UNIVERSIDADE FEDERAL DE UBERLÂNDIA

REBECCA VIEIRA RODRIGUES

EFEITO DO TRATAMENTO DO EXTRATO BRUTO DE AYAHUASCA EM CULTURA DE ASTRÓCITOS HUMANOS

UBERLÂNDIA

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EFEITO DO TRATAMENTO DO EXTRATO BRUTO DE AYAHUASCA EM CULTURA DE ASTRÓCITOS HUMANOS

Dissertação apresentada ao Programa de Pós-Graduação em Biologia Celular e Estrutural Aplicadas (PPGBC), vinculado ao Instituto de Ciências Biomédicas (ICBIM) da Universidade Federal de Uberlândia (UFU), como requisito parcial à obtenção do título de mestre em Biologia Celular e Estrutural Aplicadas

Área de Concentração: Biologia Celular

Orientadora: Profa. Dra. Renata Graciele Zanon

Linha de Pesquisa: Mecanismos de Reparo e Plasticidade Tecidual

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UNIVERSIDADE FEDERAL DE UBERLÂNDIA
Coordenação do Programa de Pós-Graduação em Biologia Celular e Estrutural Aplicadas
Rua Piauí, Bloco 2B, Sala 2B218 - Bairro Umuarama, Uberlândia-MG, CEP 38400-902
Telefone: 34 3225-8484 - ppgbc@icbim.ufu.br

ATA DE DEFESA - PÓS-GRADUAÇÃO

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Reuniu-se, por Videoconferência, a **Banca Examinadora** designada pelo Colegiado do Programa de Pós-graduação em Biologia Celular e Estrutural Aplicadas/PPGBC, assim composta: Prof. Dr. **Claudemir Kuhn Faccioli** - UFUR; Profa. Dra. **Rúbia Regina Gonçalves Sivieri** – UNIPAC; e **Renata Graciele Zanon** orientadora da candidata.

Iniciando os trabalhos a presidente da mesa, Dra. Renata Graciele Zanon, apresentou a Comissão Examinadora e a candidata, agradeceu a presença do público, e concedeu à discente **Rebecca Vieira Rodrigues** a palavra para a exposição do seu trabalho. A duração da apresentação da discente e o tempo de arguição e resposta foram conforme as normas do Programa.

A seguir a senhora presidente concedeu a palavra, pela ordem sucessivamente, aos examinadores, que passaram a arguir a candidata. Ultimada a arguição, que se desenvolveu dentro dos termos regimentais, a Banca, em sessão secreta, atribuiu o resultado final, considerando a candidata:

Aprovada

Esta defesa faz parte dos requisitos necessários à obtenção do título de Mestre.

O competente diploma será expedido após cumprimento dos demais requisitos,

conforme as normas do Programa, a legislação pertinente e a regulamentação interna da UFU.

Nada mais havendo a tratar foram encerrados os trabalhos. Foi lavrada a presente ata que após lida e lavrada conforme foi assinada pela Banca Examinadora.

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SUMÁRIO

APRESENTAÇÃO

Esta dissertação está organizada em dois capítulos. O primeiro apresenta o referencial teórico que fundamenta a pergunta científica do estudo, e o segundo é composto por um artigo científico que contém os resultados experimentais, estruturado para publicação na Revista Brasileira de Psiquiatria.

CAPÍTULO I: REFERENCIAL TEÓRICO

2.1 Transtornos Psiquiátricos e Terapias Psicodélicas

A Organização Mundial da Saúde (OMS) classifica os transtornos psiquiátricos como transtornos mentais, comportamentais ou do neurodesenvolvimento, que incluem condições como transtornos de ansiedade, depressivos, psicóticos, relacionados ao estresse e ao uso de substâncias (OMS, 2019). Além de afetarem de maneira significativa a saúde física e mental dos indivíduos, os transtornos mentais impõem um expressivo ônus social, refletido em prejuízos nas relações interpessoais, redução do desempenho acadêmico e profissional, maior envolvimento em comportamentos de risco e sobrecarga dos sistemas públicos de saúde (VASCONCELOS et al., 2008; UNODC, 2023). Os transtornos psiquiátricos estão entre as principais causas de morbidade global, atingindo aproximadamente 1,1 bilhão de pessoas em todo o mundo, segundo o estudo de Carga Global de Doenças (GBD) (GLOBAL BURDEN OF DISEASE COLLABORATIVE NETWORK et al., 2024). O GBD 2019 não encontrou evidências de uma redução global dessa morbidade desde 1990, apesar de pesquisas demonstrarem que intervenções podem alcançar uma redução nesse ônus (GBD 2019 MENTAL DISORDERS COLLABORATORS et al., 2019).

Apesar dos progressos obtidos na formulação e na ampliação das opções terapêuticas de psicofármacos e abordagens clínicas, as terapias atualmente disponíveis para os transtornos psiquiátricos apresentam limitações significativas. Uma parcela substancial dos pacientes não alcança resposta clínica adequada, mesmo após múltiplas tentativas terapêuticas, caracterizando a chamada resistência ao tratamento. Além disso, muitos indivíduos exibem apenas resposta parcial, com persistência de sintomas residuais que comprometem o desempenho funcional e a qualidade de vida. A eficácia limitada das intervenções é frequentemente agravada pela ocorrência de efeitos adversos prolongados, que podem reduzir a adesão ao tratamento e restringir o uso de doses ou combinações potencialmente mais eficazes. Esses desafios refletem, em parte, a complexidade clínica e biológica dos transtornos psiquiátricos e a limitada compreensão dos mecanismos neurobiológicos subjacentes, reforçando a necessidade de estratégias terapêuticas mais eficazes, seguras e direcionadas (HOWES, THASE, PILLINGE, 2021).

Diante de um cenário marcado por limitações terapêuticas persistentes, observa-se um interesse crescente por terapias alternativas no campo da psiquiatria, motivado pela necessidade de ampliar as possibilidades de intervenção, superar a resistência ao tratamento e contemplar a diversidade de perfis clínicos. Nas últimas décadas, tem-se verificado um renovado interesse científico pelas terapias psicodélicas, impulsionado por evidências de seu

potencial terapêutico em transtornos psiquiátricos, especialmente em quadros de difícil manejo clínico (CALDER, HASLER, 2022).

A noção de psicodélico, proposta pelo psiquiatra Humphry Osmond, está associada à expressão ou revelação de estados da mente, sem implicar necessariamente um mecanismo biológico definido (NICHOLS, NICHOLS, HENDRICKS, 2023). Os psicodélicos clássicos são compostos agonistas do receptor de serotonina 5-HT2A diretamente, como o dietilamida do ácido lisérgico (LSD), a psilocibina, a 2,5-dimetoxi-4-iodoanfetamina (DOI), a 5-metoxi-N,N-dimetiltriptamina (5-MeO-DMT) e a N,N-dimetiltriptamina (DMT) (CALDER, HASLER, 2022; MITCHELL, ANDERSON, 2023). A ingestão de substâncias psicodélicas produz um estado mental alterado que se manifesta por intensas modificações na percepção sensorial, no processamento cognitivo e no estado emocional, frequentemente incluindo experiências visuais incomuns e a evocação vívida de memórias emotivas (HOVMAND, POULSEN, ARNFRED, 2024).

2.2 Ayahuasca

A ayahuasca (AY) é uma bebida psicoativa obtida por meio da decocção da liana *Banisteriopsis caapi* (Malpighiaceae), geralmente preparada em associação com outras espécies vegetais, sendo a associação mais comum com *Psychotria viridis* (Rubiaceae) (SOUSA et al., 2022). O preparado é um líquido espesso, de coloração marrom-escura e sabor amargo característico. Seu uso está historicamente inserido em contextos ritualísticos e religiosos, especialmente entre povos indígenas da Amazônia e, mais recentemente, em religiões ayahuasqueiras brasileiras, como o Santo Daime, a União do Vegetal e a Barquinha, nas quais a bebida é empregada como sacramento com finalidades espirituais, terapêuticas e de autoconhecimento, em estruturas cerimoniais específicas (RUFFELL et al., 2023).

