Biocatalysis and Agricultural Biotechnology 56 (2024) 103033

Contents lists available at ScienceDirect

Biocatalysis and Agricultural Biotechnology

journal homepage: www.elsevier.com/locate/bab

Psilocybin and eugenol prevent DSS-induced neuroinflammation in mice

Timur Zanikov[a,1], Marta Gerasymchuk[a,1], Gregory Ian Robinson[a,1], Esmaeel Ghasemi Gojani[a], Shima Asghari[a], Alyssa Groves[a], Mackenzie Cameron[a], Rocio Rodriguez-Juarez[a], Alexandra Snelling[b], Darryl Hudson[b], Anna Fiselier[c], Olga Kovalchuk[a,*], Igor Kovalchuk[a,**]

[a] Department of Biological Sciences, University of Lethbridge, 4401 University Drive West, Lethbridge, AB, T1K 3M4, Canada
[b] GoodCap Pharmaceuticals, 520 3rd Avenue SW, Suite 1900, Calgary, AB, T2P 0R3, Canada
[c] Department of Family Medicine, Cumming School of Medicine, University of Calgary, Calgary, T2N 4N1, Canada

ARTICLE INFO

Handling Editor: Dr. Ching Hou

Keywords:
Psilocybin
Eugenol
Dextran
Inflammation
Brain

ABSTRACT

Neuroinflammation has emerged as a central pathology common to several acute and chronic brain diseases. Recent studies have displayed the anti-inflammatory properties of naturally occurring compounds derived from mushrooms and plants could potentially reduce neuroinflammation and disease progression. In this study, we aimed to investigate the impact of psilocybin and eugenol, as well as their combinations, on neuroinflammation. To induce inflammation through the gut-brain axis, we employed a colitis mouse model via oral feeding of dextran sulfate sodium (DSS). By administering various concentrations and combinations of treatments, both before and after inducing inflammation, we sought to assess the synergistic anti-inflammatory effects of psilocybin and eugenol. Our findings revealed oral psilocybin and eugenol post-treatment significantly reduced the expression of pro-inflammatory cytokines and inflammatory mediators in the brain, including IL-1β, IL-6, and COX-2. Notably, combined treatment of psilocybin and eugenol exhibited the strongest reduction in IL-6 levels when compared to the DSS group. While both psilocybin and eugenol possess anti-inflammatory effects, the combined treatment overall did not demonstrate synergistic reductions in neuroinflammation across all markers. This study adds to the growing body of evidence supporting the therapeutic potential of psilocybin and eugenol in psychiatric and neurodegenerative inflammatory disorders. Further research is necessary to elucidate the underlying mechanisms of their anti-inflammatory effects and to evaluate their efficacy in clinical settings.

1. Introduction

Neuroinflammation is an inflammatory response within the brain or spinal cord involving the activation of neuroimmune cells into pro-inflammatory states, accompanied by the release of multiple pro-inflammatory cytokines, chemokines, secondary messengers (Ca^{2+}, inositol trisphosphate, and NO), and reactive oxygen species (ROS) (DiSabato et al., 2016; Norden et al., 2016; Shabab et

* Corresponding author.
** Corresponding author.
E-mail addresses: olga.kovalchuk@uleth.ca (O. Kovalchuk), igor.kovalchuk@uleth.ca (I. Kovalchuk).
[1] These authors contributed equally.

https://doi.org/10.1016/j.bcab.2024.103033
Received 15 July 2023; Received in revised form 16 January 2024; Accepted 17 January 2024
Available online 25 January 2024
1878-8181/© 2024 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

al., 2017). The primary function of neuroinflammation is to inhibit the harmful effects of brain insults (Wyss-Coray and Mucke, 2002) by repairing tissue, supporting the blood-brain barrier, and facilitating the removal of cellular debris (Kempuraj et al., 2016). At the same time, excessive or prolonged inflammation can induce and exacerbate neurogenic disease progression, and even cause nerve damage, DNA fragmentation, and cell death (Chen et al., 2016). While neuroinflammation can be induced via brain or spine injuries, other factors including toxins, infections, and immune system dysregulation (Ebert et al., 2019) can lead to cognitive degeneration and neurodegenerative diseases, like Alzheimer's disease (AD). In addition, there is evidence indicating that inflammation has a notable impact on psychiatric disorders and is associated with depression, schizophrenia, post-traumatic stress disorder (PTSD), and mood disorders (Chen et al., 2016; Bauer and Teixeira, 2019).

Intestinal inflammation can impact brain function and contribute to neuroinflammatory processes through the gut-brain axis. Dextran sodium sulfate (DSS) is the most widely used chemical to induce inflammation of the bowels (Chassaing et al., 2014) causing damage to the intestinal epithelium barrier function and DSS-induced inflammatory bowel disease (IBD) (Cochran et al., 2020). In addition, DSS causes a disruption in the integrity of the gut barrier resulting in endotoxemia. Multiple studies demonstrate IBD and endotoxemia can induce cognitive impairment, alterations in serotonin (5-hydroxytryptamine, 5-HT)-producing enterochromaffin cells (EC), inflammation and oxidative stress within the brain (Jain et al., 2015; Emge et al., 2016). While DSS is commonly used to study IBD, it can also be used to study neuroinflammation due to its ability to disrupt the intestinal barrier causing endotoxemia, neuroinflammation and inflammatory-related neurodegenerative diseases (Kurita et al., 2020). Ongoing research is focused on investigating the therapeutic potential of natural compounds derived from mushrooms (Elsayed et al., 2014) and plants (Fürst and Zündorf, 2014) as possible treatment options due to their anti-inflammatory and antioxidant potential.

Eugenol (4-allyl-2-methoxyphenol) (Eug) is an aromatic compound, commonly found in essential oils of plants such as cloves, bay leaves, and allspice. Some studies demonstrated that eugenol is usually used as a treatment for dental caries and periodontal disease (Cai, 1996). Moreover, pharmacological studies reported that Eug has anticonvulsant, bactericidal, antifungal, analgesic, antiseptic, hepatoprotective, and antioxidant properties. It inhibits the expression of tumor necrosis factor α (TNF-α) and reduces production of nitrous oxide radicals (Kumar et al., 2021; Esmaeili et al., 2022). Eug, in addition to its effects on the CNS, has been shown to act as a neuroprotector, mitigating delayed neuronal death caused by ischemic damage in the CNS (Won et al., 1998). Additionally, rats treated with 0.01 mg/kg Eug showed improved memory and had a significant decrease in amyloid plaques, suggesting an effect on AD (Taheri et al., 2019). In the experimental epilepsy models, it was capable of reducing severity of seizures and neuronal excitability (Huang et al., 2012). Eug has been shown to effectively modulate microglia, inducing a shift from a pro-inflammatory to an anti-inflammatory state, thereby demonstrating its effects on microglial polarization (Revi and Rengan, 2020). Furthermore, oral application of Eug has recently been shown to be extremely effective in reducing inflammatory markers, including cadmium-induced inflammation (Kumar et al., 2021).

Psychedelic mushrooms have been used for centuries due to their purported healing properties, however, have not been thoroughly studied for their anti-inflammatory potential. Psychedelic mushroom extracts have demonstrated the ability to alter the inflammatory profile of macrophages (Nkadimeng et al., 2021), suggesting psilocybin (Psi), the principal bioactive compound found in psychedelic mushrooms, may have anti-inflammatory potential. Despite this finding, little research has been performed on whether psilocybin can prevent neuroinflammation.

In vivo, psilocybin undergoes rapid dephosphorylation to form psilocin (4-hydroxy-*N*,*N*-dimethyltryptamine). The acute pharmacological effects commonly associated with the administration of psilocybin/psilocin have traditionally been attributed to psilocin's binding to 5-HT2A receptors (5HT2AR). *In vitro* studies conducted on human receptor-expressing cells have demonstrated that psilocin exhibits a strong affinity for binding to 5HT2AR. Additionally, activation of 5HT2AR has shown robust anti-inflammatory effects in animal models (Yu et al., 2008; Erkizia-Santamaría et al., 2022). Moreover, psychedelics have been shown in human macrophages to inhibit inflammation induced by TNF-α *in vitro* (Nkadimeng et al., 2021).

Our previous studies demonstrated Psi and Eug can synergistically reduce inflammation in intestinal cells (Robinson et al., 2023a) and restore the intestinal barrier function in 3D models (Robinson et al., 2023b). Due to our recent findings showing Psi and Eug reduce neuroinflammation induced by LPS in mice (Zanikov et al., 2023), growing evidence of Psi acting through the gut-brain axis (Reed and Foldi, 2024), and the fact that multiple neuroinflammatory diseases are exacerbated or induced through the gut-brain axis including multiple sclerosis, Alzheimer's, and Parkinson's disease, as well as anxiety and depressive-like disorders (Rutsch et al., 2020), we aimed to inquire if psilocybin and eugenol acts through the gut-brain axis to reduce neuroinflammation. In this study, we utilized DSS to induce gut dysbiosis-mediated neuroinflammation and tested the anti-inflammatory effects of Psi and Eug. We hypothesize that psilocybin and eugenol have neuro-protective effects in DSS-induced neuroinflammation in mice, suggesting psilocybin and eugenol have anti-inflammatory effects within the brain that are mediated through the gut-brain axis.

2. Materials and methods

2.1. Animals

For this study, we used C57BL/6 J mice (Charles River Laboratories, Laval, Canada) following the Guide to Care and Use of Animals of the Canadian Council of Animal Care, which was approved by the Animal Care Committee at the University of Lethbridge, Canada (Protocol No. 2113, approved April 25, 2022).

2.2. Animal handling

8–10-week-old mice were assigned into groups that received doses of DSS or vehicle for 5, 6 or 7 days. Mice received either vehicle, 2.5 % or 3 % DSS dissolved in drinking water and were weighed daily. The number of days refers to the time between the starting

day of treatment and tissue harvesting for each group. After each time point, mice were anesthetized with Isoflurane and decapitated using the mouse guillotine. The brains were extracted, cut, washed in 1 × PBS and placed in 1.5 ml microtubes, frozen using liquid nitrogen or dry ice and stored at −80 degrees C.

