Brazilian Journal of Biology®

ISSN 1519-6984 (Print)
ISSN 1678-4375 (Online)

Original Article

Effects of ortho-eugenol on anxiety, working memory and oxidative stress in mice

Efeitos do orto-eugenol na ansiedade, memória de trabalho e estresse oxidativo em camundongos

R. Godoy[a], A. B. Macedo[a], K. Y. Gervazio[a,b], L. R. Ribeiro[a], J. L. F. Lima[a,b] and M. G. S. S. Salvadori[a,b*]

[a]Universidade Federal da Paraíba, Instituto de Pesquisa em Fármacos e Medicamentos, Laboratório de Psicofarmacologia, João Pessoa, PB, Brasil
[b]Universidade Federal da Paraíba, Centro de Ciências da Saúde, Programa de Pós-graduação em Produtos Bioativos Naturais e Sintéticos – PgPNSB, João Pessoa, PB, Brasil

Abstract

Ortho-eugenol is a synthetic derivative from eugenol, the major compound of clove essential oil, which has demonstrated antidepressant and antinociceptive effects in pioneering studies. Additionally, its effects appear to be dependent on the noradrenergic and dopaminergic systems. Depression and anxiety disorders are known to share a great overlap in their pathophysiology, and many drugs are effective in the treatment of both diseases. Furthermore, high levels of anxiety are related to working memory deficits and increased oxidative stress. Thus, in this study we investigated the effects of acute treatment of ortho-eugenol, at 50, 75 and 100 mg/kg, on anxiety, working memory and oxidative stress in male Swiss mice. Our results show that the 100 mg/kg dose increased the number of head-dips and reduced the latency in the hole-board test. The 50 mg/kg dose reduced malondialdehyde levels in the prefrontal cortex and the number of Y-maze entries compared to the MK-801-induced hyperlocomotion group. All doses reduced nitrite levels in the hippocampus. It was also possible to assess a statistical correlation between the reduction of oxidative stress and hyperlocomotion after the administration of ortho-eugenol. However, acute treatment was not able to prevent working memory deficits. Therefore, the present study shows that ortho-eugenol has an anxiolytic and antioxidant effect, and was able to prevent substance-induced hyperlocomotion. Our results contribute to the elucidation of the pharmacological profile of ortho-eugenol, as well as to direct further studies that seek to investigate its possible clinical applications.

Keywords: essential oils, anxiolytic, lipid peroxidation, hyperlocomotion, psychopharmacology.

Resumo

Ortho-eugenol é um derivado sintético do eugenol, composto principal do óleo essencial de cravo, que demonstrou efeitos antidepressivos e antinociceptivos em estudos pioneiros. Além disso, seus efeitos parecem ser dependentes dos sistemas noradrenérgico e dopaminérgico. Sabe-se que os transtornos de depressão e ansiedade compartilham uma grande sobreposição em sua fisiopatologia, e muitas drogas são eficazes no tratamento de ambas as doenças. Além disso, altos níveis de ansiedade estão relacionados a déficits de memória de trabalho e aumento do estresse oxidativo. Assim, o presente estudo investigou os efeitos do tratamento agudo de orto-eugenol, nas doses de 50, 75 e 100 mg/kg, na ansiedade, memória de trabalho e estresse oxidativo em camundongos Swiss machos. Nossos resultados mostram que a dose de 100 mg/kg aumentou o número de mergulhos e reduziu a latência no teste da placa perfurada. A dose de 50 mg/kg reduziu os níveis de malondialdeído no córtex pré-frontal e o número de entradas no labirinto em Y em comparação com o grupo de hiperlocomoção induzida por MK-801. Todas as doses reduziram os níveis de nitrito no hipocampo. Também foi possível avaliar uma correlação estatística entre a redução do estresse oxidativo e a hiperlocomoção após a administração do orto-eugenol. No entanto, o tratamento agudo não foi capaz de prevenir os déficits de memória de trabalho. Portanto, o presente estudo mostra que o orto-eugenol tem efeito ansiolítico e antioxidante, sendo capaz de prevenir a hiperlocomoção induzida pela substância. Nossos resultados contribuem para a elucidação do perfil farmacológico do orto-eugenol, bem como para direcionar novos estudos que busquem investigar suas possíveis aplicações clínicas.

Palavras-chave: óleos essenciais, ansiolítico, peroxidação lipídica, hiperlocomoção, psicofarmacologia.

*e-mail: mirian.salvadori@gmail.com
Received: February 5, 2023 – Accepted: June 21, 2023

1. Introduction

Essential oils are volatile compounds extracted from various plant parts and whose composition encompasses a great complexity of bioactive metabolites (Machado and Fernandes Junior, 2011). Their use in therapies has been of increasing interest due to their ease of extraction, low cost of obtaining and great diversity of biological effects (Zhang et al., 2021), such as anxiolytic (Chioca et al., 2013), antioxidant (Barboza, 2018) and working memory improvement effects (Amine et al., 2020).

The phenylpropanoid eugenol is the major compound of clove (*Syzygium aromaticum)* essential oil. Many studies show its wide bioactive potential, including antioxidant effect through the inhibition of lipid peroxidation (Ogata et al., 2000), neuroprotection (Irie et al., 2004) and antidepressant activity (Irie et al., 2004; Tao et al., 2005). Its mechanisms of action include the inhibition of monoamine oxidase (Tao et al., 2005), an enzyme that degrades monoaminergic neurotransmitters, and activation of alpha-2 noradrenergic receptors (Park et al., 2011). Furthermore, treatment with clove essential oil has shown promise in reversing scopolamine-induced memory deficits, as reported by Halder et al. (2011), and Siyal et al. (2023) provided evidence of a possible anxiolytic effect of eugenol.

In this scenario, ortho-eugenol consists of a synthetic isomer of eugenol which is still poorly investigated. Pioneering studies have shown that ortho-eugenol has antidepressant and antinociceptive effects in mice and, after the investigation of their mechanisms of action through the administration of receptor antagonists, it was observed a possible activation of dopaminergic D1, and noradrenergic alpha-1 and alpha-2 receptors (unpublished data; Fonsêca et al., 2016).