Banisteriopsis caapi, também conhecida como jagube, mariri, yagé ou caapi, é um cipó nativo da região amazônica, rico nos alcaloides β-carbolínicos harmina, harmalina e tetraidro-harmina. Esses compostos atuam como inibidores reversíveis da monoamina oxidase do subtipo A (MAO-A), enzima responsável pela degradação de monoaminas endógenas e aminas exógenas, incluindo o DMT. O DMT, presente nas folhas de *P. viridis*, não apresenta biodisponibilidade significativa quando administrado isoladamente por via oral, devido à sua rápida metabolização pela MAO-A. Dessa forma, a inibição dessa enzima pelas β-carbolinas de *B. caapi* permite a absorção sistêmica do DMT, aumentando consideravelmente sua exposição e resultando em efeitos psicodélicos mais intensos e prolongados (SCHIMMELPFENNIG, JANKOWIAK-SIUDA, 2025).

As folhas do arbusto *P. viridis*, popularmente conhecido como chacrona, contêm DMT. O DMT é um alcaloide indólico com propriedades psicodélicas, capaz de induzir um estado alterado de consciência intenso e imersivo, sem provocar redução do estado de vigília. A administração aguda dessa substância está associada a profundas alterações na percepção e na cognição, frequentemente acompanhadas por imagens vívidas e complexas, emoções intensas e evocação de memórias autobiográficas (TIMMERMANN et al., 2023). O DMT apresenta um perfil farmacológico complexo, atuando como agonista em diferentes receptores, com destaque para os subtipos serotoninérgicos 5-HT1A, 5-HT2A e 5-HT2C, além do receptor sigma-1. A ativação do receptor 5-HT1A está relacionada à modulação do humor, da ansiedade e de respostas autonômicas, desempenhando papel regulador nos estados emocionais. O receptor 5-HT2A, por sua vez, constitui o principal mediador dos efeitos psicodélicos, estando associado a alterações na percepção, cognição e processamento sensorial. Enquanto o receptor 5-HT2C participa da regulação do humor e de funções cognitivas, exercendo influência moduladora sobre circuitos emocionais. Adicionalmente, o DMT interage com o receptor sigma-1, uma proteína intracelular envolvida na regulação da plasticidade neuronal, na promoção da sobrevivência celular e na resposta ao estresse celular, o que sugere que seus efeitos transcendem a neurotransmissão serotoninérgica clássica e envolvem mecanismos neurobiológicos mais amplos (CARBONARO, GATCH, 2016).

Essa decocção tem sido utilizada há séculos, possivelmente milênios, em contextos rituais por povos indígenas da Amazônia. Contudo, observa-se que a AY deixou de ser uma prática restrita à bacia amazônica, tradicionalmente associada a países como Brasil, Peru, Colômbia, Equador, Bolívia e Venezuela, passando a ser consumida em diferentes contextos culturais ao redor do mundo. Atualmente, seu uso se expandiu para regiões como a América do Norte, a Europa e a Austrália, incluindo ambientes religiosos, terapêuticos e neo-xamânicos (PERKINS et al., 2021). Nesse cenário, a AY tem despertado interesse como potencial ferramenta no manejo de transtornos psiquiátricos, especialmente os relacionados ao humor, à ansiedade e ao uso de substâncias. Paralelamente a essa expansão, observa-se um crescimento expressivo da pesquisa científica, abrangendo estudos observacionais e clínicos com seres humanos, investigações pré-clínicas em modelos animais e experimentos in vitro, voltados à elucidação de seus efeitos celulares (MAIA et al., 2023).

2.3 Evidências Experimentais e Clínicas

Ensaios clínicos randomizados e controlados por placebo demonstram que a AY promove reduções rápidas e sustentadas dos sintomas depressivos em pacientes com

depressão resistente ao tratamento, acompanhadas por alterações em marcadores neurobiológicos associados à regulação do estresse e do humor, incluindo níveis de cortisol e componentes do eixo hipotálamo–hipófise–adrenal (PALHANO-FONTES et al., 2019; SOUSA, 2022). Além disso, estudos clínicos exploratórios indicam que a AY modula o sistema endocanabinoide tanto em voluntários saudáveis quanto em indivíduos com transtorno de ansiedade social (SANTOS et al., 2022). No contexto do uso de substâncias, investigações observacionais com usuários religiosos de AY relatam redução significativa ou cessação do consumo de álcool e tabaco (BARBOSA et al., 2018; DALDEGAN-BUENO et al., 2022a).

Ratos e camundongos são amplamente utilizados como modelos experimentais na pesquisa biomédica, assim, estudos utilizando esses roedores contribuíram para elucidar alguns dos mecanismos de ação da AY. Werle et al. (2024) demonstraram que a AY favorece a redução de respostas associadas ao estresse e ao trauma, promovendo a reorganização e a extinção de memórias aversivas, o que sugere um impacto positivo sobre mecanismos de regulação emocional. De forma complementar, Daldegan-Bueno et al. (2022b) observaram efeitos comportamentais compatíveis com redução da ansiedade, associados ao aumento da ativação de áreas cerebrais envolvidas no processamento emocional e na modulação de sistemas neuroquímicos relacionados ao humor. Em consonância, Almeida et al. (2022) relataram que a AY foi capaz de atenuar alterações comportamentais induzidas pela exposição ao etanol, indicando um possível papel na modulação de processos neurobiológicos envolvidos na dependência alcoólica. Ademais, Joaquim et al. (2025) demonstraram efeitos neuroprotetores da AY em um modelo experimental de acidente vascular cerebral, evidenciados pela redução de processos inflamatórios e de estresse oxidativo, o que reforça seu potencial terapêutico em condições associadas a lesão neural.

Estudos in vitro com células cerebrais humanas ainda são limitados na literatura sobre a AY e seus componentes. Até o momento, poucos trabalhos investigaram diretamente seus efeitos em modelos celulares humanos, sendo um deles conduzido com neurônios humanos indiferenciados da linhagem SH-SY5Y (KATCHBORIAN-NETO et al., 2020). Outros estudos concentraram-se na avaliação de compostos isolados presentes na AY, como a harmina, analisada em células progenitoras neurais humanas, e o DMT, investigado especificamente em culturas primárias de astrócitos humanos (DAKIC et al., 2016; ZANDONADI et al., 2023). Nesse último modelo experimental, as análises metabólicas em astrócitos humanos indicaram que o DMT pode modular vias bioquímicas relacionadas à neurotransmissão dopaminérgica, ao metabolismo de vitaminas do complexo B e ao

metabolismo de aminoácidos, sugerindo efeitos sobre processos celulares associados à função e à homeostase do sistema nervoso central (ZANDONADI, 2023).

2.4 Disfunção Astrocitária em Transtornos Psiquiátricos

Apesar do avanço das investigações sobre os efeitos da AY, ainda permanecem lacunas importantes no entendimento das respostas dos astrócitos a esse composto. Os astrócitos constituem o tipo celular glial mais abundante e heterogêneo do Sistema Nervoso Central, exercendo funções essenciais para a manutenção do equilíbrio do ambiente cerebral e para o funcionamento adequado das redes neuronais. Essas células atuam no suporte metabólico aos neurônios, na captação e reciclagem de neurotransmissores, na liberação de sinais químicos que modulam a comunicação sináptica, bem como na formação, organização e remodelação das sinapses. Além disso, contribuem para a manutenção da barreira hematoencefálica e para o controle do fluxo sanguíneo local, adequando a oferta de nutrientes e oxigênio às necessidades do tecido neural (VERKHRATSKY, NEDERGAARD, 2014; VERKHRATSKY, NEDERGAARD, 2018; ESCARTIN et al., 2021). Em situações de lesão, estresse prolongado ou em diferentes condições neurológicas e psiquiátricas, os astrócitos podem assumir um estado reativo, caracterizado por alterações morfológicas e funcionais que podem exercer tanto efeitos protetores quanto efeitos deletérios para o tecido nervoso (VERKHRATSKY, NEDERGAARD, 2014; ESCARTIN, 2021).