In addition, 8–10-week-old mice were assigned to 2 groups and received Psi and Eug treatment either prior to or after receiving DSS. Gavage was the chosen method of treatment. Pretreated mice received treatments 24 h and 1 h before providing DSS in drinking water and tissues were harvested 6 days post DSS treatment. Post-treated mice received the treatment once after providing DSS for 6 days and tissues were harvested 4 h later. We collected brains from animals at 5, 6, and 7 days after providing the solution to analyze cytokine content.

2.3. Chemicals and treatment groups

The dose of psilocybin (CAS No. 520-52-50, Applied Pharmaceutical Innovation, Edmonton, AB, Canada) used was calculated from the common dose used in humans (5 mg, based on the average weight of 70 kg) and prorated to mice using a factor of 12.3. For the combination with eugenol (CAS No. 97-53-0, Sigma-Aldrich, Saint Louis, MI, USA), three different ratios were used: 1:10, 1:20, and 1:50.

Pre-treatment groups included: Control—vehicle; Psilocybin (0.88 mg/kg); Eugenol (17.6 mg/kg); Psilocybin + eugenol (1:20; 0.88 mg/kg psilocybin and 17.59 mg/kg eugenol); DSS (0.83 mg/kg); Psilocybin (0.88 mg/kg) + DSS; Eugenol (17.6 mg/kg) + DSS; Psilocybin + eugenol (1:10; 0.88 mg/kg psilocybin and 8.8 mg/kg eugenol) + DSS; Psilocybin + eugenol (1:20; 0.88 mg/kg psilocybin and 17.6 mg/kg eugenol) + DSS; Psilocybin + eugenol (1:50; 0.88 mg/kg psilocybin and 44.0 mg/kg eugenol) + DSS.

Post-treatment groups included: Control - Vehicle; DSS (0.83 mg/kg); DSS + psilocybin (0.88 mg/kg); DSS + eugenol (17.6 mg/kg); DSS + psilocybin + eugenol (1:10); DSS + psilocybin + eugenol (1:20); DSS + psilocybin + eugenol (1:50).

These doses were chosen based on previous data demonstrating strong and additive anti-inflammatory effects of psilocybin and eugenol at physiological levels that correspond to these oral doses (Robinson et al., 2023b). Furthermore, no cytotoxic effects were seen at these doses for psilocybin and eugenol (Robinson et al., 2023a).

2.4. Protein extraction

Frozen brain tissue was homogenized via pestle on liquid nitrogen and then protein was extracted with 400 μL of RIPA lysis buffer in 1.5 ml microtubes. Zirconium beads (Cat# D1032-15, Cole-Parmer, QC, Canada) was used to homogenize mixtures 3 times for 3 min on a shaker with 2 min on ice in between sets. Next, 200 μL of RIPA lysis buffer was added to each microtube and put on the shaker at 4 °C for 2 h, then centrifuged and supernatant was collected. NanoDrop 2000/2000c Spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA) was used for protein quantification, using the Bradford protein assay with bovine serum albumin as the standard.

2.5. RNA isolation

RNA was isolated from brain tissue using TRIzol® Reagent (Invitrogen, Carlsbad, CA, USA); purified using an RNAesy kit (Qiagen), according to the manufacturer's instructions, and quantified using NanoDrop 2000c (Thermo Fisher Scientific, Wilmington, DE, USA). RNA was converted to cDNA using iScriptTM Select cDNA synthesis kit (Cat# 1,708,897, BioRad, Hercules, CA, USA).

2.6. Quantitative real-time PCR (qRT-PCR)

Quantitative real-time PCR (qRT-PCR) was performed on brain tissue from all experimental groups with SsoFastTM EvaGreen® Supermix (Cat# 1,725,202, BioRad, Hercules, CA, USA) in a C1000TM Thermo Cycler equipped with a CFX96 Touch™ Real-Time PCR Detection System (BioRad). Primers were designed using the https://www.idtdna.com/Primerquest platform. Reference gene (*GAPDH*) was analyzed with the GeNorm method. The comparative CT method (ΔΔCt method) was used to calculate relative fold expression levels using the BioRad Software (CFX Manager). Each experiment included three biological replicates for each group and two technical replicates per sample. The genes used for qRT-PCr were *IL-1β, TNF-α, IL6, COX2, α-Amylase, TRP1, TRPM8, TRPV1, HTR2A, HTR2B, MCP1, GAPDH.*

2.7. Enzyme-linked immunoassay (ELISA)

Three samples from each group were selected randomly for ELISA and were prepared using extracted protein from brain tissue. Samples were centrifuged for 10 min at 5000 rpm, supernatant was aliquoted into 0.2 μL labeled microtubes, put in a box and sent to Eve Technologies (Calgary, AB, Canada) for enzyme-linked immunoassay. All procedures were done on ice.

2.8. Synergy scoring

To determine synergy, the average negative fold change compared to the DSS group was calculated for IL-1β, IL-6, COX-2, TNF-α, MCP-1 and α-Amylase for each treatment group. The negative fold changes were inputted into the R package webapp SynergyFinder+ (SynergyFinder, Helsinki, Finland) to determine the Loewe synergy score for inhibition (Yadav et al., 2015; Zheng et al., 2022). The imputation mode was utilized due to missing combinations, and 2D maps were developed to graphically demonstrate the synergistic interactions between Psi and Eug. Mean scores and p-values were calculated and noted on the figures.

2.9. Statistical analysis

GraphPad Prism 9 (GraphPad Software, San Diego, CA) was used for statistical analysis and is presented as means with standard error of the mean (SEM) error bars. A one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test was used. *P*-values less than 0.05 were considered statistically significant. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; and ****$p < 0.0001$.

3. Results

3.1. Verification of DSS-induced inflammation model

Initially, multiple doses and durations of orally fed DSS were tested on mice to determine which model adequately recapitulates neuroinflammation (Fig. 1). DSS was fed for 4, 5, or 6 days as previous research suggested between 3 and 7 days would induce a mouse model of colitis and neuroinflammation (Do and Woo, 2018). Concentrations at 0 %, 2.5 %, and 3 % were tested as both 2.5 % and 3 % have been previously used to induce different severities of colitis (Liu et al., 2021; Wu et al., 2021). mRNA samples from treated mice were isolated, quantified and analyzed via RT-qPCR. While no significant differences were seen, likely due to low statistical power ($n = 3$), a common trend was seen. Mice treated for 6 days at 3 % DSS had higher levels of all four tested markers, including IL-1β, IL-6, COX-2, and TNF-*α* (Fig. 1A,B,C,D, $p =$ N.S.). In contrast, 2.5 % DSS treated for 6 days did not increase levels of any marker of inflammation. While mice treated for 5 days did see increased levels of markers, this trend was not consistent. IL-6 and TNF-*α* levels were higher at 5 days of 2.5 % and 3 % DSS (Fig. 1B–D, $p =$ N.S.), however, IL-1β and COX-2 levels were unaffected (Fig. 1A–C, $p =$ N.S.). Lastly, no changes were seen after 4 days (Fig. 1A,B,C,D, $p =$ N.S.).

3.2. RT-qPCR analysis of cytokines in pre- and post-treatment animals

Next, we utilized mice treated with 3 % DSS for 6 days to test the anti-inflammatory effects of psilocybin and eugenol as this consistently induced neuroinflammation. Animals treated with psilocybin and/or eugenol either pre- or post-treatment were euthanized, brains were collected and homogenized, and mRNA was isolated. The expression of pro-inflammatory cytokines and enzymes were quantified and analyzed by RT-qPCR.

mRNA levels of *IL-1β* were significantly higher in the DSS group than the control ($p < 0.05$), however, no treatments of Psi and/or Eug significantly downregulated *IL-1β* compared to the DSS group (Fig. 2A). Similar to IL-1β, expression of cyclooxygenase-2 (COX-2) was significantly higher for the DSS group compared to the control ($p < 0.01$, Fig. 2C), however, no treatments of Eug and/or Psi significantly reduced COX-2 levels compared to the DSS group. Changes in expression of the other two cytokines were insignificant, although TNF-α trended lower for all treatments ($p =$ N.S., Fig. 2D) and IL-6 trended higher for all treatments compared to the DSS, except DSS + psilocybin ($p =$ N.S., Fig. 2B). Based on these results, pre-treatment with psilocybin and eugenol do not appear to reduce inflammation.

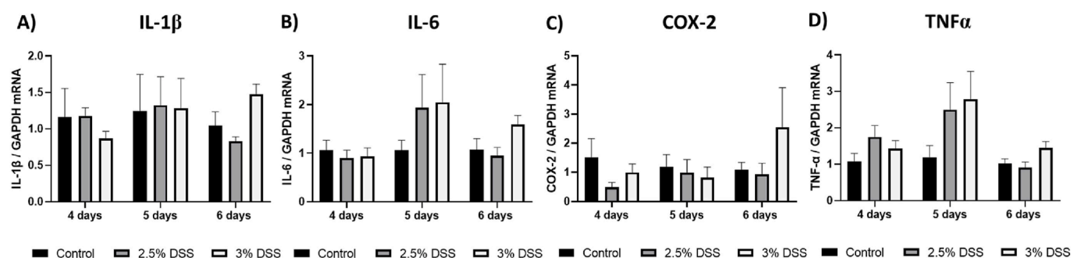

Fig. 1. The effect of 0, 2.5, and 3 % DSS concentration on mRNA expression in brain tissue after 4, 5, and 6 days. Changes in mRNA expression were measured by RT-qPCR for: (**A**) *IL-1β*, (**B**) *IL-6*, (**C**) *COX-2*, (**D**) *TNF-α*. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a loading control. Data were analyzed with a One-way ANOVA followed by Dunnett's post hoc test ($n = 3$). No comparisons are significant. Bars represent mean ± SEM. DSS, Dextran Sodium Sulfate.