Data from the World Health Organization (2017) indicate that anxiety disorders affect more than 264 million people and are an increasingly urgent global concern. Anxiety consists of a set of responses that arise from the anticipation of potentially dangerous situations (LeDoux and Pine, 2016). In addition to subjective feelings, anxiety is also associated with physiological responses, such as muscle tension, sweating and tachycardia, and with behavioral responses, which includes avoiding a certain situation or environment (Craske et al., 2011). It is important to emphasize that anxious responses are natural and necessary for a healthy life. However, in cases where there is a prolonged or recurrent exacerbation of these responses, to the point of undermining the patient's capabilities and quality of life, it may be configured as an anxiety disorder (Kaur and Singh, 2017).

The neurobiology of anxiety disorders is associated with dysregulated levels of monoaminergic neurotransmitters such as serotonin, norepinephrine and dopamine. These neurotransmitters, also related to depression, act diffusely through the nervous system, influencing neuronal arousal, wakefulness, attention, and modulating the responsiveness of the amygdala, the limbic system and the prefrontal cortex to emotionally salient stimuli (Morilak and Frazer, 2004).

As for the pharmacological treatment of anxiety disorders, selective serotonin reuptake inhibitors (SSRIs) and serotonin and norepinephrine reuptake inhibitors (SNRIs) are among the first-line drugs (Murrough et al., 2015). These drugs act by blocking presynaptic serotonergic (SSRIs and SNRIs) and noradrenergic transporters (only SNRIs), therefore increasing the availability of serotonin and noradrenaline in the synaptic cleft (Telles-Correia et al., 2007). The use of these drugs, however, is limited by their long-term tolerability and by their adverse effects, such as sexual impotence, drowsiness, nausea, diarrhea and headache (Goodwin, 2015; Murrough et al., 2015). The SSRI and SNRI classes are frequently used to treat both depression and anxiety disorders (Cummings et al., 2014). The high incidence of anxiety disorders, added to the large number of adverse effects and the long-term tolerability of current anxiolytic drugs, reinforces the need to develop new pharmacological alternatives.

In turn, working memory (WM) is a type of declarative memory that is part of the executive functions of the brain (Clark and Beck, 2010). It allows the individual to keep in mind, for a brief time, information that can be manipulated or stored by the brain (Izquierdo, 2018). WM is modulated by monoaminergic neurotransmitters and depends on an adequate balance between noradrenaline (NA) and dopamine (DA) levels (Wang et al., 2019). For instance, optimal levels of NA improve WK through the activation of alpha-2 adrenergic receptors in the prefrontal cortex; whilst higher NA levels increase the activation of alpha-1 receptors, leading to WK impairment (Li et al., 1999). Dopamine also positively modulates working memory and improves overall cognitive performance (Bäckman and Nyberg, 2013). Not surprisingly, administration of either D1 or alpha-2 receptor antagonists produces dysfunction in working memory performance (Birnbaum et al., 2000).

A relationship between anxiety and working memory was shown by Otto et al. (2016), where high levels of anxiety impair working memory, and deficits in working memory increase individual susceptibility to anxiety and stress. Other mental disorders are also related to working memory deficits, such as depression (Nikolin et al., 2021), attention deficit hyperactivity disorder (ADHD) (Millan et al., 2012), schizophrenia (Millan et al., 2012), Alzheimer's disease (Gilmour et al., 2019), obsessive-compulsive disorder (OCD) (Meram et al., 2021) and autism spectrum disorders (ASD) (Rabiee et al., 2018); conditions in which working memory deficits are detrimental to patients' quality of life.

Oxidative stress consists in natural cellular processes that give rise to free radicals, such as reactive oxygen species. Although cells have mechanisms to counteract with it, certain conditions can result in exacerbated levels that damage cellular structures (Barbosa et al., 2010). Elevated levels of oxidative stress, which can be detected by nitrate and malondialdehyde (Chakraborty et al., 2009) levels, are associated with both anxiety (Bouayed et al., 2009) and working memory deficits (Al-Amin et al., 2019). In fact, Bouayed et al. (2009) demonstrated that mice treated with substances that induce oxidative stress develop anxiety-like behaviors.

Prefrontal cortex and hippocampus are brain structures that play important roles in both anxiety and working memory (Albani et al., 2014; Bishop et al., 2004; Lalonde, 2002). It has been reported that induced oxidative stress in

the prefrontal cortex, assessed by enhanced malondialdehyde levels, provokes anxiety-like behavior and working memory impairments in mice (Namgyal et al., 2021). Moreover, murine anxiety models present higher levels of reactive oxygen species in the hippocampus (Hassan et al., 2014; Hovatta et al., 2010), and exacerbated levels of nitric oxide mediate memory dysfunction, including the induction of working memory deficits (Udayabanu et al., 2008; Yamada et al., 1999). Finally, dysregulation of dopamine and noradrenaline levels is also associated with the imbalance of oxidative stress in the brain (Álvarez-Diduk and Galano, 2015; Ben-Shachar et al., 1995).

In summary, ortho-eugenol consists of a poorly studied synthetic isomer of eugenol whose mechanisms of action appear to act on the dopaminergic and noradrenergic systems. Taking into account the pharmacological profile of eugenol, as well as data from pioneering works on ortho-eugenol, the present project proposed to investigate the effects of ortho-eugenol on anxiety, working memory and oxidative stress.

2. Materials and Methods

2.1. Animal feeding and housing conditions

All experiments were conducted with male albino Swiss mice about 3 months old and weighing 25–35 grams. The tests were carried out at the Psychopharmacology Laboratory localized in the Institute of Research in Drugs and Medicines (IPeFarM) of the Federal University of Paraíba (UFPB), where the animals were weighed and housed in polyethylene cages, containing between 7 and 10 animals each, and kept under controlled temperature conditions (21 ± 1ºC), with free access to pellets (Purina®) and water available in polyethylene bottles. Sixty minutes before the tests, the animals were deprived of food and water. The benches and equipment were sanitized with 70% alcohol, however, during the tests, low-grade ethanol (10%) was used in an attempt to reduce possible odors that could interfere with the behavior of the animals. All procedures from the beginning to the moment of euthanasia were performed trying to avoid the suffering and pain of the animals as much as possible. The project was approved by the Ethics Committee on the Use of Animals (CEUA/UFPB) under the numbers 6541260419 and 1828070621.

2.2. Substances and doses

The substances and their respective doses were prepared minutes before their administration, using decimal concentrations in order to allow the injection of 0.1 mL/10 g of the animal's weight. Immediately before the tests, ortho-eugenol was dissolved in tween 80 and the other drugs (diazepam and MK-801) were diluted in saline solution. All injections were administered intraperitoneally and acutely. The doses used were 50, 75 and 100 mg/kg for ortho-eugenol, 1 mg/kg for diazepam, and 0.15 mg/kg for MK-801.