Astrócitos humanos normais (Normal Human Astrocytes – NHA) representam um modelo in vitro de elevada relevância para a investigação de potenciais estratégias terapêuticas voltadas ao sistema nervoso central (SNC), uma vez que se trata de células humanas não transformadas que mantêm propriedades fisiológicas típicas dos astrócitos encontrados no tecido cerebral saudável (RUDNYTSKA et al., 2022). Esse modelo tem sido frequentemente empregado como uma ferramenta experimental relevante em investigações voltadas à compreensão de processos associados à neurotoxicidade, à neurogênese, às lesões cerebrais e às doenças neurodegenerativas (SHAO et al., 2012; SUTINEN et al., 2012; AZIZI, KRYNSKA, 2013). Portanto, as células NHA constituem um sistema in vitro adequado para a avaliação de compostos com potencial ação no sistema nervoso central, pois permitem a investigação de respostas celulares humanas diretamente relevantes aos processos fisiológicos e patológicos do SNC, superando algumas das limitações inerentes aos modelos animais, cujas diferenças bioquímicas, estruturais e funcionais em relação aos astrócitos humanos podem comprometer a tradução dos achados à clínica (RZEPKA et al., 2020).

Estudos conduzidos em pacientes com diferentes diagnósticos psiquiátricos têm demonstrado alterações funcionais relevantes nos astrócitos, corroborando sua participação direta nos mecanismos patofisiológicos desses transtornos. Em condições como esquizofrenia, transtornos de humor, dependência química e transtornos do neurodesenvolvimento, essas alterações envolvem disfunções na captação e no metabolismo do glutamato, na produção de D-serina, na liberação de fatores neurotróficos e na comunicação intercelular (KOYAMA, 2015). Tais disfunções comprometem a homeostase sináptica, a plasticidade neural e a modulação da neurotransmissão, processos essenciais para o funcionamento adequado do sistema nervoso central. Cabras et al. (2010) demonstraram que a imipramina exerce efeitos diretos sobre astrócitos humanos in vitro, promovendo alterações morfológicas e a expressão de marcadores associados a um fenótipo neuronal, sugerindo que esse fármaco pode influenciar a plasticidade e o estado funcional dos astrócitos. Silva et al. (2024) demonstraram que os antipsicóticos risperidona e haloperidol modulam de forma distinta a função astroglial em culturas de astrócitos e fatias hipocampais de ratos, sendo a risperidona associada a respostas anti-inflamatórias e à modulação de fatores tróficos, enquanto o haloperidol induziu um perfil pró-inflamatório, evidenciando ações diretas desses fármacos sobre a inflamação e o suporte trófico mediado por astrócitos.

Considerando o aumento do consumo de AY nas últimas décadas e a relevância crescente dos astrócitos como elementos ativos na fisiopatologia dos transtornos psiquiátricos, este estudo investigou os efeitos do extrato bruto de AY em astrócitos humanos, utilizando um modelo in vitro. Apesar de evidências prévias apontarem para ações neuromoduladoras de *B. caapi*, *P. viridis* e de seus principais constituintes, os mecanismos subjacentes a esses efeitos permanecem parcialmente elucidados; assim, o objetivo foi avaliar se a AY promove alterações funcionais e moleculares em astrócitos, com potenciais implicações em processos relacionados à inflamação, à proteção celular e à manutenção da homeostase e da plasticidade do Sistema Nervoso Central.

2.5 Marcadores Estudados e sua Relação com os Astrócitos no Contexto de Doenças

- A interleucina-6 (IL-6) é uma citocina pleiotrópica central na interface entre inflamação periférica e neuroinflamação, exercendo papel relevante na fisiopatologia de diversos transtornos psiquiátricos. Evidências clínicas robustas indicam que níveis elevados de IL-6 circulante estão consistentemente associados a diferentes diagnósticos psiquiátricos, incluindo transtorno depressivo maior, transtornos de ansiedade, esquizofrenia e transtorno bipolar, sugerindo a existência de um eixo inflamatório comum subjacente a essas condições (LI et al.,

2020; ZHANG et al., 2023). A administração de AY tem sido associada a efeitos antidepressivos e imunomodulatórios, porém seus impactos sobre a IL-6 variam conforme o contexto experimental. Em estudos clínicos, apesar da melhora rápida dos sintomas depressivos, as alterações nos níveis de IL-6 nem sempre são consistentes ou significativas (PALHANO-FONTES et al., 2019). Em modelos animais, especialmente sob condições inflamatórias prévias, a AY pode reduzir a produção de citocinas pró-inflamatórias, incluindo a IL-6 (KOYAMA, 2015; SOUSA et al., 2025).

- A proteína fibrilar glial ácida (GFAP) é frequentemente empregada em análises histológicas e imunocitoquímicas de astrócitos. A GFAP é uma proteína estrutural pertencente à família dos filamentos intermediários tipo III, responsável pela sustentação e integridade do citoesqueleto dos astrócitos (PEKNY, 2014). Em condições fisiológicas, a expressão de GFAP reflete o grau de diferenciação e a estabilidade estrutural das células gliais; contudo, sob estímulos de lesão, inflamação ou estresse metabólico, ocorre uma superexpressão dessa proteína, fenômeno amplamente reconhecido como astrogliose reativa (SOFRONIEW, 2010). O aumento da expressão de GFAP é apenas um indicador da astrogliose que também se caracteriza pela intensificação de suas funções de suporte.Evidências indicam que o aumento da expressão de GFAP não deve ser interpretado exclusivamente como um marcador de disfunção astrocitária, podendo refletir respostas adaptativas associadas a efeitos funcionais benéficos, especialmente em contextos de reparo tecidual e suporte à plasticidade neural (TOOPS et al., 2012; BELAYA et al., 2020). Toops et al. (2012) observaram que o aumento da expressão de GFAP em astrócitos esteve associado à maior extensão do crescimento axonal em explantes de retina mantidos em condições favoráveis, indicando que níveis elevados dessa proteína podem contribuir para o suporte estrutural necessário ao crescimento neurítico. Belaya et al. (2020) observaram que o aumento da reatividade astrocitária marcada por GFAP decorrente de exercício físico voluntário em modelo de Alzheimer foi acompanhado por alterações morfológicas de astrócitos e aumentos de fatores associados à plasticidade sináptica, como BDNF e PSD-95, sugerindo que a modulação de GFAP pode estar ligada a efeitos neuroprotetores e de melhora cognitiva nesse contexto.
- O receptor do fator de crescimento nervoso NGFRp75 é um receptor de baixa afinidade para neurotrofinas, incluindo NGF, BDNF e suas formas precursoras, atuando como um importante modulador da sinalização neurotrófica no Sistema Nervoso Central. O NGFRp75 exerce funções contextuais e altamente dependentes do microambiente celular, podendo regular processos como sobrevivência celular, remodelação estrutural, resposta ao estresse e plasticidade tecidual. Evidências experimentais demonstram que a ativação dessa

via participa ativamente da adaptação celular frente a insultos neurológicos, como observado em modelos de doença de Parkinson e epilepsia, nos quais a modulação da via proNGF mediada por NGFRp75 e sortilina ou a indução isolada de NGFRp75 está associada a respostas neuroprotetoras e reorganização do tecido neural (SAMOYLENKO et al., 2009; VONDRAN et al., 2014). Em astrócitos, a expressão de NGFRp75 tem sido consistentemente relacionada a estados de ativação funcional, particularmente em condições de lesão ou desafio ao tecido nervoso. Chen et al. (2022) demonstraram que a indução de NGFRp75 em astrócitos após lesão cortical penetrante promove a proliferação dessas células, sugerindo um papel direto desse receptor na expansão e reorganização do compartimento glial durante processos de reparo. De forma complementar, Cragnolini et al. (2009) demonstraram que a sinalização do NGF mediada por NGFRp75 regula a proliferação de astrócitos, atenuando o ciclo celular de maneira dependente do contexto, atuando tanto como regulador negativo quanto positivo do ciclo celular, a depender do estado de ativação e do ambiente molecular circundante. Em conjunto, esses achados reforçam a noção de que o NGFRp75 não atua exclusivamente como um marcador de estresse ou dano, mas integra redes de sinalização que conferem aos astrócitos um fenótipo mais plástico e responsivo.