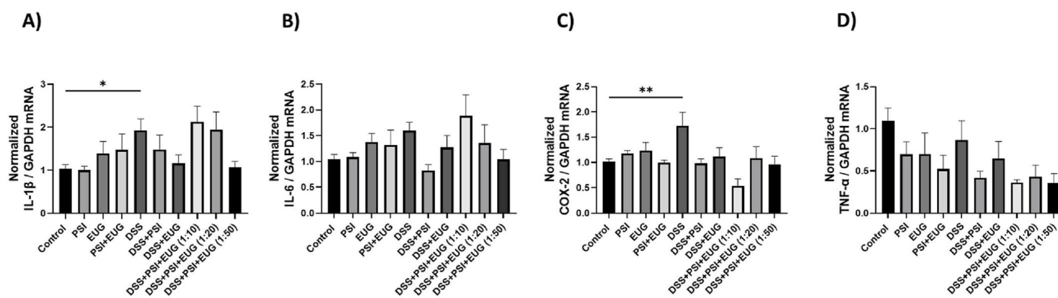

Fig. 2. The effect of pre-treatment on mRNA expression in brain tissue. Changes in mRNA expression as measured by RT-qPCR for: (**A**) *IL-1β*, (**B**) *IL-6*, (**C**) *COX-2*, (**D**) *TNF-α*. Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) was used as a housekeeping gene. Data were analyzed with ANOVA and Dunnett's post hoc test. Significance (p) is indicated within the figures using the following scale: *, $p < 0.05$, **, $p < 0.01$. Bars represent mean ± SEM. PSI, psilocybin; EUG, eugenol; DSS, Dextran Sodium Sulfate.

In contrast, all post-treatment groups significantly downregulated *IL-1β* expression including DSS + Psi ($p < 0.001$), DSS + Eug ($p < 0.0001$), DSS + Psi + Eug (1:10) ($p < 0.01$), DSS + Psi + Eug (1:20) ($p < 0.001$), DSS + Psi + Eug (1:50) ($p < 0.01$) compared to the DSS group (Fig. 3A). Similar results shown for the *IL-6* and *COX-2* levels of in the DSS group compared to the control ($p < 0.01$) and all post-treatment groups compared to the DSS group ($p < 0.01$, Figs. 3B and 2C). DSS did not upregulate *TNF-α* and there was no significant difference in expression of *TNF-α* in any of the treatment groups (Fig. 3D). In addition, DSS + psilocybin + eugenol (1:50) appeared to increase *TNF-α* levels (p = N.S., Fig. 3D).

3.3. RT-qPCR analysis of MCP-1 and α-Amylase in pre- and post-treatment animals

Next, we measured levels of *MCP-1*, which recruits and activates monocytes, memory T cells, and dendritic cells, and α-*Amylase,* which is used to determine the prognosis of inflammatory diseases, in the pre-treatment and the post-treatment groups. DSS significantly increased *MCP-1* levels compared to the control ($p < 0.0001$, Fig. 4A). The Psi + Eug + DSS (1:10) group showed a significant increase in levels of *MCP-1* expression in the pre-treatment compared to the DSS group ($p < 0.0001$, Fig. 4A), whereas Psi + Eug + DSS (1:50) demonstrated significantly lower levels of *MCP-1* compared to the DSS group ($p < 0.01$, Fig. 4A). Also, the expression of *MCP-1* was significantly lower in the Psi, and eugenol groups, compared to the DSS group ($p < 0.0001$, Fig. 4A), and was similar to the control.

The highest levels of α-*Amylase* were demonstrated in the DSS group compared to the control ($p < 0.0001$, Fig. 4B). All other treatment groups showed significantly lower expression of α-*Amylase* compared to the DSS group ($p < 0.001$, Fig. 4B). Interestingly, psilocybin appeared to have the largest decrease in *α-Amylase* levels ($p < 0.0001$, Fig. 4B).

The post-treatment groups demonstrated similar patterns. DSS had the highest levels of *MCP-1* compared to the control ($p < 0.01$, Fig. 5A). Post-treatment groups demonstrated significantly lower levels of *MCP-1*, with DSS + Eug and Psi + Eug + DSS (1:10) being the most significant ($p < 0.0001$, Fig. 5A). Interestingly, psilocybin did not significantly reduce MCP-1 levels compared to DSS group (p = N.S. Fig. 5A).

Similarly, α-*Amylase* expression levels were upregulated in DSS group compared to the control ($p < 0.001$, Fig. 5B), whereas psilocybin ($p < 0.01$), eugenol ($p < 0.001$), and 1:10 ($p < 0.001$) and 1:20 ($p < 0.01$) psilocybin to eugenol post-treatments significantly reduced *α-Amylase* levels compared to the DSS group (Fig. 5B).

3.4. Cytokines in pre- and post-treatment groups measured by ELISA

Next, we performed an array of ELISAs to look at multiple different cytokine protein levels. While many cytokines were outside of the limit of detection, IL-2, IL-4, and IL-10 levels were measured.

Relative levels of Interleukin 2 (IL-2) in the DSS + Eug group were shown to be downregulated compared to the DSS group ($p < 0.05$, Fig. 6A). No significant changes were shown for expression of Interleukin 4 (IL-4) or Interleukin 10 (IL-10), although IL-10 post-treatment groups tended to be lower than the DSS group (p = N.S., Figs. 6B and 7C respectively).

Next, we measured levels of IL-6 and MCP-1 (Fig. 7A and B respectively) in blood using ELISA. Although no significant differences were shown, IL-6 levels appeared higher in DSS + Psi group compared to the DSS group (p = N.S., Fig. 7A) and MCP-1 levels in DSS + Eug group appeared to be higher compared to the control group ($p = 0.0926$, Fig. 7B).

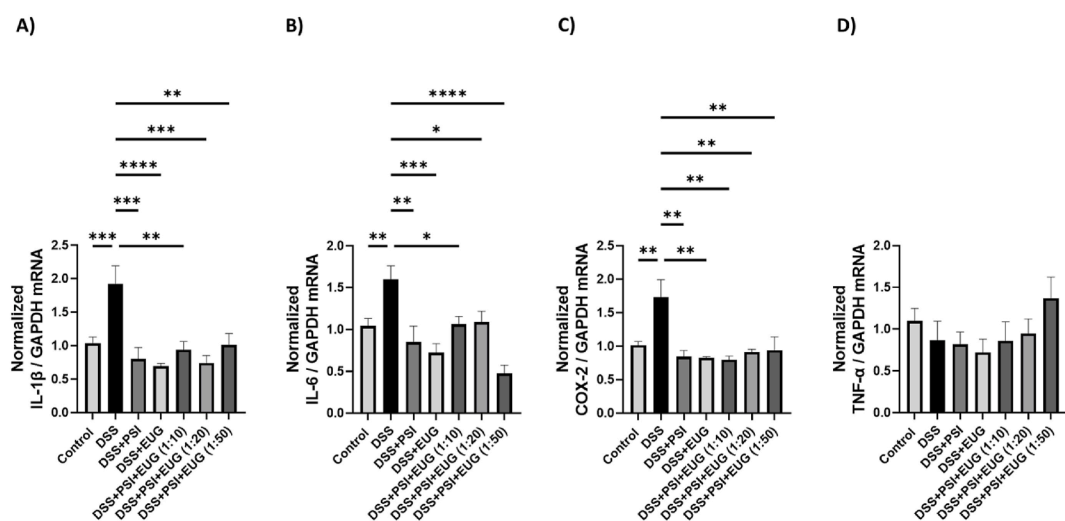

Fig. 3. The effect of post-treatment on mRNA expression in brain tissue. Changes in mRNA expression as measured by RT-qPCR for: (**A**) *IL-1β*, (**B**) *IL-6*, (**C**) *COX2*, (**D**) *TNF-α*. Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) was used as a housekeeping gene. Data were analyzed with ANOVA and Dunnett's post hoc test. Significance (p) is indicated within the figures using the following scale: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$. Bars represent mean ± SEM. PSI, psilocybin; EUG, eugenol; DSS, Dextran Sodium Sulfate.

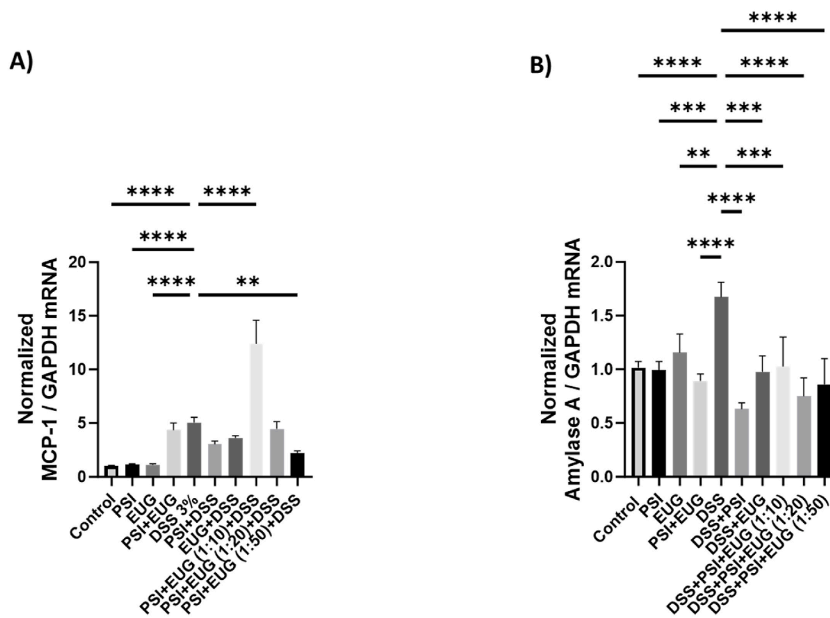

Fig. 4. The effect of pre-treatment on mRNA expression in brain tissue. Changes in mRNA expression as measured by RT-qPCR for: (**A**) *MCP-1*, (**B**) α*-Amylase*. *Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)* was used as a housekeeping gene. Data were analyzed with ANOVA and Dunnett's post hoc test. Significance (*p*) is indicated within the figures using the following scale: **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$. Bars represent mean ± SEM. PSI, psilocybin; EUG, eugenol; DSS, Dextran Sodium Sulfate.