2.3. Hole-board test

The hole-board test was originally developed by Boissier et al. (1960) in the 1960s, and can be used to investigate anxiety-like behavioral patterns. This test consists of a flat platform containing 16 holes, which the animal is free to explore for a predetermined time (Casarrubea et al., 2009). As for the rationale of the test, it is theorized that it generates an anxiogenic situation in which mice, naturally curious animals, show less exploration of the holes, avoiding exposure to potential predators. Nevertheless, the administration of anxiolytic drugs is able to reduce fear and increase mice exploratory behavior, increasing the number of head-dips (Casarrubea et al., 2017; Crawley, 1985).

Diazepam is a drug of the benzodiazepine class already validated for the clinical treatment of anxiety. Thus, this drug is widely used in preclinical studies as a positive control group, since its anxiolytic effects can also be observed in rats and mice through several behavioral tests (Griebel et al., 2000; Lepicard et al., 2000; Pellow et al., 1985).

Groups containing seven animals were used: a negative control group, treated with vehicle (2% tween solution), a positive control group treated with diazepam (1 mg/kg) and three groups treated with ortho-eugenol (50, 75 and 100 mg/kg). After 30 minutes (for control and diazepam groups) and 45 minutes (for ortho-eugenol groups), the animals were placed individually in the center of the plate. During the five-minute period of free exploration, the parameters recorded were the time elapsed until the first head-dip in any hole (latency) and the total number of head-dips. Head-dips were considered when the animal's head was entirely inside the board hole.

2.4. Y-maze test

The Y-maze can be used to assess working memory in mice from the analysis of spontaneous alternation, which is based on the fact that mice are naturally curious and tend to explore new environments. Individuals with intact working memory will remember the most recently visited arms, and will tend to visit all three arms more or less uniformly (spontaneous alternation); while mice with dysfunctional working memory will show reduced rates of spontaneous alternation (Kraeuter et al., 2019).

In the experimental stage, each arm was identified as A, B or C; the animals were placed at the end of arm A and recorded while exploring the maze freely for 8 minutes. During the experiment, an experimenter registered the pattern of the mice's entries. In the evaluation stage, the recorded videos were also analyzed and the entry patterns in each arm were counted once again, in order to ensure uniformity with simultaneous analysis. Each entry was considered when all four limbs of the animal were within the explored arm.

A spontaneous alternation is defined as three consecutive entries into different arms. The maximum number of alternations is the total of entries minus two. Therefore, the alternation percentage is calculated by: (number of alternation/number of entries – 2) x 100 (Crouzier et al., 2018; Wong et al., 2020; Kraeuter et al., 2019). Noteworthy, the more data (number of entries) are provided to the presented formula, the better will be the statistical strength of the calculated entry patterns. In this sense, for the evaluation of working memory, a five-day habituation protocol was

implemented, with the objective of minimizing the animals' stress and anxiety during the test and mitigating possible negative influences of these factors on exploratory behavior.

The adopted protocol was established after consulting several studies in the specialized literature (Gouveia and Hurst, 2017; Henderson et al., 2020; Hurst and West, 2010; Marcotte et al., 2021). In the habituation stage, and later in the experimental stage, the animals were handled with 10 cm PVC pipes. The pipes remained in the cages during the five days of habituation, so that the animals could become familiar with them. The protocol is based on the mice's natural affinity for tunnels and closed environments and, compared to the usual method of handling by the base of the tail, it is able to considerably reduce the mice's stress and anxiety levels (Gouveia and Hurst, 2019; Hurst and West, 2010). In fact, a study by Gouveia and Hurst (2019) showed that animals that underwent tunnel manipulation and received subcutaneous injections still showed a considerably higher voluntary interaction with the researcher compared to animals that were only handled by the base of the tail, but that did not receive any injections.

MK-801 (dizocilpine) is a non-competitive antagonist of NMDA glutamate channels (Zemanova et al., 2013). Several studies use MK-801 to induce working memory deficits, in many cases seeking to analyze whether or not this situation could be reversed by test drugs. In these cases, the group that receives only MK-801 constitutes the positive control group of the experiments (Brown et al., 2013; Lainiola et al., 2014; Zemanova et al., 2013; Ohno and Watanabe, 1995; Chino et al., 2019; Shiraishi et al., 2016; Rosenbrock et al., 2019).

In previous experiments conducted by our group, it was performed the standardization of the Y-maze and the ideal dose of MK-801 (non-competitive antagonist of NMDA channels) to be used with the aim of impairing the Swiss mice working memory. After these, the defined dose was 0.15 mg/kg, also used by several studies in the literature (Lainiola et al., 2014; Maurice et al., 1997; Zemanova et al., 2013).

The animals were divided into five groups: negative control, treated with saline; positive control, treated with MK-801 at a dose of 0.15 mg/kg; and three experimental groups treated with both MK-801 and ortho-eugenol at doses of 50, 75 or 100 mg/kg, all intraperitoneally, as standardized by Fonsêca et al. (2016). It is worth noting that the assessment of working memory was conducted on days and with animals different from the hole-board test, preventing the same animals from undergoing both behavioral tests. In the experimental groups, ortho-eugenol and MK-801 were administered intraperitoneally 45 and 30 minutes before the tests, respectively. In the positive control group, only the administration of MK-801 was performed 30 minutes before the behavioral tests, as described in several studies (Bruin et al., 2010; Maurice et al., 1994; Miki et al., 2019; Rychtyk et al., 2019). The negative control group received only saline 30 minutes before the tests.

2.5. Evaluation of in vivo antioxidant activity

All the antioxidant evaluation tests were performed with the animals that underwent the working memory test. The methodologies used were the TBARS and nitrite determination, using the structures of the prefrontal cortex and hippocampus, respectively. Euthanasia by decapitation and removal of structures took place immediately after the animals left the Y-maze.

2.5.1. Lipid peroxidation by the TBARS assay

According to Draper and Hadley (1990), the formation of lipid peroxides during lipid peroxidation is accompanied by the measurement of thiobarbituric acid reactive substances (TBARS). Brain structures (prefrontal cortex) were dissected and a 10% homogenate (w/v, in 1.15% KCl) was prepared. Briefly, the samples were mixed with 1 ml of trichloroacetic acid (10%) and 1 ml of thiobarbituric acid (0.6%). The reaction was heated in a water bath for 15 min and n-butanol (2:1 v/v) was added to the medium. After centrifugation (800 x g for 15 min) the TBARS content was determined at 535 nm and the result expressed as grams of MDA/grams of sample.