- A proteína S100β é amplamente reconhecida como um biomarcador de lesão cerebral, pois sua concentração se eleva em resposta a danos gliais e ao comprometimento da integridade do tecido nervoso. Pertencente à família das proteínas S100 ligadoras de cálcio, a S100β é predominantemente expressa por astrócitos maduros e participa de múltiplos processos celulares relacionados à homeostase e à comunicação glia–neurônio (DONATO et al., 2013). Diferentemente da GFAP, que exerce função estrutural, a S100β é uma proteína solúvel e ativamente secretada, podendo ser liberada no meio extracelular e alcançar a circulação sistêmica, especialmente em condições associadas à lesão cerebral e à disrupção da barreira hematoencefálica (KAPURAL et al., 2002).Em concentrações fisiológicas, a S100β desempenha efeitos neurotróficos e citoprotetores, contribuindo para a sobrevivência neuronal, a modulação da plasticidade sináptica e o suporte metabólico. Contudo, quando presente em níveis elevados, essa proteína pode adquirir propriedades pró-inflamatórias e neurotóxicas, ativando vias associadas à inflamação e ao estresse oxidativo e, assim, favorecendo a progressão de processos neurodegenerativos (DONATO, 2013; MICCHETTI et al., 2023).

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CAPÍTULO II: ARTIGO CIENTÍFICO

Ayahuasca increases GFAP and NGFRp75 expression in human astrocytes demonstrating potentially beneficial effects to the astrocytic viability

Rebecca Vieira Rodrigues[1], Renata Graciele Zanon[1],*

[1]Laboratory of Morphology and Cell Culture, Department of Anatomy, Institute of Biomedical Sciences, Federal University of Uberlândia, MG, Brazil.

** Correspondence: Renata Graciele Zanon, Laboratory of Morphology and Cell Culture, Department of Anatomy, Institute of Biomedical Sciences, Federal University of Uberlândia, Av. Pará, CEP 38405-318, Uberlândia, MG, Brasil. E-mail: rezanon@ufu.br*

Abstract

Objective: Ayahuasca, a potent psychedelic containing N,N-dimethyltryptamine (DMT), harmine, harmaline, and tetrahydroharmine, has been associated with neural plasticity and therapeutic effects in psychiatric disorders. This study aimed to investigate whether ayahuasca induces astrocytic alterations with potential beneficial effects on cellular and molecular mechanisms involved in central nervous system homeostasis and synaptic plasticity.

Methods: A normal human astrocyte cell line was used. Crude ayahuasca extract at concentrations ranging from 0.5 to 300 μg/mL was applied in cell viability assays, assessed by resazurin reduction and calcein/propidium iodide staining after 24 and 72 hours. Concentrations of 10 and 50 μg/mL were selected for reactive oxygen species assays, evaluated after 1, 3, and 24 hours, and for immunofluorescence analyses of S100β, GFAP, IL-6, and NGFRp75 after 24 hours of exposure.

Results: Treatment with 50 μg/mL of ayahuasca significantly increased GFAP and NGFRp75 expression, indicating changes in astrocytic structural organization and associated signaling pathways. The extract did not affect cell viability, did not induce oxidative stress, and did not alter S100β or IL-6 expression, suggesting preservation of astrocytic integrity under the experimental conditions.

Conclusion: Ayahuasca appears to positively modulate adaptive and plasticity-related mechanisms in astrocytes while demonstrating a favorable safety profile in vitro. These findings add to the growing evidence on the neurobiological effects associated with ayahuasca exposure.

Keywords: Psychedelic Agents; Neuroplasticity; Cell Signaling; Central Nervous System

Introduction

Ayahuasca (AY) is a psychoactive beverage obtained through the decoction of the Amazonian liana *Banisteriopsis caapi* (Malpighiaceae), used in combination with other plant species.[1] Traditionally consumed by shamans in ritualistic and Indigenous ceremonial contexts in the Amazon, its use has expanded markedly over the past four decades, becoming incorporated into religious, therapeutic, and spiritual practices in different regions worldwide. The most common formulation of AY consists of *B. caapi*, which is rich in β-carbolines: harmine, harmaline, and tetrahydroharmine, combined with the leaves of the shrub *Psychotria viridis* (Rubiaceae), which contain N,N-dimethyltryptamine (DMT).[2,3] Acute administration induces an altered mental state characterized by changes in perception and cognition, visionary experiences, intense emotions, and the retrieval of autobiographical memories.[3]

AY has been investigated in clinical and observational studies as a potential therapeutic approach for psychiatric disorders, including depression, anxiety, substance use disorders, alcoholism, and tobacco use. Evidence indicates that AY may promote rapid and sustained reductions in depressive and anxiety symptoms, including in individuals with treatment-resistant depression, as well as being associated with decreased alcohol and tobacco use. In light of these findings, its effects have been considered comparable to, and in some contexts superior to, those observed with conventional pharmacological and psychotherapeutic approaches, particularly in refractory populations.[4-8]

Rats and mice are widely used as experimental models due to their high genetic and functional similarity to humans.[9,10] Accordingly, studies using these rodents have contributed to elucidating some of the mechanisms of action of AY. AY demonstrated utility in stress- and trauma-related disorders by promoting the reconsolidation and extinction of aversive memories through the activation of serotonergic receptors.[11] Complementarily, AY showed anxiolytic effects associated with increased activation of brain regions involved in emotional processing and the serotonergic pathway.[12] AY was able to attenuate ethanol-induced withdrawal syndrome through the modulation of serotonergic receptors and prodynorphin, suggesting a potential therapeutic role in the treatment of alcohol use disorder.[13] Moreover, AY may represent a promising therapeutic approach for stroke by reducing levels of inflammatory and oxidative stress markers in rats subjected to a middle cerebral artery occlusion model.[14]

In vitro studies using human brain cells are scarce. To date, only one article has used undifferentiated neurons (SH-SY5Y).[15] Two others examined AY components: harmine in

human neural progenitor cells (hNPCs) and DMT in primary astrocytes.[16,17] The latter study examined the metabolic effects of DMT on human astrocytes, identifying modulation of pathways associated with dopamine biosynthesis, vitamin B6 and B3 metabolism, and branched chain amino acid (BCAA) metabolism.[17]

Significant gaps remain in understanding astrocytic responses to AY exposure. Astrocytes are the most abundant and heterogeneous glial cell type in the central nervous system (CNS) and maintain homeostasis and brain defense through multiple mechanisms: regulation of neuronal energy metabolism, uptake and recycling of neurotransmitters, release of gliotransmitters (e.g., ATP), coordination of synaptogenesis and synaptic plasticity, maintenance of blood–brain barrier integrity, and local blood flow modulation.[18,19] Under conditions of injury, chronic stress, or neuropsychiatric disease, astrocytes adopt a reactive phenotype, characterized by morphological and functional changes that can be either protective or detrimental.[20]

Although some neuromodulatory properties of *B. caapi*, *P. viridis*, and their components have been demonstrated, further experimental evidence is required to elucidate the mechanisms underlying these effects. In the present study, we investigated whether AY is capable of inducing relevant astrocytic alterations, with potential beneficial effects on cellular and molecular mechanisms involved in inflammation, cellular protection, and the maintenance of homeostasis and plasticity of the central nervous system in human astrocytes.