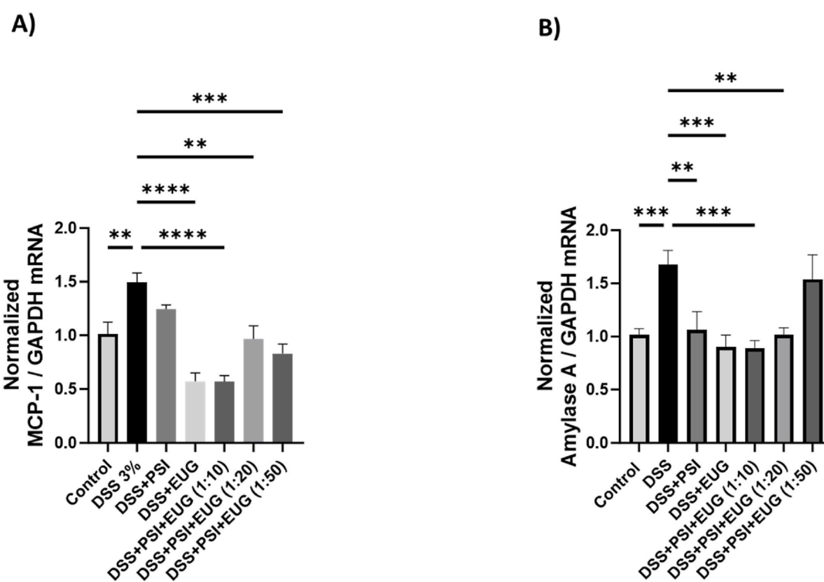

Fig. 5. The effect of post-treatment on mRNA expression in brain tissue. Changes in mRNA expression as measured by RT-qPCR for: (**A**) *MCP-1*, (**B**) α*-Amylase*. *Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)* was used as a housekeeping gene. Data were analyzed with ANOVA and Dunnett's post hoc test. Significance (p) is indicated within the figures using the following scale: **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$. Bars represent mean ± SEM. PSI, psilocybin; EUG, eugenol; DSS, Dextran Sodium Sulfate.

3.5. Synergism demonstrated in pre- and post-treatment

Since Psi and Eug demonstrate a potent ability to reduce inflammation by themselves, we wanted to test if the combination of the two compounds at different doses resulted in synergism. To test this, we utilized the Loewe calculation of synergism within SynergyFinder + to calculate synergy in the average negative fold change of the tested mRNAs.

In the pre-treatment groups, synergy was dose-dependent as both positive and negative synergy was demonstrated in multiple cytokines depending on the concentration of psilocybin and eugenol. Overall, the mean Loewe synergy score was −0.27 for *IL-1β* (Fig. 8A, $p = 0.454$) suggesting antagonism, however potentiation was demonstrated at 0.88 mg/kg of psilocybin combined with the high-

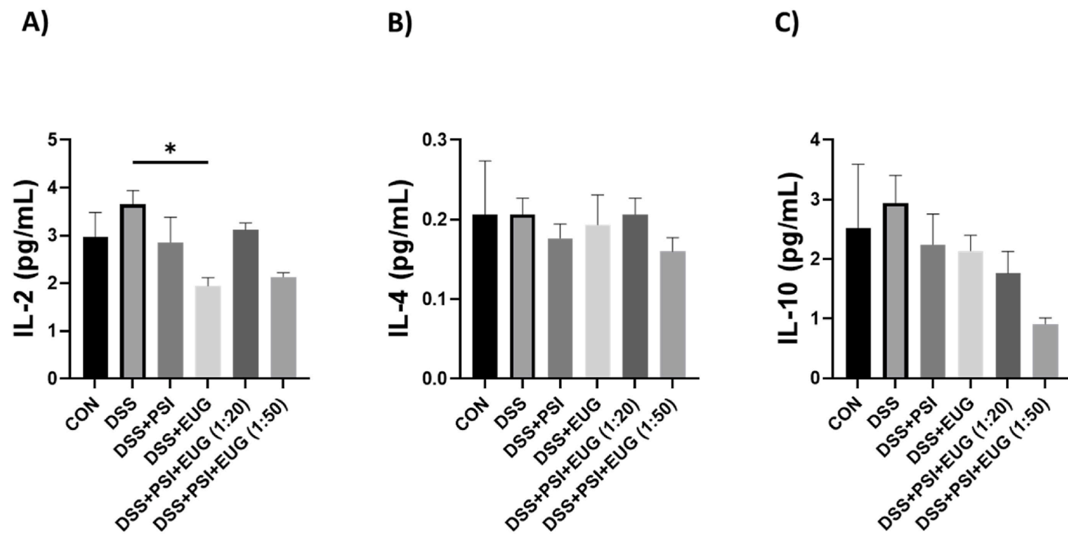

Fig. 6. Pro-inflammatory cytokine levels in the post-treatment DSS-induced brain inflammation. The levels of (**A**) IL-2, (**B**) IL-4, (**C**) IL-10 were measured by an ELISA. Data were analyzed with ANOVA and Tukey's post hoc test ($n = 3$). Significance (p) is indicated within the figures using the following scale: *, $p < 0.05$. Bars represent mean ± SEM. PSI, psilocybin; EUG, eugenol; DSS, Dextran Sodium Sulfate.

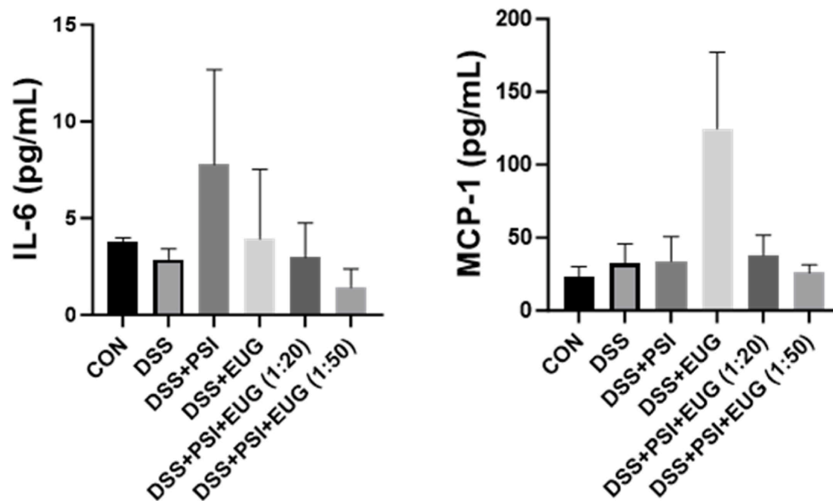

Fig. 7. The content of pro-inflammatory cytokines in post-treatment DSS-induced inflammation in blood. The amounts of (**A**) IL-6, (**B**) MCP-1 were determined by ELISA. Data were analyzed with ANOVA and Tukey ($n = 3$). Bars represent mean ± SEM. PSI, psilocybin; EUG, eugenol; DSS, Dextran Sodium Sulfate.

est dose of eugenol (44 mg/kg), which corresponds to the 1:50 ratio (Fig. 8A). In contrast, antagonism was demonstrated at both 8.8 (1:10 ratio) and 17.6 mg/kg (1:20 ratio) of eugenol when combined with 0.88 mg/kg of psilocybin (Fig. 8A).

Although *IL-6* only demonstrated antagonism between psilocybin and eugenol, with a mean synergy score was −0.75 (Fig. 8B, $p = 0.0646$), *COX-2* only demonstrated potentiation with a mean score of 0.42 (Fig. 8C, $p = 0.496$). Specifically, the highest synergy to reduce *COX-2* levels was with 0.88 mg/kg of psilocybin and 8.8 mg/kg of eugenol, corresponding to the 1:10 ratio of psilocybin to eugenol. Similarly, *TNF-α* showed a mean synergy of 0.19 (Fig. 8D, $p = 0.303$), however, potentiation was minimal and strongest in the 1:50 and 1:10 eugenol to psilocybin ratios.

In contrast, *MCP-1* had a mean synergy score of −0.38 (Fig. 8E, $p = 0.562$), but demonstrated both potentiation and antagonism. Pre-treatment with the 1:50 psilocybin to eugenol dose (44 mg/kg of eugenol and 0.88 mg/kg of psilocybin) resulted in synergy to reduce *MCP-1* levels, whereas, both 1:20 and 1:10 ratios demonstrated antagonism (Fig. 8E). Lastly, *α-Amylase* only demonstrated antagonism with a mean Loewe synergy score of −0.72 (Fig. 8F, $p = 0.0531$).

Overall, pre-treatments with psilocybin and eugenol resulted in dose-dependent potentiation and antagonism; specifically, the 1:50 psilocybin to eugenol ratio (0.88 mg/kg of psilocybin and 44 mg/kg of eugenol) demonstrated the strongest results with synergy for *IL-1β*, *TNF-α*, and *MCP-1* (Fig. 8).

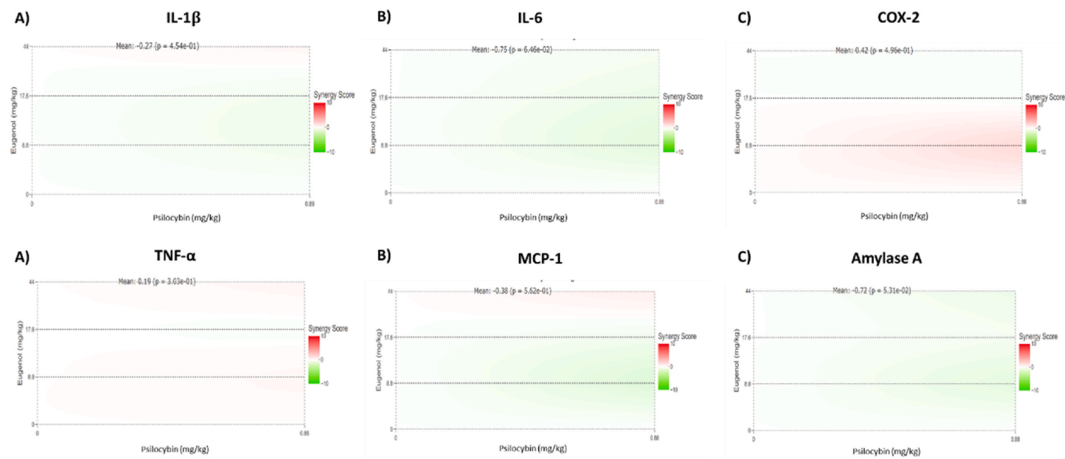

Fig. 8. Loewe synergy scores for negative fold change of cytokines and markers from the brains of mice pre-treated with 0 or 0.88 mg/kg of psilocybin combined with 0, 8.8, 17.6, and 44 mg/kg of eugenol compared to the DSS group. Synergy scores are indicated for: (**A**) *IL-1β*, (**B**) *IL-6*, (**C**) *COX2*, (**D**) *TNF-α*, (**E**) *MCP-1*, and (**F**) *α-Amylase.* Synergy scores are calculated by SynergyFinder + using imputation. Red represents potentiation. Green represents antagonism. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Similarly, we measured the synergism of post-treatment groups. Interestingly, synergism of post-treatments (Fig. 9) appeared to be opposite of the synergy in the pre-treatment groups (Fig. 8). As in pre-treatment groups, both potentiation and antagonism were dose-dependent.