2.5.2. Determination of nitrite levels

To determine the effects of ortho-eugenol on nitric oxide (NO) production, we quantified the nitrite levels in the hippocampus using the Griess reagent test (Green et al., 1981; Radenovic and Selakovic, 2005). The structure (hippocampus) homogenate was centrifuged (800 x g for 10 min), 100 µL of the supernatant was collected and 100 µL of Griess reagent (1% sulfanilamide / 0.1% N-(1-naphthyl)ethylenediamine hydrochloride / 5% phosphoric acid / distilled water – 1:1:1:1) and incubated at room temperature for 10 min. The calibration curve was prepared under the same conditions, using different concentrations of $NaNO_2$ (0.75–100 mM). The blank was prepared using 100 µL of the Griess reagent with 100 µL of the buffer used to prepare the homogenate. The absorbance of the samples was determined at a wavelength of 560 nm, and the concentration of nitrite was expressed as nM nitrite/grams of the sample.

2.6. Statistical analysis

Firstly, the normality of the samples was assessed by the Shapiro-Wilk test. For parametric data, statistical analysis was performed using one-way ANOVA followed by Dunnett's *post hoc* test. For nonparametric data, the Kruskal-Wallis test was performed followed by Dunn's *post hoc* test. To investigate possible correlations between results, Pearson's correlation test was used. The values were represented by the mean ± standard error of the mean (SEM), with differences considered statistically significant when $p < 0.05$.

3. Results

3.1. Assessment of anxious-like behavior

The number of head-dips performed during the hole-board test are shown in Figure 1. It was possible to find a statistically significant increase in the number of head-dips between the diazepam-treated group (30.7 ± 1.5; $p < 0.0001$) and the control group (16.1 ± 1.0). Among the groups treated with ortho-eugenol, only the dose of

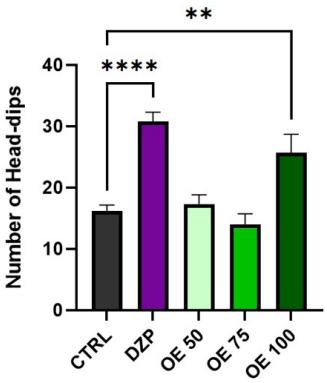

Figure 1. Number of dips in the perforated plate test. Effects of ortho-eugenol (50, 75 and 100 mg/kg), diazepam (1 mg/kg) and vehicle on the number of head-dips in the hole-board test. Values expressed as mean ± SEM (n = 5–8). One-way ANOVA followed by Dunnett's test. **p < 0.01, ****p < 0.0001.

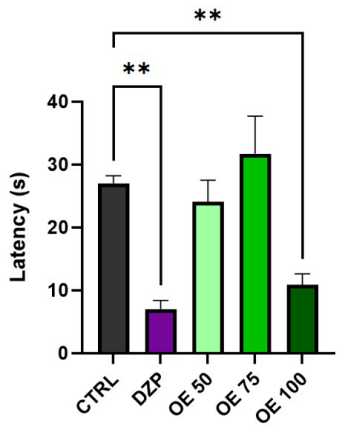

Figure 2. Latency time in the hole-board test. Effects of ortho-eugenol (50, 75 and 100 mg/kg), diazepam (1 mg/kg) and vehicle on latency time (in seconds) in the perforated plate test. Values expressed as mean ± SEM (n = 5–8). One-way ANOVA followed by Dunnett's test. **p < 0.01.

100 mg/kg (25.6 ± 3.0; p = 0.003) was able to significantly increase the number of head-dips. Meanwhile, 50 mg/kg (17.3 ± 1.4; p = 0.97) and 75 mg/kg (14.0 ± 1.7; p = 0.81) doses showed no significant differences.

The latency for the first head-dip in the hole-board test is shown in Figure 2. In this parameter, there was a statistically significant reduction between the diazepam-treated group (7.0 ± 1.3; p = 0.003) and the control group (27.0 ± 1.2). In the groups treated with ortho-eugenol, only the dose of 100 mg/kg (10.8 ± 1.7; p = 0.006) was able to significantly reduce the latency. However, the doses of 50 mg/kg (24.0 ± 3.4; p = 0.93) and 75 mg/kg (31.7 ± 5.9; p = 0.72) did not show significant differences.

3.2. Assessment of working memory

The percentage of spontaneous alternations in the Y-maze test is shown in Figure 3. All groups were compared to the control group and to the group treated with MK-801. The administration of MK-801 (56.6 ± 2.0; p = 0.005) was able to reduce the percentage of spontaneous alternations in relation to the control group (67.9 ± 1.5). However, when compared with the MK-801 groups, none of the groups treated with ortho-eugenol at doses of 50 (61.5 ± 1.4; p = 0.44), 75 (62.3 ± 3.8; p = 0.30) or 100 (59.5 ± 2.7; p = 0.80) mg/kg showed any statistically significant changes in this parameter. Experimental groups also showed no difference in comparison with the control group.

The number of entries in the Y-maze test is shown in Figure 4. All groups were compared with the control group and to the MK-801-treated group. The data obtained indicate that both the group treated with MK-801 (76.1 ± 5.9; p = 0.002) and the group previously treated with ortho-eugenol at a dose of 75 mg/kg (74.6 ± 5.4; p = 0.005) increased the number of entries in relation to the control (47.1 ± 2.9). On the other hand, when compared with the MK-801 group, animals previously treated with ortho-eugenol at a dose of 50 mg/kg (56.2 ± 4.5; p = 0.04) showed a statistically significant reduction in the number of entries. Finally, in both scenarios, the 100 mg/kg dose (67.0 ± 6.8; p = 0.05 [vs Ctrl]; p = 0.56 [vs MK]) was not able to reach statistical significance.

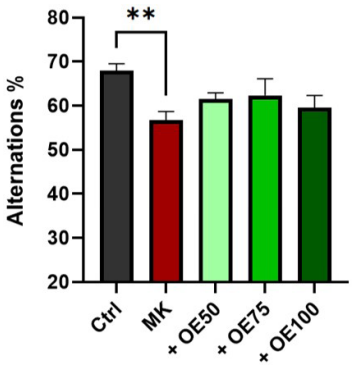

Figure 3. Percentage of spontaneous alternations in the Y-maze. Effects of ortho-eugenol (50, 75 and 100 mg/kg) + MK-801 (0.15 mg/kg), MK-801 (0.15 mg/kg) and vehicle on the percentage of spontaneous alternations in the Y-maze. Values expressed as mean ± SEM (n = 8–10). One-way ANOVA followed by Dunnett's test. **p < 0.01.