Methods

Cell culture

Normal human astrocytes (NHA) consist of primary, normal, non-transformed, and non-immortalized cells that preserve the physiological mechanisms of cell cycle control, genomic stability, and the functional phenotype characteristic of astrocytes.[21] The NHA cell line, obtained from the European Collection of Authenticated Cell Cultures (ECACC, UK), was provided to our research group by Prof. Dr. Rosy Iara Maciel de Azambuja Ribeiro, Experimental Pathology Laboratory, Federal University of São João del-Rei (UFSJ). The cells were maintained in a humidified incubator (AutoFlow NU-8500, NuAire, USA) with 5% CO_2 at 37 °C and cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin (P/S). All reagents were obtained from Sigma-Aldrich (USA). This study did not require ethical approval, as the data were obtained exclusively from commercially acquired cell lines, in accordance with Communication No. 13/2012 of the Research Ethics Committee involving Human Subjects of

the Federal University of Uberlândia (CEP/UFU), which states that research of this nature does not require ethical review.

Plant material

A single batch of AY was used throughout the study. It was prepared and provided free of charge by the Centro Espiritual AYA Medicina, located in the city of Uberlândia, Minas Gerais, Brazil. After receipt of the prepared decoction, the material was stored at −80 °C in an ultra-low temperature freezer (CryoCube F570, Eppendorf, Germany). After 24 h, it was transferred to a lyophilizer (L101, Liobras, Brazil) and lyophilized for 36 h. This study was registered in the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SisGen; registration no. A80AC51). The lyophilized AY extract was dissolved in dimethyl sulfoxide (DMSO) (Nova Biotecnologia, Brazil) to obtain a stock solution at a concentration of 1 g/mL. For the experiments, the stock solution was diluted in DMEM to the desired concentrations.

Cell viability assay

NHA cells were seeded in 96-well plates at a density of 1×10^4 cells per well, with four wells per experimental group, under basal culture conditions, and maintained for 24 h in a humidified incubator at 37 °C with 5% CO_2 to allow cell adhesion. After this period, the experimental treatments were initiated. The AY groups were treated with the extract at concentrations of 0.5, 1, 2, 5, 10, 20, 50, 100, 200, and 300 μg/mL. The control group received DMEM only, whereas the vehicle control groups were treated with DMSO at concentrations of 0.3, 0.05, and 0.01%, corresponding to the final DMSO concentrations present in the AY-treated groups at 300, 50, and 10 μg/mL, respectively. DMSO was used as a solvent at concentrations below 0.5%, based on literature evidence indicating the absence of relevant effects on cell viability, morphology, and GFAP expression in astrocytes under these conditions.[22]

In parallel, an additional experiment with a 72 h treatment period was performed, including the AY10 and AY50 groups, control, and vehicle control groups (0.05 and 0.01% DMSO), with the aim of investigating potential cytotoxic effects resulting from prolonged exposure. The assessment of cell viability at 24 and 72 h allowed the identification of acute and prolonged effects, respectively, providing a more comprehensive evaluation of toxicity.[23] Cell viability was assessed using the resazurin reduction assay, which is directly proportional to the number of metabolically active cells.[24] Briefly, four hours before the end of the

treatment period, resazurin (Sigma-Aldrich) was added to each well at a final concentration of 10%. After four hours of incubation, the absorbance of the reduced dye, resorufin, was measured at wavelengths of 570 and 600 nm. Cell viability was expressed as a percentage relative to the control group (DMEM only). Data were analyzed using one-way ANOVA followed by Tukey's post hoc test.

Cytochemistry: calcein and propidium iodide

NHA cells were seeded in 96-well plates at a density of 1×10^4 cells per well, with two wells per group, and maintained until adhesion. Subsequently, cells were treated for 24 or 72 h. The experimental groups AY10 and AY50 were treated with 10 and 50 μg/mL of the extract, respectively, whereas the control group and the vehicle control corresponding to the AY50 group received DMEM medium alone and 0.05% DMSO, respectively. Cells were then incubated for 30 min with 3 μM calcein-AM and 2.5 μM propidium iodide (both from Sigma-Aldrich). Calcein-AM is a lipophilic molecule that permeates the membrane of living cells and is hydrolyzed by intracellular esterases, generating green fluorescentcalcein and thereby selectively labeling metabolically active cells. In contrast, propidium iodide penetrates only cells with compromised membranes, intercalates into nuclear DNA, and emits red fluorescence, enabling the identification of dead cells.[25] After incubation, cells were analyzed using an inverted fluorescence microscope (Nikon Tsi, Nikon, Japan) at 200× magnification. Cell counting was performed in four distinct fields from two wells using ImageJ software to determine the proportion of live and dead cells under each condition. Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test.

Oxidative stress assessment: measurement of reactive oxygen species

Cells were seeded in black 96-well plates at a density of 1×10^4 cells per well, with four wells per group, and maintained until adhesion. Cells were then treated for 1, 3, or 24 h. The experimental groups AY10 and AY50 received 10 and 50 μg/mL of the extract, respectively, whereas the control group and the vehicle control groups corresponding to AY10 and AY50 received DMEM medium alone and DMSO at concentrations of 0.05% and 0.01%, respectively. After treatment, the medium was removed, and cells were incubated with 10 μM 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA) (Cayman Chemical, USA) for 30 min. For autofluorescence control (blank group), 100 μL of PBS was added to six wells. DCFH-DA is internalized by cells and cleaved by intracellular esterases to form dichlorodihydrofluorescein, which, upon oxidation by reactive oxygen species (ROS), is

converted into the fluorescent molecule dichlorofluorescein.[26] After incubation, fluorescence was measured using a microplate fluorescence reader (Synergy LX, BioTek, USA) with an excitation filter at 495/20 nm and an emission filter at 530/30 nm. Results were expressed as the mean fluorescence of each group after subtraction of the mean blank value, and values are presented in units of 10^6. The Kruskal–Wallis test followed by Dunn's post hoc test was applied.

Immunofluorescence

Cells were seeded in 96-well plates at a density of 1×10^4 cells per well, with three wells per group, and maintained until adhesion. They were then treated for 24 h. The extract was administered to the AY10 and AY50 groups at concentrations of 10 and 50 μg/mL, respectively, whereas the control and AY50 vehicle control groups received only DMEM medium and 0.05% DMSO, respectively. After this period, the treatment was removed, and cells were washed twice with 0.01 M phosphate-buffered saline (PBS, pH 7.4) and fixed with 4% paraformaldehyde (ÊxodoCientífica, Brazil) for 15 min.

Primary antibodies [rabbit anti-GFAP (Dako, Denmark), rabbit anti-NGFRp75 (Imuny, Brazil), rabbit anti-IL-6 (Santa Cruz Biotechnology, USA), and rabbit anti-S100β (Imuny)] were incubated for 24 h in 1% BSA (Sigma-Aldrich) and 0.1% Tween at 4 °C. After two washes with PBS, the secondary antibody [goat anti-rabbit Alexa Fluor 594 (Thermo Fisher Scientific, USA)] was incubated for 2 h at room temperature in the dark. Following two additional PBS washes, the nuclear marker DAPI (Sigma-Aldrich), diluted in PBS and glycerol (Imbralab, Brazil) (1:1), was added and incubated for 10 min in the dark. After a final wash, five images per well were acquired using an inverted Nikon Tsi fluorescence microscope at 200× magnification.

Microscope settings were adjusted to achieve an optimal signal-to-noise ratio and standardized based on the control group, and then maintained for all other groups. Mean fluorescence intensity was determined by image processing using ImageJ software, and results were expressed as pixel density in units of 10^5. Data were analyzed using one-way ANOVA followed by Tukey's post hoc test.

Statistical analysis

Graphs and statistical analyses were performed using GraphPad Prism software (version 8.4.0; GraphPad Software, USA). Data normality was assessed using the Shapiro–Wilk test. All data are presented as mean ± SEM, and results were considered statistically

significant when $p \leq 0.05$, as determined by one-way ANOVA followed by Tukey's multiple comparisons test for parametric data, or by the Kruskal–Wallis test followed by Dunn's multiple comparisons test for nonparametric data.