For *IL-1β*, only antagonism was demonstrated with a mean Loewe score of −2.05 (Fig. 9A, $p = 0.0022$), however, no effect was observed at the 1:20 ratio of Psi to Eug. In contrast, *IL-6* demonstrated synergism at the 1:50 ratio, antagonism at both the 1:20 and 1:10 ratios with mean Loewe score of −0.18 (Fig. 9B, $p = 0.763$). For *COX-2*, post-treatments groups had neither potentiation nor antagonism, with a mean score of −0.12 (Fig. 9C, $p = 0.42$). Similarly, *TNF-α* was relatively neutral but trended toward antagonism with a mean score of −0.24 (Fig. 9D, $p = 0.189$). Lastly, both *MCP-1* and *α-Amylase* demonstrated synergy at the 1:10 ratio, while antagonism at the 1:50 ratio, with a mean Loewe score of 0.013 (Fig. 9E, $p = 0.973$) and −0.041 (Fig. 9F, $p = 0.880$), respectively.

Overall, there are no post-treatment combinations that clearly demonstrate strong potentiation across multiple markers that suggested to be better than other post-treatments based on synergy. Instead, any synergy gained for one cytokine or enzyme at a set dose or ratio has multiple other markers demonstrate antagonism.

4. Discussion

The inflammatory response in the brain and spinal cord has been a central concern of many researchers. Aspects of neuroinflammation, such as context, course, duration, and progression of primary cause, affect degree of inflammatory response. This response

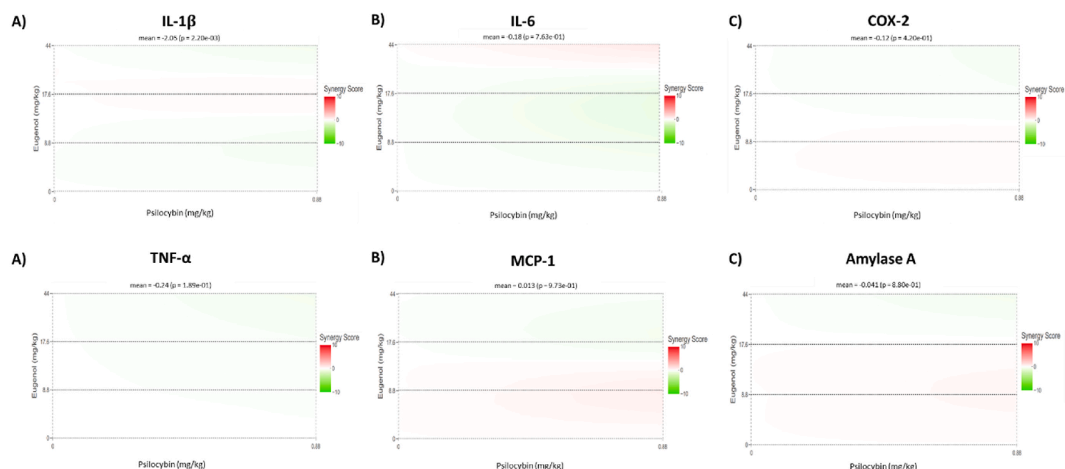

Fig. 9. Loewe synergy scores for negative fold change of cytokines and markers from the brains of mice post-treated with 0 or 0.88 mg/kg of psilocybin combined with 0, 8.8, 17.6, and 44 mg/kg of eugenol compared to the DSS group. Synergy scores are indicated for: (**A**) *IL-1β*, (**B**) *IL-6*, (**C**) *COX2*, (**D**) *TNF-α*, (**E**) *MCP-1*, and (**F**) *α-Amylase.* Synergy scores are calculated by SynergyFinder + using imputation. Red represents potentiation. Green represents antagonism. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

may result in recruitment of immune response, tissue damage or potential cell death. In addition, properties of natural compounds of mushrooms and plants and their effect on inflammation have been subject of interest in recent studies.

In this study, we utilized psilocybin and eugenol in combination to reduce neuroinflammation induced via the gut-brain axis. Psilocybin and eugenol were chosen based on previous studies that demonstrated superior and synergistic anti-inflammatory effects compared to other pairings with capsaicin or curcumin, and did not demonstrate any cytotoxic effects (Robinson et al., 2023a). Subsequently, psilocybin and eugenol were found to prevent gut dysbiosis in 3D models (Robinson et al., 2023b), while preventing neuroinflammation in an LPS-induced model of neuroinflammation (Zanikov et al., 2023).

As our previous study using LPS induced septic shock and resulted in severe and systemic inflammation, we decided to study another model utilizing DSS to induce gut-brain axis inflammation. While DSS is commonly used to study colitis, and results in endotoxemia, DSS does not induce severe septic shock similar to LPS. Furthermore, this model would better simulate the gut-brain axis contributions to neuroinflammatory diseases including multiple sclerosis, Alzheimer's, and Parkinson's disease, as well as anxiety and depressive-like disorders (Rutsch et al., 2020). As a result, we could determine if psilocybin and eugenol appear to provide anti-inflammatory effects by acting on the gut and potentially be useful in the treatment of the aforementioned diseases.

Previous studies have shown that DSS treatment elevates expression of IL-6, and TNF-α in hippocampus (Takahashi et al., 2019), while inflammation in the bowel is associated with alterations in the central nervous system by the activation of TNF-α signaling and microglia in the brain (Riazi et al., 2008). We established DSS-induced inflammation models in mice, which are supported by the enhanced expression of pro-inflammatory cytokines in the brain. Interestingly, we did not see consistent and upregulated levels of *TNF-α* (Figs. 2 and 3). Next, we demonstrated the effect of administrating different treatments of Eug and Psi, as well as their combinations, prior to and after inflammation induction. The effects of pre-treatment with either psilocybin and/or eugenol appeared to be minimal. In contrast, post-treatment demonstrated promise in inhibiting inflammation and decreasing cytokines.

Evidence suggests that psychedelics can stimulate neurogenesis and gliogenesis, reduce inflammation, and ameliorate oxidative stress, which could be used for therapeutics in psychiatric, neurodegenerative, and movement disorders (Kargbo, 2023). One study demonstrated anti-inflammatory and antidepressant effects of psilocybin in the human U937 macrophage cell line, by decreasing levels of TNF–α and IL–1β (Nkadimeng et al., 2021). IL-1β is a pro-inflammatory cytokine with multiple roles. It is involved in the regulation of pain, inflammation, homeostasis and differentiation of monocytes into conventional dendritic cells (Ren and Torres, 2009; Kaneko et al., 2019). TNF-α is a multifunctional cytokine, it has a pleiotropic effect on inflammation and immune response. TNF-α is involved in the regulation of immune–inflammatory reactions of host defense against infectious, autoimmune, and endocrine diseases and cancer, and its actions help determine the survival or death of various cells (Hsieh et al., 2022). The reduction of such pro-inflammatory cytokines is associated with improved health outcomes in chronic inflammation (Nkadimeng et al., 2020). Authors of this study also demonstrated that mushroom extracts contained components, such as *n*-hexadecanoic acid, 4 h-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl, 3-octanone, and dibutyl phthalate, can induce natural anti-inflammatory and antioxidant effects. Psilocybin's role in inflammation is a new topic and has yet to be fully studied, therefore more research is needed to explore its therapeutic potential.

Compared to psilocybin, eugenol's effects on inflammation have been more thoroughly studied. For instance, eugenol can alleviate DSS-induced colonic inflammation by downregulating pro-inflammatory cytokine expression, such as TNF-α, recover intestinal barrier and beneficially affect gut microbiota (Chen et al., 2021). Eugenol shows antidepressant effects by promoting metallothionein-III production in the hippocampus (Liaqat et al., 2022). Eugenol can also be used as a damage-preventing agent in oxidative stress (Barboza et al., 2018). Since eugenol ameliorates DSS-induced colitis, there is a possibility that the observed reduction in brain inflammation is not a direct result of addressing brain inflammation. Instead, it may be due to alleviating colitis, which would prevent endotoxemia resulting in the failure to induce brain inflammation. Therefore, this study does not provide explanation of the precise mechanism of action of psilocybin and eugenol in relation to the location of anti-inflammatory action, however, due to the gut-brain axis method of inflammation induction and previous papers showing amelioration of intestinal inflammation, we believe these effects are likely in part mediated through the gut (Robinson et al., 2023a, 2023b; Reed and Foldi, 2024). That being said, our previous data studying LPS-induced brain inflammation demonstrated similar anti-inflammatory results (Zanikov et al., 2023), suggesting psilocybin and eugenol does act directly on the brain. Further research should study the contributions of the anti-inflammatory effects of Psi and Eug on each the gut and the brain.

It is worth noting that there are neuropsychiatric and neurological diseases, in which gut-brain axis plays important role, such as depression, schizophrenia, Parkinson's disease, epilepsy and migraine (Socała et al., 2021). These diseases, whereby inflammation plays a large role in the pathogenesis of the disease, would likely benefit from psilocybin and eugenol co-treatment. While other neurological and gut diseases may be of benefit as well, this study in particular indicates that psilocybin and eugenol can beneficially influence the gut-brain axis.

Some studies report eugenol's ability to inhibit apoptosis and secretion of pro-inflammatory cytokines (Said and Abd Rabo, 2017), and can reduce *IL-1β* and *COX-2* gene expression (Hussain et al., 2011). COX-2 is activated in response to various factors, such as TNF-α. COX-2 can synthesize pro-inflammatory mediators and prostaglandins, which can function as immune-suppressors. It was also shown that upregulation of COX-2-associated with inflammation (Gandhi et al., 2017). In our previous study we described the effects of combined treatment involving both eugenol and psilocybin on LPS-induced brain inflammation in mice model (Zanikov et al., 2023).

