Med. Vet. Márcio de Azevedo Figueiredo
Coordenador da CEUA-FURG

AUTORIZAÇÃO DE ALTERAÇÃO DE VIGÊNCIA Nº 002/2021

PROCESSO Nº	23116.004920/2019-13
CEUA Nº	Pq009/2019
UNIDADE	Instituto de Ciências Biológicas
TÍTULO DO PROJETO	Investigação dos efeitos antioxidantes e neuroprotetores do resveratrol e suas formas glicosiladas em modelo experimental in vivo da Doença de Parkinson
NÚMERO DE ANIMAIS	15 adultos e 1854 embriões
ENVIO DOS RELATÓRIOS PARCIAIS	Janeiro de 2022; janeiro de 2023
ENVIO DO RELATÓRIO FINAL	Março de 2024
PROFESSOR RESPONSÁVEL	Mariana Appel Hort

PARECER DA CEUA:

Após a análise de sua solicitação de alteração de vigência, enviada a essa comissão em 23 de março de 2021, a CEUA-FURG considera **APROVADA** a data de 31 de março de 2024 como término da vigência do projeto.

Rio Grande, 11 de abril de 2021.

Med. Vet. Alice T. Meirelles Leite
Coordenadora da CEUA-FURG



SERVIÇO PÚBLICO FEDERAL
MINISTÉRIO DA EDUCAÇÃO
UNIVERSIDADE FEDERAL DO RIO GRANDE - FURG
PROPESP - COMISSÃO DE ÉTICA EM USO ANIMAL



PARECER N° 14, DE 29 DE FEVEREIRO DE 2024

PROCESSO N°	23116.006597/2023-07
CEUA N°	Pq009/2019
COLABORADORES AUTORIZADOS À MANIPULAR OS ANIMAIS	Dennis Guilherme da Costa Silva; Lucia Emanuelli Schimith; Linda Karolynne Seregni Monteiro; Paola Xiomara Briñez Gallego
TÍTULO DO PROJETO	Investigação dos efeitos antioxidantes e neuroprotetores do resveratrol e suas formas glicosiladas em modelo experimental <i>in vivo</i> da Doença de Parkinson
VIGÊNCIA	31/03/2025
ESPÉCIE/ LINHAGEM / RAÇA	<i>Danio rerio</i> (zebrafish)
NÚMERO DE ANIMAIS	15 Adultos e 1854 Embriões
PESO/ IDADE	Adultos - 600 mg; Embriões 240 ug
SEXO	10 machos e 5 Fêmeas Adultos
ORIGEM	Loja Toca dos Bichos, Rio Grande, RS
ENVIO DO RELATÓRIO PARCIAL	Janeiro de 2025
ENVIO DO RELATÓRIO FINAL	Abril de 2025

PROFESSOR RESPONSÁVEL	Mariana Appel Hort
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PARECER DA CEUA:

Após a análise de sua solicitação de alteração de vigência, enviada a essa comissão em 22 de fevereiro de 2024, a CEUA-FURG considera **APROVADA** a data de 31 de março de 2025 como término da vigência do projeto.



Documento assinado eletronicamente por **Marcio de Azevedo Figueiredo, Servidor**, em 29/02/2024, às 11:32, conforme horário oficial de Brasília, com fundamento no art. 6º, § 1º, do [Decreto nº 8.539, de 8 de outubro de 2015](#).



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Referência: Caso responda este documento Parecer, indicar o Processo nº 23116.006597/2023-07

SEI nº 0185904