Results

AY did not induce cytotoxic effects in NHA cells at concentrations up to 50 µg/mL.

Cell viability was initially assessed to determine whether the samples exerted toxic effects on NHA cells. Overall, most tested concentrations did not induce significant cytotoxicity, as determined by the 24 h resazurin reduction viability assay (Figure 1A). The control group exhibited a mean viability of 100.0 ± 1.1%, whereas cells exposed to increasing doses of AY (µg/mL) showed the following values: 0.5, 96.4 ± 2.3%; 1, 97.8 ± 2.2%; 2, 99.9 ± 0.6%; 5, 99.0 ± 2.5%; 10, 99.0 ± 0.5%; 20, 95.0 ± 0.4%; and 50, 95.7 ± 0.7%.

In contrast, higher concentrations of AY (100, 200, and 300 µg/mL), which yielded viabilities of 88.3 ± 3.0%, 86.0 ± 1.6%, and 83.4 ± 1.8%, respectively, resulted in reductions in cell viability exceeding 10% and were statistically significant compared with the control group ($p \leq 0.05$). The vehicle DMSO proved to be safe, as it did not affect cell viability at any tested condition. Vehicle control values corresponding to AY doses of 300, 50, and 10 µg/mL were 100.8 ± 0.8%, 101.7 ± 1.2%, and 103.4 ± 1.2%, respectively.

Based on the determination that 50 µg/mL was the highest non-cytotoxic dose, concentrations of 10 and 50 µg/mL of crude AY extract were selected for subsequent experiments. At a dose of 10, a different response from the control was initiated, and from a dose of 50 onwards, cell viability began to be compromised; therefore, the chosen doses were 10 and 50 µg/mL.Over a 72 h treatment period, AY at 10 and 50 µg/mL likewise did not affect cell viability (Figure 1B). The control group showed a viability of 100.0 ± 1.1%, whereas cells treated with AY at 10 and 50 µg/mL exhibited viabilities of 97.1 ± 0.3% and 95.4 ± 1.7%, respectively. The corresponding vehicle control groups presented viabilities of 97.1 ± 0.3% and 98.6 ± 1.3%, respectively.

Cell viability was also assessed using calcein-AM and propidium iodide staining. As expected, the treatment was well tolerated at the tested doses, both after 24 hours (Figure 2A and C) and after 72 hours of exposure (Figure 2B), as the proportions of live and dead cells across all experimental groups were comparable to those of the control, with no statistically significant differences. After 24 hours, cell viability in the control group reached 98.0 ± 0.5%. Cells treated with AY at concentrations of 10 and 50 µg/mL exhibited viabilities of 97.9 ±

0.6% and 95.1 ± 0.7%, respectively, whereas the vehicle group presented a viability of 98.3 ± 0.6%. After 72 hours, the control group displayed a viability of 95.5 ± 2.1%. Under the same conditions, AY-treated groups at 10 and 50 µg/mL achieved viabilities of 99.3 ± 0.2% and 98.3 ± 0.4%, respectively, while the vehicle group reached 96.9 ± 0.7%.

ROS quantification was also not affected by AY. Analyses were performed at 1, 3, and 24 hours (Figure 2D). Fluorescence results are expressed in units of 10^6. At the 1-hour time point, values of 6.7 ± 1.1 were observed for the control group, 6.5 ± 0.8 and 5.5 ± 0.5 for AY doses of 10 and 50 µg/mL, respectively, and 7.1 ± 0.8 for the vehicle group. At 3 hours, the values were 6.2 ± 0.5 for the control, 7.0 ± 0.7 for the 10 µg/mL dose, 6.2 ± 0.4 for the 50 µg/mL dose, and 6.3 ± 0.7 for the vehicle. At 24 hours, the results were 5.1 ± 0.3 for the control, 5.1 ± 0.2 for the 10 µg/mL dose, 5.0 ± 0.3 for the 50 µg/mL dose, and 5.2 ± 0.3 for the vehicle group.

AY increases GFAP expression without altering S100β levels

Astrocytes treated with 50 µg/mL AY exhibited a significant increase in GFAP levels (Figure 3F–J), reaching 2.8 ± 0.2 compared with the control group ($p = 0.0036$). The remaining groups showed values similar to the control (1.8 ± 0.2); treatment with 10 µg/mL AY resulted in 2.2 ± 0.2, and the vehicle group showed 1.8 ± 0.2. In contrast, S100β levels remained unchanged at both AY doses (Figure 3A–E). The control group (8.1 ± 0.5) was comparable to all other groups: AY 10 µg/mL (6.8 ± 0.5), AY 50 µg/mL (7.2 ± 0.4), and vehicle (7.0 ± 0.5).

IL-6 and NGFRp75 indicate positive effects of AY treatment

Administration of AY and the vehicle resulted in significant reductions in interleukin-6 (IL-6) expression ($p \leq 0.01$), as illustrated in Figure 3K–O. The control group exhibited a value of 3.2 ± 0.2, whereas treatment with AY at 10 and 50 µg/mL decreased this parameter to 2.4 ± 0.1 and 2.3 ± 0.1, respectively, an effect comparable to that observed in the vehicle group (2.2 ± 0.1). Notably, the vehicle-treated group displayed a reduction similar to that of the AY-treated groups relative to the control, indicating that this effect may be attributable to the vehicle DMSO.

In contrast, exposure to AY at 50 µg/mL markedly increased NGFRp75 expression ($p < 0.0001$), as shown in Figure 3P–T. Expression levels were 5.6 ± 0.5 in the control group, 5.9 ± 0.5 in the vehicle group, 5.8 ± 0.6 in the AY10 group, and 10.8 ± 0.7 in the AY50 group.

Discussion

Normal Human Astrocytes (NHA) constitute a relevant in vitro model for investigating CNS-targeted therapies, as they are non-transformed human cells that preserve key physiological characteristics of astrocytes found in normal brain tissue.[27] This model has been widely used in studies on neurotoxicity, neurogenesis, brain injury, and neurodegenerative disorders.[28-30] Therefore, NHA cells provide an appropriate system for evaluating CNS-directed compounds, as they recapitulate human-specific responses, avoid species-related limitations of animal models, and allow more direct and controlled analyses of cellular effects.[31]

The use of AY has increased markedly in recent decades, reflecting its growing consumption and stimulating scientific interest in its biological effects and therapeutic potential. AY may exert significant effects on astrocytes, which is particularly relevant given that psychiatric disorders such as depression, anxiety, and stress-related conditions—where AY has been investigated—are associated with astroglial dysfunction.[4-8] In these conditions, astrocytes show impairments in neurotransmitter modulation, inflammatory regulation, and synaptic homeostasis.[32] Thus, AY may influence key molecular mechanisms underlying these disorders, highlighting the importance of understanding its effects on astroglial function to better elucidate its therapeutic potential.

In our study, treatment with AY promoted a significant increase in the expression of GFAP and NGFRp75. In addition, its safety was evidenced by the absence of changes in cell viability, ROS production, and the inflammatory markers evaluated.

In this context, treatment of astrocytes with AY did not alter cell viability at any time point, indicating that AY neither induces cell death nor stimulates proliferation, as it did not decrease or increase viability values. Cell viability was also assessed by calcein and propidium iodide staining, expressed as the difference between live and dead cells.[33] Analyses performed under the same time and dose conditions confirmed the previous findings, further supporting the safety of the treatment.