After establishing the DSS-induced inflammation model in male mice, we administered psilocybin and eugenol as a pre-treatment or as a post-treatment. Apart from upregulation of *IL-1β* and *COX-2* in the DSS, relative to the control, RT-qPCR results for the pre-treatment did not show any significant changes (Fig. 2A and C, respectively). Although not significant, *TNF-α* trends to be downregulated for all groups compared to the control (Fig. 2D). On the other hand, post-treatment results of RT-qPCR demonstrated notable

downregulation of all treatment groups, relative to the DSS, for *IL-1β, IL-6* and *COX-2* (Fig. 3A, B and C, respectively). The most significant change was shown for downregulation of pro-inflammatory cytokine *IL-6* in the DSS + Psi + Eug (1:50), relative to the DSS groups (Fig. 3B). Interestingly, all treatment groups showed similar downregulation of *COX-2* compared to the DSS (Fig. 3C).

We also measured the effect of treatments for markers *MCP-1* and *α-Amylase.* MCP-1 is a chemokine and its main role in inflammation is to attract monocytes, memory T cells and dendric cells to the inflammatory sites. MCP-1 can also increase expression of cytokines TNF-α and IL-β (Tong et al., 2020). Surprisingly, DSS + psilocybin + eugenol (1:10) showed the most notable upregulation of *MCP-1* in the pre-treatment relative to the DSS group (Fig. 4A). Apart from that, all other groups showed a decrease in expression of *MCP-1* and most noticeable it was demonstrated for psilocybin, eugenol and DSS + Psi + Eug (1:50) groups compared to the DSS (Fig. 4A). On the other hand, the DSS + Psi + Eug (1:10) and DSS + Eug groups demonstrated lowest levels of *MCP-1* in the post-treatment compared to the DSS (Fig. 5A). All other groups also demonstrated a decrease in amounts of *MCP-1* compared to the DSS (Fig. 5A). *α-Amylase* is an enzyme that participates in the degradation of polysaccharides. It is used for the prognosis and treatment of inflammatory diseases, actinomycetes infection and against some bacteria (Gutiérrez-Corrales et al., 2017). Pre-treatment group of psilocybin + DSS demonstrated the most significant decrease of *α-Amylase* expression compared to the DSS group, while all other groups also showed relative downregulation of this marker (Fig. 4B). On the other hand, DSS + Psi + Eug (1:50) group in the post-treatment demonstrated similar upregulation of *α-Amylase* to the DSS group (Fig. 5B).

ELISA results demonstrated a decrease in levels of IL-2 compared to the DSS group (Fig. 6A). Apart from that, post-treatment did not show any effect on levels of IL-4 and IL-10 (Fig. 6B and C, respectively). IL-2 is a cytokine that is involved in the activation and regulation of the immune response (Hoyer et al., 2008). It is a key growth and survival factor of T-cells, it promotes differentiation of the memory T cell phenotype (Kanagalingam et al., 2019). It is produced by T cells, specifically $CD4^+$ helper cells and IL-2 can induce the proliferation of T cells and T-helper 1 (Th1) and Th2 effector (Abbas, 2020). IL-4 is a signature type II inflammatory response cytokine. It belongs to the Th2-type cytokines and is a key player in the inflammatory response triggered by allergy or parasite infection. It stimulates B cell proliferation and activation of eosinophils, basophils, and mast cells. Moreover, it also can activate immunoglobulin (Ig) class of IgE and IgG4, and it also plays a role in the fibrosis of internal organs and skin (Junttila, 2018). IL-10 is a cytokine that can affect multiple cell types. Its anti-inflammatory role is to suppress monocytes and macrophage ability, as well as production of pro-inflammatory cytokines, such as IL-6, by inhibiting Th1 and stimulating B lymphocytes and Th2 lymphocytes. Thus, leading to the suppression of inflammatory response (Rong et al., 2018). We also found a significant increase of MCP-1 amounts in blood for the DSS + Eug group compared to the control (Fig. 7B).

While psilocybin has potent anti-inflammatory effects, the exact mechanism has not been determined. Although, psilocybin's effects are likely mediated through 5-HT2A receptor, it could also act on the other receptors including 5-HT2B, 5-HT2C, or 5-HT1A (Tylš et al., 2014). The most plausible signaling of psilocybin involves the biased activation of the 5-HT2A receptor resulting in β-arrestin 2-dependent signaling, which negatively regulates NF-κB signaling (Koopman et al., 2021; Cheshmehkani et al., 2017); however, psilocybin can also act through $G\alpha_{q/11}$ and $G\alpha_{i/o}$. In contrast, recent evidence indicates that some effects may be mediated through glucocorticoid signaling pathways (Jones et al., 2023). While psilocybin can act directly on neuronal cells, it is expected the psilocybin acts on macrophages to reduce inflammatory cytokine production (Nkadimeng et al., 2021). Furthermore, based on our previous research showing psilocybin ameliorates gut dysbiosis (Robinson et al., 2023b), psilocybin is very likely to inhibit the DSS-induced colitis, thereby preventing neuroinflammation within this model. As a result, psilocybin's effects are likely multi-modal by inhibiting colitis and the resulting gut-brain axis inflammation, but also directly acting on neuronal and immune cells within the brain.

In contrast, eugenol acts on different receptors and is believed to activate different signaling pathways, which could explain why Eug and Psi have been shown to have synergy to reduce inflammation (Robinson et al., 2023a). Eugenol can interact with multiple TRP receptors including TRPV1 to induce CaMKK2 signaling (Huang et al., 2023), thereby suppressing chemokine production in myeloid subsets (Racioppi et al., 2019). Due to these differing anti-inflammatory mechanisms, it is not surprising that eugenol and psilocybin can have potent and synergistic anti-inflammatory effects (Robinson et al., 2023a). While we did see synergy for pre-treatment with psilocybin and eugenol, there were minimal effects on cytokine expression in the pre-treatment compared to controls. In contrast, post-treatments appeared to be antagonistic, however, strong anti-inflammatory effects were seen. It is not known why these differing effects occur between pre- and post-treatment. Regardless, post-treatment is a more likely clinical application as pre-treatment would require continuous consumption of psilocybin and the long-term effects of microdosing are not known (Ona and Bouso, 2020).

Safety concerns for psilocybin should also be mentioned (Moreno et al., 2006; Gukasyan et al., 2022). Despite common opinion, psilocybin has not been shown to be neurotoxic for enteral or parenteral administration (Hernandez-Leon et al., 2024). Furthermore, the calculated LD_{50}, which is over 2 g/kg, is significantly higher than the clinical dose of 25 mg (Hernandez-Leon et al., 2024). In contrast, there is reason to believe psilocybin might induce valvular disease due 5-HT2B agonism, which is known to cause thickening of cardiac valve leaflets, subvalvular apparatus, and impaired motion of the valves (Lam and Balachandran, 2015). Due to the potential risk of 5-HT2B agonism by psilocybin, the FDA has issued a First Draft Guidance on Clinical Trials with Psychedelic Drugs to encourage the evaluation of psilocybin on inducing valvular heart disease (McIntyre, 2023). Current studies are unsure if psilocin, the active ingredient and a conversion production of psilocybin, is strong enough agonist to induce valvular disease. The EC_{50} of psilocin is known to be higher than 20 μM (Rickli et al., 2016), which is approximately 1000-fold higher than the mean maximal psilocin blood concentration of 3.82 nM of the oral clinical dose (25 mg). Therefore, it is unlikely that the levels of psilocin after a clinical dose induce considerable 5-HT2B agonism, if any at all. However, future psilocybin clinical trials should rule out any association between psilocybin use and valvular hear disease prior to adoption as a therapeutic. In contrast, eugenol has been declared by the World Health Organization to be generally recognized as safe and non-mutagenic (Nisar et al., 2021).

5. Conclusions

This study established DSS-induced neuroinflammation in mice. Eugenol and psilocybin demonstrated potent abilities to reduce the expression of pro-inflammatory cytokines and other inflammatory markers. In addition, our study demonstrated the anti-inflammatory effects of combined treatment with psilocybin and eugenol in brain tissue, while implicating the gut-brain axis as a mechanism of action. While synergism has been shown in previous studies, psilocybin and eugenol demonstrated minimal synergy in this model. With growing interest in psilocybin applications for medical purposes, this study provides useful insights into its effect on inflammation, which will help guide future research in this area.

Statements and declarations

Research costs were supported via MITACS and in partnership with GoodCap Pharmaceuticals, however, the funders had no role in the collection, analysis, or interpretation of the data.

Funding

This work was supported by MITACS Accelerate to I.K.

CRediT authorship contribution statement

Timur Zanikov: Conceptualization, Methodology, Writing – original draft. **Marta Gerasymchuk:** Data curation, Methodology, Project administration, Writing – review & editing. **Gregory Ian Robinson:** Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – review & editing. **Esmaeel Ghasemi Gojani:** Investigation, Methodology. **Shima Asghari:** Methodology. **Alyssa Groves:** Methodology. **Mackenzie Cameron:** Methodology. **Rocio Rodriguez-Juarez:** Methodology, Resources. **Alexandra Snelling:** Conceptualization, Resources. **Darryl Hudson:** Conceptualization. **Anna Fiselier:** Writing – original draft, Writing – review & editing. **Olga Kovalchuk:** Conceptualization, Supervision, Validation, Writing – review & editing. **Igor Kovalchuk:** Conceptualization, Funding acquisition, Supervision, Validation, Writing – review & editing.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:While research costs were supported via MITACS and in partnership with GoodCap Pharmaceuticals, the funders had no role in the collection, analysis, or interpretation of the data. Any conflicts of interest have been mitigated.

Data availability

Data will be made available on request.

Acknowledgements

We thank Justin Dubiel and Hunter Johnson for helping with the organ collection in second part of the study. Also, thanks to all employers of the Vivarium for the help with anesthesia of animals and gavage.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bcab.2024.103033.

References

Abbas, A.K., 2020. The surprising story of IL-2: from experimental models to clinical application. Am. J. Pathol. 190 (9), 1776–1781. https://doi.org/10.1016/j.ajpath.2020.05.007.