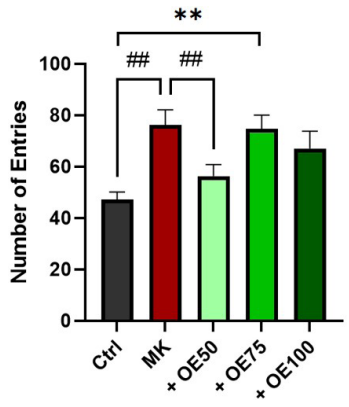

Figure 4. Number of entries in the Y-Maze test. Effects of ortho-eugenol (50, 75 and 100 mg/kg) + MK-801 (0.15 mg/kg), MK-801 (0.15 mg/kg) and vehicle on the number of entries in the Y-maze. Values expressed as mean ± SEM (n = 8–10). One-way ANOVA followed by Dunnett's test. **p < 0.01; when compared to the control group. ##p < 0.01; when compared to the MK-801 group.

3.3. Assessment of oxidative stress

The levels of lipid peroxidation in the prefrontal cortex by the TBARS assay, with the results expressed in grams of MDA/grams of sample, is shown in Figure 5. All groups were compared to the control group and to the group treated with MK-801. The results indicate increased MDA levels in the MK-801 group (3.880 ± 0.007; p = 0.003) in relation to the control group (3.770 ± 0.02). When comparing the other groups with the group treated with MK-801, the OE50 group (3.796 ± 0.035, p = 0.041) showed a reduction in the concentration of MDA, which was not seen in the doses of ortho-eugenol at 75 (3.857 ± 0.004; p = 0.21) and 100 mg/kg (3.865 ± 0.006; p = 0.42).

The results of the hippocampal nitrite determination test, with the results expressed in nM nitrite/grams of tissue, is shown in Figure 6. All groups were compared to the control group and to the group treated with MK-801. In this test, the animals treated with MK-801 (1194.0 ± 138.3; p = 0.0001) increased the levels of nitrite in relation to the control (456.8 ± 24.4). As for the experimental groups, all three doses of 50 mg/kg (743.4 ± 154.7; p = 0.01), 75 mg/kg (764 ± 84.0; p = 0.02) and 100 mg/kg (698.6 ± 57.1; p = 0.003) were able to reduce the concentration of nitrite in comparison with the group treated with MK-801. However, none of them presented differences when compared to control (OE50, p = 0.2; OE75, p = 0.1; OE100, p = 0.3).

3.4. Correlation between the effects of ortho-eugenol on hyperlocomotion and oxidative stress

The results of Pearson's correlation between the number of entries and the levels of malondialdehyde (g MDA/g of tissue) and nitrite (nM nitrite/g of tissue) are presented in Table 1. Besides the OE50 group, no other group obtained any statistically significant correlation. For the 50 mg/kg ortho-eugenol dose, a negative correlation was found between the number of entries and the levels of lipid peroxidation in the prefrontal cortex (p = 0.044); and also a positive correlation between the number of entries and nitrite levels in the hippocampus (p = 0.029).

4. Discussion

The present study aimed to investigate the effects of ortho-eugenol on anxiety, working memory and oxidative stress. Taken together, our results show that ortho-eugenol reduced anxiety-like behavior in the hole-board test, reduced hyperlocomotion in the Y-maze test, and presented an antioxidant profile in both the nitrite determination test and the lipid peroxidation levels by the TBARS assay. Additionally, a correlation was found between the antioxidant effects of ortho-eugenol and the hyperlocomotive behavior. However, our data were not able to demonstrate improvement of working memory deficits by ortho-eugenol.

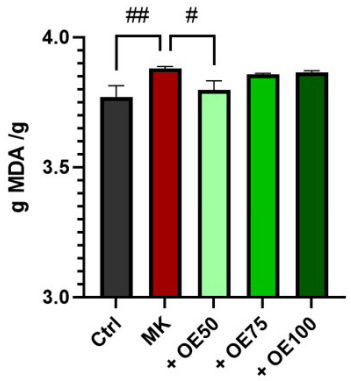

Figure 5. Lipid peroxidation by the TBARS method in g MDA/g sample. Malondialdehyde concentration in the groups treated with ortho-eugenol (50, 75 and 100 mg/kg) + MK-801 (0.15 mg/kg), MK-801 (0.15 mg/kg) and vehicle. Values expressed as mean ± SEM (n = 8–10). Kruskal-Wallis test followed by Dunn's test. #p < 0.05, ##p < 0.01; when compared to the MK-801 group.

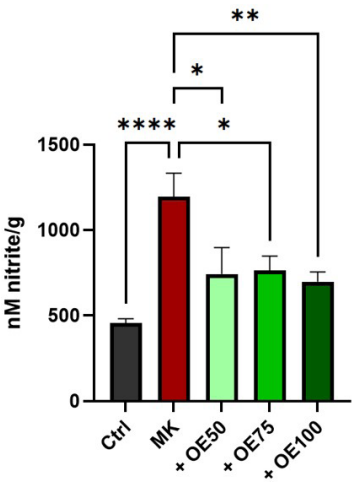

Figure 6. Nitrite determination in nM of nitrite/g of tissue. Nitrite levels in the groups treated with ortho-eugenol (50, 75 and 100 mg/kg) + MK-801 (0.15 mg/kg), MK-801 (0.15 mg/kg) and vehicle. Values expressed as mean ± SEM (n = 8–10). One-way ANOVA followed by Dunnett's test. *p < 0.05, **p < 0.01, ****p < 0.0001.

Table 1. Pearson's correlation coefficient (r) between the number of Y-maze entries and the levels of malondialdehyde in the prefrontal cortex and nitrite in the hippocampus. *p < 0.05.

	Ctrl	MK	+ OE50	+ OE75	+ OE100
Prefrontal cortex	-0.49	-0.28	-0.71 *	-0.36	-0.33
Hippocampus	0.42	0.18	0.75 *	-0.42	0.22

In the literature, well-established studies have already shown that drugs that reduce anxiety (anxiolytics), such as diazepam, are able to increase the exploratory behavior of mice, resulting in a greater number of head-dips and a reduction in latency to the dip in the hole-board test (Takeda et al., 1998). Convergently, the present study was also able to replicate the effects of diazepam, as observed through increased number of head-dips and reduced latency.