ROS play essential roles in the regulation of several physiological processes in all cells, including intracellular signaling, modulation of signal transduction pathways, and gene expression. However, excessive ROS production promotes oxidative stress, compromising cellular integrity through macromolecular damage, alterations in mitochondrial function, and activation of pathways that may culminate in apoptosis or necrosis.[34] Treatment with AY did not affect ROS production at 1, 3, or 24 hours, as no statistically significant differences were observed among the experimental groups at any of the evaluated time points. Nevertheless,

the 24-hour time point exhibited overall ROS levels lower than those observed at 1 and 3 hours, suggesting cellular adaptation over the incubation period, independently of the treatment.[35] Additionally, DMSO, although described in the literature as an antioxidant, did not exert a measurable antioxidant effect at the 0.05% concentration, as the vehicle control group presented ROS levels statistically equivalent to those of the control group.[36] These findings were consistent with the cell viability data, since the maintenance of physiological ROS levels was accompanied by preservation of cellular integrity and metabolism, indicating that the treatment did not induce oxidative stress.

Interleukin-6 (IL-6) is a pro-inflammatory cytokine implicated in neuroinflammation and psychiatric disorders, with elevated levels reported in patients with mental disorders and in astrocytes exposed to stress.[37,38] In the present study, AY treatment did not significantly alter IL-6 levels compared with the vehicle group. This finding should be interpreted with caution, as DMSO, used as a vehicle, exhibits well-documented anti-inflammatory properties that may have masked extract-specific effects, suggesting that the reduction observed relative to the untreated control is predominantly attributable to the vehicle.[39] Overall, evidence indicates that AY-related effects on IL-6 are heterogeneous and depend on the experimental model, inflammatory context, and concentration employed.[4,40,41]

GFAP, a structural protein belonging to the type III intermediate filament family, is responsible for maintaining the structural support and cytoskeletal integrity of astrocytes and reflects their level of activity; in this context, increased GFAP expression indicates adaptive astrocytic activation.[42,43] The increase in GFAP expression observed at the dose of 50 µg/mL suggests the activation of an astroglial response consistent with a functional state of astrogliosis, associated with adaptive mechanisms of structural support, neural plasticity, and maintenance of tissue homeostasis, rather than processes of injury or cellular damage.

NGFRp75 is a low-affinity receptor for neurotrophins, including nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF), and plays a relevant role in the regulation of glial plasticity, cellular adaptation to stress, and signaling pathways associated with neural survival and remodeling.[44,45] In astrocytes, the expression of this receptor has been associated with states of functional activation, in which these cells may acquire a phenotype that is more responsive to the lesional microenvironment.[46] In addition, NGFRp75 has been implicated in injury-related responses, including astrocyte proliferation and neuroprotective signaling, as well as in pathological conditions such as Parkinson's disease and epilepsy, where its expression is upregulated following neural damage or hyperexcitable states.[44-46] In this context, the increase in NGFRp75 expression observed concomitantly with

the elevation of GFAP indicates that the treatment induced not only structural changes but also potentially functional modifications in astrocytes. Thus, the concomitant upregulation of GFAP and NGFRp75 suggests that the treatment promoted structural and possibly functional alterations in astrocytes, consistent with an adaptive response of the nervous tissue.

S100β acts as a biomarker of brain injury, as its concentration increases in response to glial damage. It is a member of the S100 family of calcium-binding proteins and is also widely used as a marker of mature astrocytes.[47] Unlike GFAP, S100β is a soluble and secreted protein, released into the extracellular milieu mainly under conditions of tissue damage, which makes it an important serum biomarker of brain injury.[48,49] At physiological levels, S100β exerts neurotrophic and protective effects; however, at elevated concentrations, it may act in a pro-inflammatory and neurotoxic manner, contributing to the progression of degenerative processes.[47] In the present study, no changes in S100β levels were observed in human astrocytes treated with AY, corroborating the safety profile observed in the other assays, including cell viability, ROS production, and IL-6 expression.

Some limitations should be considered when interpreting these findings. The experimental design was based primarily on short exposure periods of 24 hours, with only one viability assay extended to 72 hours, which restricts conclusions to acute effects and does not allow assessment of long-term responses. In addition, DMSO was used as the solvent, and its known anti-inflammatory properties may have interfered with the evaluation of the inflammatory marker IL-6, potentially masking specific effects attributable to AY.

In conclusion, AY's action on astrocytes, evidenced by the increased expression of GFAP and NGFRp75, reinforces its potential as a modulator of glial function. Notably, this study provides the first evidence demonstrating that ayahuasca modulates the astrocytic markers p75NGFR and GFAP, representing a novel contribution to thefield.These findings are relevant, as reduced levels of these markers have been associated with astrocytic dysfunction, a recurring component in the pathophysiology of different psychiatric disorders.Thus, the results broaden the understanding of the cellular mechanisms of AY and support its possible therapeutic role in conditions associated with astrocytic dysfunction. The versatility and broad capacity of AY to modulate astrocytic functions underscore promising prospects for future research on this compound. It is expected that this initiative will contribute to the development of more effective therapies capable of restoring glial homeostasis and attenuating dysfunctions associated with various psychiatric and neurological disorders, thereby expanding the translational potential of AY.

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Disclosure

The authors report no conflicts of interest.

Data Availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Author Contributions

RGZ was responsible for conceptualization, methodology, formal analysis, resources, supervision, and writing – review and editing. RVR was responsible for investigation, formal analysis and writing – original draft. All authors have read and approved the final version submitted and take public responsibility for all aspects of the work.

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Figure and Legends

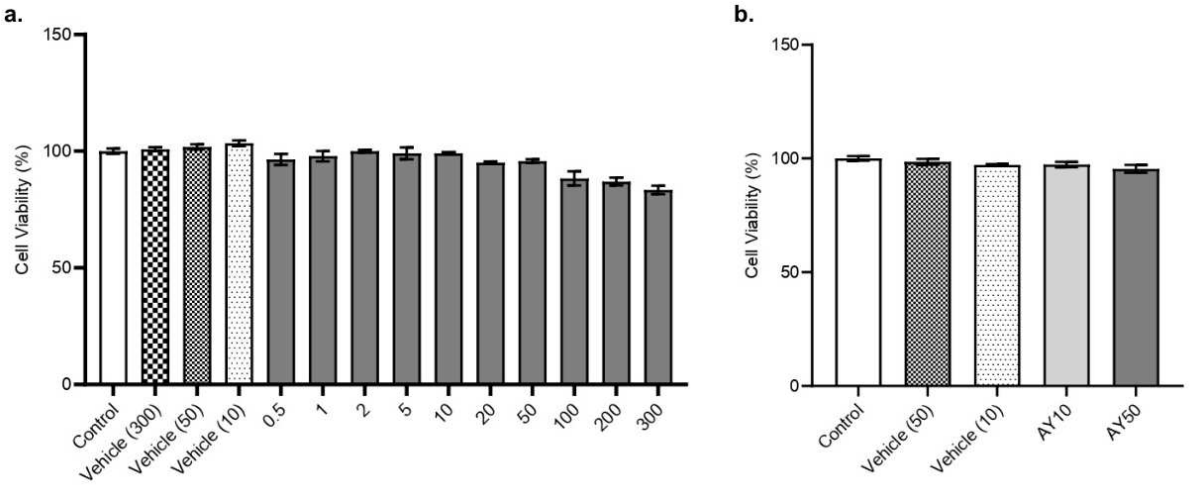

Figure 1. Analysis of cell viability of human astrocytes treated with different concentrations of ayahuasca. A) 24-hour resazurin reduction test with increasing doses of AY (0.5 to 300 µg/mL), control (DMEM only), and vehicle (0.3, 0.05, and 0.01% DMSO). B) 72-hour resazurin reduction test with two non-toxic doses (10 and 50 µg/mL, identified in A), control (DMEM only), and DMSO vehicle (0.05 and 0.01%). * indicates $p \leq 0.05$