Barboza, J.N., da Silva Maia Bezerra Filho, C., Silva, R.O., Medeiros, J.V.R., de Sousa, D.P., 2018. An overview on the anti-inflammatory potential and antioxidant profile of eugenol. Oxid. Med. Cell. Longev. 2018, 3957262. https://doi.org/10.1155/2018/3957262.

Bauer, M.E., Teixeira, A.L., 2019. Inflammation in psychiatric disorders: what comes first? Ann. N. Y. Acad. Sci. 1437 (1), 57–67. https://doi.org/10.1111/nyas.13712.

Cai, L., 1996. Compounds from syzygium aromaticum possessing growth inhibitory activity against oral pathogens. J. Nat. Prod. 59 (10), 987–990. https://doi.org/10.1021/np960451q.

Chassaing, B., Aitken, J.D., Malleshappa, M., Vijay-Kumar, M., 2014. Dextran sulfate sodium (DSS)-Induced colitis in mice. Curr. Protoc. Im. 104. https://doi.org/10.1002/0471142735.im1525s104. Unit-15.25.

Chen, W.W., Zhang, X., Huang, W.J., 2016. Role of neuroinflammation in neurodegenerative diseases. Mol. Med. Rep. 13 (4), 3391–3396. https://doi.org/10.3892/mmr.2016.4948. (Review).

Chen, S., Wu, X., Tang, S., et al., 2021. Eugenol alleviates dextran sulfate sodium-induced colitis independent of intestinal microbiota in mice. J. Agric. Food Chem. 69 (36), 10506–10514. https://doi.org/10.1021/acs.jafc.1c00917.

Cheshmehkani, A., Senatorov, I.S., Dhuguru, J., Ghoneim, O., Moniri, N.H., 2017. Free-fatty acid receptor-4 (FFA4) modulates ROS generation and COX-2 expression via the C-terminal β-arrestin phosphosensor in Raw 264.7 macrophages. Biochem. Pharmacol. 146, 139–150. https://doi.org/10.1016/j.bcp.2017.09.008.

Cochran, K.E., Lamson, N.G., Whitehead, K.A., 2020. Expanding the utility of the dextran sulfate sodium (DSS) mouse model to induce a clinically relevant loss of intestinal barrier function. PeerJ 8, e8681. https://doi.org/10.7717/peerj.8681.

DiSabato, D.J., Quan, N., Godbout, J.P., 2016. Neuroinflammation: the devil is in the details. J. Neurochem. 139 (Suppl. 2), 136–153. https://doi.org/10.1111/jnc.13607. Suppl 2.

Do, J., Woo, J., 2018. From gut to brain: alteration in inflammation markers in the brain of dextran sodium sulfate-induced colitis model mice. Clin. Psychopharmacol. Neurosci. 16 (4), 422–433. https://doi.org/10.9758/cpn.2018.16.4.422.

Ebert, S.E., Jensen, P., Ozenne, B., et al., 2019. Molecular imaging of neuroinflammation in patients after mild traumatic brain injury: a longitudinal 123 I-CLINDE single photon emission computed tomography study. Eur. J. Neurol. 26 (12), 1426–1432. https://doi.org/10.1111/ene.13971.
Elsayed, E.A., El Enshasy, H., Wadaan, M.A.M., Aziz, R., 2014. Mushrooms: a potential natural source of anti-inflammatory compounds for medical applications. Mediat. Inflamm. 2014, 805841. https://doi.org/10.1155/2014/805841.
Emge, J.R., Huynh, K., Miller, E.N., et al., 2016. Modulation of the microbiota-gut-brain axis by probiotics in a murine model of inflammatory bowel disease. Am. J. Physiol. Gastrointest. Liver Physiol. 310 (11), G989–G998. https://doi.org/10.1152/ajpgi.00086.2016.
Erkizia-Santamaría, I., Alles-Pascual, R., Horrillo, I., Meana, J.J., Ortega, J.E., 2022. Serotonin 5-HT2A, 5-HT2c and 5-HT1A receptor involvement in the acute effects of psilocybin in mice. In vitro pharmacological profile and modulation of thermoregulation and head-twich response. Biomed. Pharmacother. 154, 113612. https://doi.org/10.1016/j.biopha.2022.113612.
Esmaeili, F., Zahmatkeshan, M., Yousefpoor, Y., Alipanah, H., Safari, E., Osanloo, M., 2022. Anti-inflammatory and anti-nociceptive effects of Cinnamon and Clove essential oils nanogels: an in vivo study. BMC Compl. Med. Ther. 22 (1), 143. https://doi.org/10.1186/s12906-022-03619-9.
Fürst, R., Zündorf, I., 2014. Plant-derived anti-inflammatory compounds: hopes and disappointments regarding the translation of preclinical knowledge into clinical progress. Mediat. Inflamm. 2014, 146832. https://doi.org/10.1155/2014/146832.
Gandhi, J., Khera, L., Gaur, N., Paul, C., Kaul, R., 2017. Role of modulator of inflammation cyclooxygenase-2 in gammaherpesvirus mediated tumorigenesis. Front. Microbiol. 8, 538. https://doi.org/10.3389/fmicb.2017.00538.
Gukasyan, N., Davis, A.K., Barrett, F.S., et al., 2022. Efficacy and safety of psilocybin-assisted treatment for major depressive disorder: prospective 12-month follow-up. J. Psychopharmacol. 36 (2), 151–158. https://doi.org/10.1177/02698811211073759.
Gutiérrez-Corrales, A., Campano-Cuevas, E., Castillo-Dalí, G., Torres-Lagares, D., Gutiérrez-Pérez, J.L., 2017. Ability of salivary biomarkers in the prognostic of systemic and buccal inflammation. J. Clin. Exp. Dent. 9 (5), e716–e722. https://doi.org/10.4317/jced.53776.
Hernandez-Leon, A., Escamilla-Orozco, R.I., Tabal-Robles, A.R., et al., 2024. Antidepressant- and anxiolytic-like activities and acute toxicity evaluation of the Psilocybe cubensis mushroom in experimental models in mice. J. Ethnopharmacol. 320, 117415. https://doi.org/10.1016/j.jep.2023.117415.
Hoyer, K.K., Dooms, H., Barron, L., Abbas, A.K., 2008. Interleukin-2 in the development and control of inflammatory disease. Immunol. Rev. 226, 19–28. https://doi.org/10.1111/j.1600-065X.2008.00697.x.
Hsieh, H.L., Yu, M.C., Cheng, L.C., et al., 2022. Quercetin exerts anti-inflammatory effects via inhibiting tumor necrosis factor-α-induced matrix metalloproteinase-9 expression in normal human gastric epithelial cells. World J. Gastroenterol. 28 (11), 1139–1158. https://doi.org/10.3748/wjg.v28.i11.1139.
Huang, C.W., Chow, J.C., Tsai, J.J., Wu, S.N., 2012. Characterizing the effects of Eugenol on neuronal ionic currents and hyperexcitability. Psychopharmacology (Berl) 221 (4), 575–587. https://doi.org/10.1007/s00213-011-2603-y.
Huang, T., Chen, X., Chen, D., et al., 2023. Eugenol promotes appetite through TRP channels mediated-CaMKK2/AMPK signaling pathway. Phytother Res. 37 (7), 2759–2770. https://doi.org/10.1002/ptr.7768.
Hussain, A., Brahmbhatt, K., Priyani, A., Ahmed, M., Rizvi, T.A., Sharma, C., 2011. Eugenol enhances the chemotherapeutic potential of gemcitabine and induces anticarcinogenic and anti-inflammatory activity in human cervical cancer cells. Cancer Biother. Radiopharm. 26 (5), 519–527. https://doi.org/10.1089/cbr.2010.0925.
Jain, P., Hassan, A., Koyani, C., et al., 2015. Behavioral and molecular processing of visceral pain in the brain of mice: impact of colitis and psychological stress. Front. Behav. Neurosci. 9. https://www.frontiersin.org/articles/10.3389/fnbeh.2015.00177. (Accessed 19 May 2023).
Jones, N.T., Zahid, Z., Grady, S.M., et al., 2023. Transient elevation of plasma glucocorticoids supports psilocybin-induced anxiolysis in mice. ACS Pharmacol. Transl. Sci. 6 (8), 1221–1231. https://doi.org/10.1021/acsptsci.3c00123.
Junttila, I.S., 2018. Tuning the cytokine responses: an update on interleukin (IL)-4 and IL-13 receptor complexes. Front. Immunol. 9, 888. https://doi.org/10.3389/fimmu.2018.00888.
Kanagalingam, T., Solomon, L., Vijeyakumaran, M., Palikhe, N.S., Vliagoftis, H., Cameron, L., 2019. IL-2 modulates Th2 cell responses to glucocorticosteroid: a cause of persistent type 2 inflammation? Immun., Inflamm. Disease 7 (3), 112–124. https://doi.org/10.1002/iid3.249.
Kaneko, N., Kurata, M., Yamamoto, T., Morikawa, S., Masumoto, J., 2019. The role of interleukin-1 in general pathology. Inflamm. Regen. 39, 12. https://doi.org/10.1186/s41232-019-0101-5.
Kargbo, R.B., 2023. Psychedelic-Assisted neuroplasticity for the treatment of mental health disorders. ACS Med. Chem. Lett. 14 (2), 133–135. https://doi.org/10.1021/acsmedchemlett.2c00546.
Kempuraj, D., Thangavel, R., Natteru, P.A., et al., 2016. Neuroinflammation induces neurodegeneration. J. Neurol. Neurosurg. Spine 1 (1), 1003.
Koopman, N., Katsavelis, D., Hove, AS ten, Brul, S., de Jonge, W.J., eppen, J., 2021. The multifaceted role of serotonin in intestinal homeostasis. Int. J. Mol. Sci. 22 (17), 9487. https://doi.org/10.3390/ijms22179487.
Kumar, A., Siddiqi, N.J., Alrashood, S.T., Khan, H.A., Dubey, A., Sharma, B., 2021. Protective effect of eugenol on hepatic inflammation and oxidative stress induced by cadmium in male rats. Biomed. Pharmacother. 139, 111588. https://doi.org/10.1016/j.biopha.2021.111588.
Kurita, N., Yamashiro, K., Kuroki, T., et al., 2020. Metabolic endotoxemia promotes neuroinflammation after focal cerebral ischemia. J. Cerebr. Blood Flow Metabol. 40 (12), 2505–2520. https://doi.org/10.1177/0271678X19899577.
Lam, N.T., Balachandran, K., 2015. The mechanobiology of drug-induced cardiac valve disease. J. Long Term Eff. Med. Implants 25 (1–2), 27–40. https://doi.org/10.1615/jlongtermeffmedimplants.2015011871.
Liaqat, H., Parveen, A., Kim, S.Y., 2022. Antidepressive effect of natural products and their derivatives targeting BDNF-TrkB in gut–brain Axis. Int. J. Mol. Sci. 23 (23), 14968. https://doi.org/10.3390/ijms232314968.
Liu, S., Xu, A., Gao, Y., et al., 2021. Graphene oxide exacerbates dextran sodium sulfate-induced colitis via ROS/AMPK/p53 signaling to mediate apoptosis. J. Nanobiotechnol. 19 (1), 85. https://doi.org/10.1186/s12951-021-00832-5.
McIntyre, R.S., 2023. Serotonin 5-HT2B receptor agonism and valvular heart disease: implications for the development of psilocybin and related agents. Expet Opin. Drug Saf. 22 (10), 881–883. https://doi.org/10.1080/14740338.2023.2248883.
Moreno, F.A., Wiegand, C.B., Taitano, E.K., Delgado, P.L., 2006. Safety, tolerability, and efficacy of psilocybin in 9 patients with obsessive-compulsive disorder. J. Clin. Psychiatry 67 (11), 1735–1740. https://doi.org/10.4088/jcp.v67n1110.
Nisar, M.F., Khadim, M., Rafiq, M., Chen, J., Yang, Y., Wan, C.C., 2021. Pharmacological properties and health benefits of eugenol: a comprehensive review. Oxid. Med. Cell. Longev. 2021, 2497354. https://doi.org/10.1155/2021/2497354.
Nkadimeng, S.M., Nabatanzi, A., Steinmann, C.M.L., Phytochemical, Eloff JN., 2020. Cytotoxicity, antioxidant and anti-inflammatory effects of psilocybe natalensis magic mushroom. Plants 9 (9), 1127. https://doi.org/10.3390/plants9091127.
Nkadimeng, S.M., Steinmann, C.M.L., Eloff, J.N., 2021. Anti-inflammatory effects of four psilocybin-containing magic mushroom water extracts in vitro on 15-lipoxygenase activity and on lipopolysaccharide-induced cyclooxygenase-2 and inflammatory cytokines in human U937 macrophage cells. J. Inflamm. Res. 14, 3729–3738. https://doi.org/10.2147/JIR.S317182.
Norden, D.M., Trojanowski, P.J., Villanueva, E., Navarro, E., Godbout, J.P., 2016. Sequential activation of microglia and astrocyte cytokine expression precedes increased Iba-1 or GFAP immunoreactivity following systemic immune challenge. Glia 64 (2), 300–316. https://doi.org/10.1002/glia.22930.
Ona, G., Bouso, J.C., 2020. Potential safety, benefits, and influence of the placebo effect in microdosing psychedelic drugs: a systematic review. Neurosci. Biobehav. Rev. 119, 194–203. https://doi.org/10.1016/j.neubiorev.2020.09.035.
Racioppi, L., Nelson, E.R., Huang, W., et al., 2019. CaMKK2 in myeloid cells is a key regulator of the immune-suppressive microenvironment in breast cancer. Nat. Commun. 10 (1), 2450. https://doi.org/10.1038/s41467-019-10424-5.
Reed, F., Foldi, C.J., 2024. Do the therapeutic effects of psilocybin involve actions in the gut? Trends in Pharmacological Sciences. Published online. https://doi.org/10.1016/j.tips.2023.12.007.
Ren, K., Torres, R., 2009. Role of interleukin-1 beta during pain and inflammation. Brain Res. Rev. 60 (1), 57–64. https://doi.org/10.1016/j.brainresrev.2008.12.020.
Revi, N., Rengan, A.K., 2020. Eugenol-encapsulated nanocarriers for microglial polarisation: a promising therapeutic application for neuroprotection. BioNanoSci. 10 (4), 1010–1017. https://doi.org/10.1007/s12668-020-00789-z.
Riazi, K., Galic, M.A., Kuzmiski, J.B., Ho, W., Sharkey, K.A., Pittman, Q.J., 2008. Microglial activation and TNFα production mediate altered CNS excitability following