The experimental groups were treated with ortho-eugenol at three different doses: 50, 75 and 100 mg/kg. Only the highest dose, 100 mg/kg, was able to increase the number of head-dips, indicating that this dose was effective in reducing anxiety and increasing the animals' exploratory behavior. Furthermore, in the latency time parameter, again the 100 mg/kg dose was the only one to present statistically significant differences in relation to the control group. The animals treated at this dose took less time for the first dip, showing another indication of the anxiolytic effect of ortho-eugenol. Thus, these results provide evidence that ortho-eugenol at 100 mg/kg has an anxiolytic profile.

Monoaminergic neurotransmitters play a key role in the manifestation of anxiety. For example, lower levels of dopamine in the prefrontal cortex of rats are associated with increased anxiety-like behavior (Mizoguchi et al., 2010). In addition, several noradrenergic drugs are used in the clinic with positive results for the treatment of anxiety disorders (Brunello et al., 2003). Pioneering studies reports that ortho-eugenol's mechanism of action involves alpha-1 and alpha-2 noradrenergic receptors, and the D1 dopaminergic receptor (unpublished data; Fonsêca et al., 2016). Therefore, it is possible to hypothesize that these mechanisms are involved in the anxiolytic effect of ortho-eugenol and, in this sense, that higher doses may lead to a more pronounced modulatory effect on the dopaminergic and noradrenergic systems, which could explain the greater efficacy of the 100 mg/kg dose.

As for the Y-maze test, several articles point out that MK-801 is effective in inducing working memory deficits in mice, as observed by the reduction of the spontaneous alternations percentage. Additionally, the ability of MK-801 to promote hyperlocomotion is also reported in the literature, as it is able to increase the number of entries in the Y-maze arms (Maurice et al., 1997; Miki et al., 2019; Hasegawa et al., 2016).

Convergently, the results obtained in the present study indicate that MK-801 was effective in inducing working memory deficits and also in increasing hyperlocomotion. However, when animals were previously treated with ortho-eugenol, at all doses tested, they did not show statistically significant working memory deficits. At the same time, although the mean percentage of spontaneous alternations increased in all cases, no dose was able to reach statistical significance when compared to the group treated with MK-801 alone.

Our results are similar to those found by Dossat et al. (2017) and Almeida et al. (2021), in the sense that, in both studies, the experimental groups did not show working memory deficits when compared to the control groups, although they did not present a statistically significant difference when compared to the positive control (group with impaired working memory). The authors' interpretation in these studies is that the test drug was able to successfully prevent substance-induced working memory deficits.

However, most articles with an experimental design similar to ours appear to consider the ability of a drug to prevent substance-induced deficits only if there is a statistical significance in comparison to the positive control (Akefe et al., 2022; Bruin et al., 2010; Cheng et al., 2011; Eduviere et al., 2015; Maurice et al., 1997; Miki et al., 2019; Roberts et al., 2010). In order to adopt the interpretation of greater statistical rigor, and although the groups previously treated with ortho-eugenol have increased the rate of spontaneous alternations, it was not possible to state its effectiveness in the prevention of working memory deficits induced by MK-801, since there was no statistically significant difference when compared to the positive control.

On the other hand, in the locomotion behavior analysis, the animals previously treated with the dose of 50 mg/kg of ortho-eugenol showed a reduced number of entries in relation to the group treated only with MK-801. Thus, the results evidence the ability of ortho-eugenol to prevent the increase in locomotor activity induced by MK-801. Interestingly, this effect was observed only at the lowest dose. A possible explanation for this observation is that higher doses of ortho-eugenol can elevate D1 receptor activation to above-optimal levels. In fact, it is classically known in the literature that the hyperactivation of the dopaminergic system is associated with hyperlocomotion behavior (Irifune et al., 1997; Irifune et al., 1991; Oades et al., 1986). Thus, although there is no evidence that high ortho-eugenol doses increase hyperlocomotion *per se*, this hypothesis could help to explain why higher doses were less effective in reducing MK-801-induced hyperlocomotion behavior.

In the evaluation of oxidative stress, both methodologies demonstrated that MK-801 was able to increase levels of oxidative stress, as seen by increased concentrations of nitrite and MDA. Confluently, it is possible to find studies in the literature that report neurotoxic effects and increased oxidative stress produced by the administration of MK-801 (Ozyurt et al., 2007a; Ozyurt et al., 2007b; Willis and Ray, 2007).

The lipid peroxidation levels were measured by the TBARS method. It was found that only the dose of 50 mg/kg was effective in reducing malondialdehyde concentration. A similar effect of eugenol has been reported by preclinical studies (Jayashree and Subramanyam, 1999; Nagababu and Lakshmaiah, 1992). The doses of 75 and 100 mg/kg, on the contrary, showed no significant differences when compared to the control or to the MK-801 group. Thus, the results appear to indicate that higher doses of ortho-eugenol were less effective in reducing oxidative stress induced by MK-801.

Several articles show that high levels of dopamine, as well as the hyperactivation of the dopaminergic system, can promote oxidative stress (Ben-Shachar et al., 1995; Grima et al., 2003; Terland et al., 1997). Similarly, drugs of abuse that act on the dopaminergic system are also

associated with neurotoxicity and free radical formation (Yamamoto and Bankson, 2005). Finally, diseases associated with high levels of the dopaminergic system in the prefrontal cortex also have high levels of oxidative stress in this region (Kim et al., 2014).

Here, the analyzed structure in the lipid peroxidation protocol was the prefrontal cortex, which is known to have an important distribution of D1 receptors and whose dysregulation is associated with several disorders (Muly III et al., 1998). Considering the action of ortho-eugenol on D1 receptors, it is possible to interpret that the effects of dose progression (50, 75 and 100 mg/kg) on the activation of the dopaminergic system may have been reflected in the progressively higher levels of MDA observed. By contrast, plenty studies show that activation of the noradrenergic system is capable of promoting neuroprotection against oxidative stress (Álvarez-Diduk and Galano, 2015; Patri and Singh, 2019; Troadec et al., 2001; Yoshioka et al., 2021). Thus, moderate activation of this system may be a possible explanation for the effectiveness of the 50 mg/kg dose in reducing MDA levels.