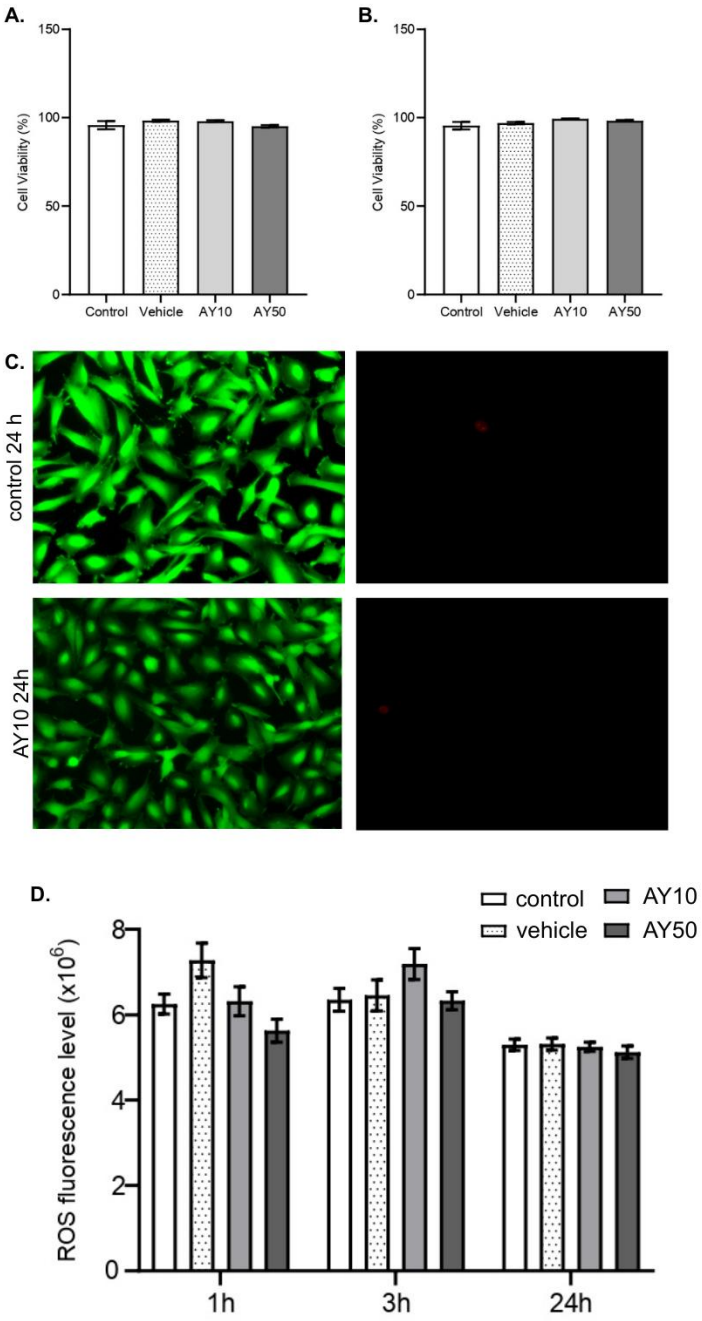

Figure 2. A–B) Effect of ayahuasca on cell viability measured by markers of live (calcein-AM) and dead (propidium iodide) cells in human astrocytes at 24 and 72 hours, respectively. Experimental groups included a DMEM-only control, ayahuasca at 10 and 50 µg/mL, and vehicle controls containing 0.05% and 0.01% DMSO. C) Representative images of calcein (green) and propidium iodide (red) labeling in control cultures and after 24 hours of treatment with AY at a dose of 10 µg/mL. D) ROS production measured with DCFH-DA in human astrocytes at 1, 3, and 24 hours. The following groups were analyzed: DMEM-only control, ayahuasca at 10 and 50 µg/mL, and a vehicle control containing 0.05% DMSO.

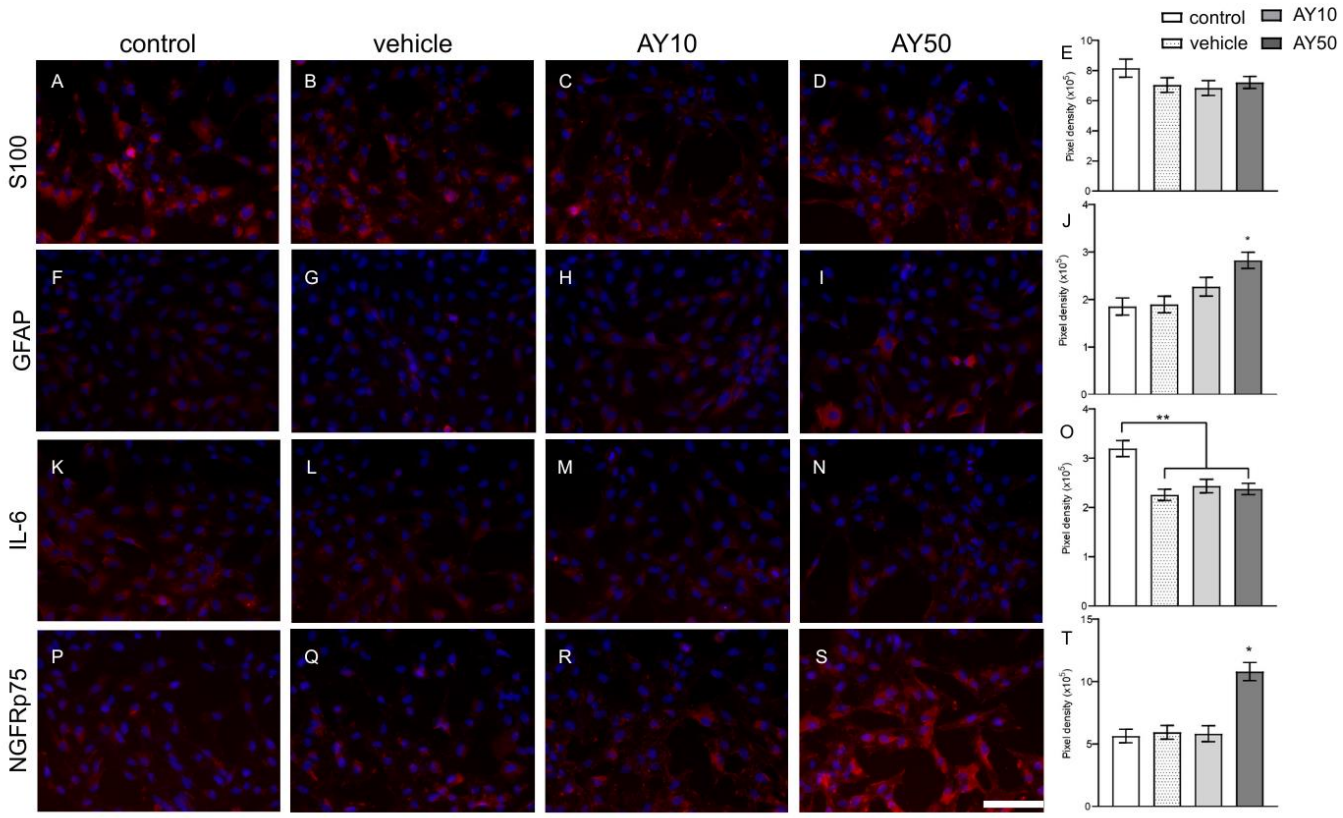

Figure 3. Immunofluorescence in human astrocytes in the following groups: control (treated only with DMEM), ayahuasca at 10 µg/mL and 50 µg/mL. A-D) Photomicrographs with blue (nucleus-DAPI) and red (S100β) labeling. E) Graph showing pixel quantification of red labeling intensity for S100β. F-I) Photomicrographs with blue (nucleus-DAPI) and red (GFAP) labeling. J) Graph showing pixel quantification of red labeling intensity for GFAP. K-N) Photomicrographs with blue (nucleus-DAPI) and red (IL-6) labeling. O) Graph showing pixel quantification of red labeling intensity for IL-6. P-S) Photomicrographs with blue (nucleus-DAPI) and red (NGFRp75) labeling. T) Graph showing pixel quantification of red labeling intensity for NGFRp75. * indicates $p \leq 0.05$ and ** indicates $p \leq 0.01$. Scale bar: 100 µm.