peripheral inflammation. Proc. Natl. Acad. Sci. USA 105 (44), 17151–17156. https://doi.org/10.1073/pnas.0806682105.
Rickli, A., Moning, O.D., Hoener, M.C., Liechti, M.E., 2016. Receptor interaction profiles of novel psychoactive tryptamines compared with classic hallucinogens. Eur. Neuropsychopharmacol 26 (8), 1327–1337. https://doi.org/10.1016/j.euroneuro.2016.05.001.
Robinson, G.I., Li, D., Wang, B., et al., 2023a. Anti-inflammatory effects of serotonin receptor and transient receptor potential channel ligands in human small intestinal epithelial cells. Curr. Issues Mol. Biol. 45 (8), 6743–6774. https://doi.org/10.3390/cimb45080427.
Robinson, G.I., Li, D., Wang, B., et al., 2023b. Psilocybin and eugenol reduce inflammation in human 3D EpiIntestinal tissue. Life 13 (12), 2345. https://doi.org/10.3390/life13122345.
Rong, Y.D., Bian, A.L., Hu, H.Y., Ma, Y., Zhou, X.Z., 2018. Study on relationship between elderly sarcopenia and inflammatory cytokine IL-6, anti-inflammatory cytokine IL-10. BMC Geriatr. 18 (1), 308. https://doi.org/10.1186/s12877-018-1007-9.
Rutsch, A., Kantsjö, J.B., Ronchi, F., 2020. The gut-brain Axis: how microbiota and host inflammasome influence brain physiology and pathology. Front. Immunol. 11, 604179. https://doi.org/10.3389/fimmu.2020.604179.
Said, M.M., Abd Rabo, M.M., 2017. Neuroprotective effects of eugenol against aluminiuminduced toxicity in the rat brain. Arh. Hig. Rad. Toksikol. 68 (1), 27–36. https://doi.org/10.1515/aiht-2017-68-2878.
Shabab, T., Khanabdali, R., Moghadamtousi, S.Z., Kadir, H.A., Mohan, G., 2017. Neuroinflammation pathways: a general review. Int. J. Neurosci. 127 (7), 624–633. https://doi.org/10.1080/00207454.2016.1212854.
Socała, K., Doboszewska, U., Szopa, A., et al., 2021. The role of microbiota-gut-brain axis in neuropsychiatric and neurological disorders. Pharmacol. Res. 172, 105840. https://doi.org/10.1016/j.phrs.2021.105840.
Taheri, P., Yaghmaei, P., Tehrani, H.S., Ebrahim-Habibi, A., 2019. Effects of eugenol on Alzheimer's disease-like manifestations in insulin- and aβ-induced rat models. Neurophysiology 51 (2), 114–119. https://doi.org/10.1007/s11062-019-09801-z.
Takahashi, K., Nakagawasai, O., Nemoto, W., et al., 2019. Effect of Enterococcus faecalis 2001 on colitis and depressive-like behavior in dextran sulfate sodium-treated mice: involvement of the brain–gut axis. J. Neuroinflammation 16 (1), 201. https://doi.org/10.1186/s12974-019-1580-7.
Tong, X., Zeng, H., Gu, P., Wang, K., Zhang, H., Lin, X., 2020. Monocyte chemoattractant protein-1 promotes the proliferation, migration and differentiation potential of fibroblast-like synoviocytes via the PI3K/P38 cellular signaling pathway. Mol. Med. Rep. 21 (3), 1623–1632. https://doi.org/10.3892/mmr.2020.10969.
Tylš, F., Páleníček, T., Horáček, J., 2014. Psilocybin – summary of knowledge and new perspectives. Eur. Neuropsychopharmacol 24 (3), 342–356. https://doi.org/10.1016/j.euroneuro.2013.12.006.
Won, M.H., Lee, J.C., Kim, Y.H., et al., 1998. Postischemic hypothermia induced by eugenol protects hippocampal neurons from global ischemia in gerbils. Neurosci. Lett. 254 (2), 101–104. https://doi.org/10.1016/S0304-3940(98)00664-8.
Wu, H., Chen, Q.Y., Wang, W.Z., et al., 2021. Compound sophorae decoction enhances intestinal barrier function of dextran sodium sulfate induced colitis via regulating notch signaling pathway in mice. Biomed. Pharmacother. 133, 110937. https://doi.org/10.1016/j.biopha.2020.110937.
Wyss-Coray, T., Mucke, L., 2002. Inflammation in neurodegenerative disease–a double-edged sword. Neuron 35 (3), 419–432. https://doi.org/10.1016/s0896-6273(02)00794-8.
Yadav, B., Wennerberg, K., Aittokallio, T., Tang, J., 2015. Searching for drug synergy in complex dose-response landscapes using an interaction potency model. Comput. Struct. Biotechnol. J. 13, 504–513. https://doi.org/10.1016/j.csbj.2015.09.001.
Yu, B., Becnel, J., Zerfaoui, M., Rohatgi, R., Boulares, A.H., Nichols, C.D., 2008. Serotonin 5-hydroxytryptamine(2A) receptor activation suppresses tumor necrosis factor-alpha-induced inflammation with extraordinary potency. J. Pharmacol. Exp. Therapeut. 327 (2), 316–323. https://doi.org/10.1124/jpet.108.143461.
Zanikov, T., Gerasymchuk, M., Ghasemi Gojani, E., et al., 2023. The effect of combined treatment of psilocybin and eugenol on lipopolysaccharide-induced brain inflammation in mice. Molecules 28 (6), 2624. https://doi.org/10.3390/molecules28062624.
Zheng, S., Wang, W., Aldahdooh, J., et al., 2022. SynergyFinder plus: toward better interpretation and annotation of drug combination screening datasets. Dev. Reprod. Biol. 20 (3), 587–596. https://doi.org/10.1016/j.gpb.2022.01.004.