In the nitrite determination method, all three ortho-eugenol doses (50, 75 and 100 mg/kg) were effective in reducing oxidative stress induced by MK-801. Likewise, eugenol is also reported to mitigate nitrite levels after substance-induced oxidative stress (Prasad and Muralidhara, 2013; Vasconcelos et al., 2020). Besides lipid peroxidation, the neurotoxic effects of D1-receptor overactivation also includes increased nitrite levels, while noradrenaline activation is neuroprotective and reduces nitrite production and accumulation (Chen et al., 2003; Dello Russo et al., 2004). However, our results show that even the higher doses of ortho-eugenol prevented the elevation of nitrite levels induced by MK-801 administration.

There are many studies in the literature that investigated the distribution of dopamine receptors in the brain. It is well known that D1 receptors are overall poorly expressed in the hippocampus (which is more closely related with D5 and D2 receptors), in contrast to higher D1 expression in regions such as the striatum and prefrontal cortex (Bahena-Trujillo and Gonzalo-Flores, 2000; Cortés et al., 1989; Hurd et al., 2001; Mishra et al., 2018; Missale et al., 1998). Considering the neurotoxic effect of dopaminergic overactivation, a lower number of D1 receptors could explain why the antioxidant effect of ortho-eugenol was not diminished by higher doses in the hippocampus, as otherwise shown in the prefrontal cortex.

In summary, ortho-eugenol at a dose of 100 mg/kg showed anxiolytic activity by increasing the number of dips and reducing the latency time in the perforated plate test. Pretreatment with all three doses (50, 75 and 100 mg/kg) of ortho-eugenol reduced the levels of oxidative stress in the hippocampus, by decreasing the concentration of nitrite in this region. The hippocampus is related to memory formation, fear conditioning, and increased oxidative stress in this structure increases anxious-like behavior (Hassan et al., 2014; Hovatta et al., 2010; Salim et al., 2011). Thus, it is possible that there is some relationship between the anxiolytic effects and the antioxidant action of ortho-eugenol on the hippocampus.

As discussed, several drugs used in the treatment of depression are also used in the treatment of anxiety (Cummings et al., 2014; Goodwin, 2015; Murrough et al., 2015). In this context, eugenol, as well as many of its isomers, are reported to show antidepressant effects in preclinical trials (Amaral et al., 2013; Duan et al., 2015; Norte et al., 2005). Therefore, our results may contribute to directing further studies to investigate potential clinical uses of ortho-eugenol.

The 50 mg/kg dose was the only to present an antioxidant profile in both the prefrontal cortex and hippocampus regions. In addition, the 50 mg/kg dose also reduced MK-801-induced hyperlocomotion. After consulting the literature, two articles may contribute to the present discussion. In the study by Kanazawa et al. (2017), the authors found a positive correlation between hyperlocomotion and lipid peroxidation levels in the prefrontal cortex and hippocampus. Similarly, a study by Mâcedo et al. (2013) was able to identify the increase in lipid peroxidation in the prefrontal cortex, hippocampus and striatum in a murine model of substance-induced hyperlocomotion.

Therefore, in an attempt to investigate whether the effects of ortho-eugenol on hyperlocomotion and oxidative stress in the prefrontal cortex and hippocampus are correlated, we performed a Pearson correlation test for all groups. As predicted, only ortho-eugenol at a dose of 50 mg/kg showed a correlation between the levels of oxidative stress, in both tests, and the number of entries (Table 1). Interestingly, however, while there was a positive correlation between locomotion behavior and nitrite levels in the hippocampus, the levels of MDA in the prefrontal cortex showed a negative correlation with locomotion behavior.

Finally, although no group previously treated with ortho-eugenol showed a significant reduction in working memory, as indicated by the percentage of spontaneous Y-maze switches, there was no significant improvement compared to the group treated with MK-801 alone. For this reason, it was not possible to state that ortho-eugenol is effective in preventing working memory deficits. Future studies may investigate whether repeated ortho-eugenol administration can improve its effectiveness on working memory deficits.

In the literature, molecules related to ortho-eugenol, such as eugenol and bis-eugenol, also showed antioxidant activity in rodents (Kabuto et al., 2007; Kumar et al., 2021; Murakami et al., 2003; Nagababu and Lakshmaiah, 1992). In this sense, our study was the first to provide evidence that ortho-eugenol, an isomer of eugenol, has antioxidant effects, as seen through the nitrite and lipid peroxidation tests.

5. Conclusion

Taken together, the results obtained in the present study provide the first evidence of the anxiolytic activity of ortho-eugenol, since the 100 mg/kg dose was able to increase the number of dips and reduce the latency time in the hole-board test. In the Y-maze test, ortho-eugenol

was not able to prevent MK-801-induced working memory deficits. On the other hand, ortho-eugenol at the 50 mg/kg dose was able to reduce the hyperlocomotion induced by MK-801 in the Y-maze. All doses of ortho-eugenol (50, 75 and 100 mg/kg) showed antioxidant activity and reduced nitrite levels in the hippocampus, and the 50 mg/kg dose also reduced lipid peroxidation in the prefrontal cortex. In addition, a correlation was found between the effects of the 50 mg/kg ortho-eugenol dose on hyperlocomotion and oxidative stress, which may contribute to the understanding of its pharmacodynamics. Thus, our results support the pharmacological potential of ortho-eugenol and can contribute to direct the development of future research.

Acknowledgements

We would like to express our gratitude to Humberto Andrade, Adriana Golzio and Ian Amaral, for their contribution in the experiments and text reviewing.

This work was supported by Public Call no. 03 of Research Productivity PROPESQ/PRPG/UFPB PVB13281-2020.

References

AKEFE, I.O., ADEGOKE, V.A., LAMIDI, I.Y., AMEH, M.P., IDOGA, E.S., UBAH, S.A. and AJAYI, I.E., 2022. Myrtenal mitigates streptozotocin-induced spatial memory deficit via improving oxido inflammatory, cholinergic and neurotransmitter functions in mice. *Current Research in Pharmacology and Drug Discovery*, vol. 3, p. 100106. http://dx.doi.org/10.1016/j.crphar.2022.100106. PMid:35570857.

AL-AMIN, M.M., CHOWDURY, M.I.A., SAIFULLAH, A.R.M., ALAM, M.N., JAIN, P., HOSSAIN, M., ALAM, M.A., KAZI, M., AHMAD, A., RAISH, M., ALQAHTANI, A. and REZA, H.M., 2019. Levocarnitine improves AlCl3-induced spatial working memory impairment in Swiss albino mice. *Frontiers in Neuroscience*, vol. 13, p. 278. http://dx.doi.org/10.3389/fnins.2019.00278. PMid:30971884.

ALBANI, S.H., MCHAIL, D.G. and DUMAS, T.C., 2014. Developmental studies of the hippocampus and hippocampal-dependent behaviors: insights from interdisciplinary studies and tips for new investigators. *Neuroscience and Biobehavioral Reviews*, vol. 43, pp. 183-190. http://dx.doi.org/10.1016/j.neubiorev.2014.04.009. PMid:24769291.

ALMEIDA, G.R.L., SZCZEPANIK, J.C., SELHORST, I., SCHMITZ, A.E., SANTOS, B., CUNHA, M.P., HEINRICH, I.A., DE PAULA, G.C., DE BEM, A.F., LEAL, R.B. and DAFRE, A.L., 2021. Methylglyoxal-mediated dopamine depletion, working memory deficit, and depression-like behavior are prevented by a dopamine/noradrenaline reuptake inhibitor. *Molecular Neurobiology*, vol. 58, no. 2, pp. 735-749. http://dx.doi.org/10.1007/s12035-020-02146-3. PMid:33011857.

ÁLVAREZ-DIDUK, R. and GALANO, A., 2015. Adrenaline and noradrenaline: protectors against oxidative stress or molecular targets? *The Journal of Physical Chemistry B*, vol. 119, no. 8, pp. 3479-3491. http://dx.doi.org/10.1021/acs.jpcb.5b00052. PMid:25646569.

AMARAL, J.F., SILVA, M.I., AQUINO NETO, M.R., MOURA, B.A., CARVALHO, A.M., VASCONCELOS, P.F., BARBOSA FILHO, J.M., GUTIERREZ, S.J., VASCONCELOS, S.M., MACÊDO, D.S. and SOUSA, F.C., 2013. Antidepressant-like effect of bis-eugenol in the mice forced swimming test: evidence for the involvement of the monoaminergic system. *Fundamental & Clinical Pharmacology*, vol. 27, no. 5, pp. 471-482. http://dx.doi.org/10.1111/j.1472-8206.2012.01058.x. PMid:22827775.

AMINE, K.M., KAHINA, C., NAWEL, H., FAIZA, Z., JEAN, G., MOHAMED, T., SAÂDIA, M. and AHSENE, B., 2020. Protective effects of Pelargonium graveolens essential oil on methomyl-induced oxidative stress and spatial working memory impairment in association with histopathological changes in the hippocampus of male wistar rats. *Basic and Clinical Neuroscience*, vol. 11, no. 4, pp. 433-446. PMid:33613881.

BÄCKMAN, L. and NYBERG, L., 2013. Dopamine and training-related working-memory improvement. *Neuroscience and Biobehavioral Reviews*, vol. 37, no. 9 Pt B, pp. 2209-2219. http://dx.doi.org/10.1016/j.neubiorev.2013.01.014. PMid:23333266.

BAHENA-TRUJILLO, R. and GONZALO-FLORES, J.A.A.-M., 2000. Dopamina: síntesis, liberación y receptores en el Sistema Nervioso Central. *Revista Bioética*, vol. 11, no. 1, pp. 39-60. http://dx.doi.org/10.32776/revbiomed.v11i1.218.

BARBOSA, K.B.F., COSTA, N.M.B., ALFENAS, R.C.G., DE PAULA, S.O., MINIM, V.P.R. and BRESSAN, J., 2010. Estresse oxidativo: conceito, implicações e fatores modulatórios. *Revista de Nutrição*, vol. 23, no. 4, pp. 629-643. http://dx.doi.org/10.1590/S1415-52732010000400013.

BARBOZA, J.N., 2018. *Potencial anti-inflamatório e perfil antioxidante do eugenol: uma revisão*. João Pessoa: Universidade Federal da Paraíba, 36 p. Trabalho de Conclusão de Curso.

BEN-SHACHAR, D., ZUK, R. and GLINKA, Y., 1995. Dopamine neurotoxicity: inhibition of mitochondrial respiration. *Journal of Neurochemistry*, vol. 64, no. 2, pp. 718-723. http://dx.doi.org/10.1046/j.1471-4159.1995.64020718.x. PMid:7830065.

BIRNBAUM, S.G., PODELL, D.M. and ARNSTEN, A.F.T., 2000. Noradrenergic alpha-2 receptor agonists reverse working memory deficits induced by the anxiogenic drug, FG7142, in rats. *Pharmacology, Biochemistry, and Behavior*, vol. 67, no. 3, pp. 397-403. http://dx.doi.org/10.1016/S0091-3057(00)00306-3. PMid:11164065.

BISHOP, S., DUNCAN, J., BRETT, M. and LAWRENCE, A.D., 2004. Prefrontal cortical function and anxiety: controlling attention to threat-related stimuli. *Nature Neuroscience*, vol. 7, no. 2, pp. 184-188. http://dx.doi.org/10.1038/nn1173. PMid:14703573.

BOISSIER, J.R., SIMON, P. and LWOFF, J.M., 1964. Use of a particular mouse reaction (hole board method) for the study of psychotropic drugs. *Therapie*, vol. 19, pp. 571-583. PMid:14182942.

BOUAYED, J., RAMMAL, H. and SOULIMANI, R., 2009. Oxidative stress and anxiety: relationship and cellular pathways. *Oxidative Medicine and Cellular Longevity*, vol. 2, no. 2, pp. 63-67. http://dx.doi.org/10.4161/oxim.2.2.7944. PMid:20357926.

BROWN, J.W., WHITEHEAD, C.A., BASSO, A.M., RUETER, L.E. and ZHANG, M., 2013. Preclinical evaluation of non-imidazole histamine H3 receptor antagonists in comparison to atypical antipsychotics for the treatment of cognitive deficits associated with schizophrenia. *The International Journal of Neuropsychopharmacology*, vol. 16, no. 4, pp. 889-904. http://dx.doi.org/10.1017/S1461145712000739. PMid:22906530.

BRUIN, N.M.W.J., PRICKAERTS, J., LANGE, J.H., AKKERMAN, S., ANDRIAMBELOSON, E., HAAN, M., WIJNEN, J., VAN DRIMMELEN, M., HISSINK, E., HEIJINK, L. and KRUSE, C.G., 2010. SLV330, a cannabinoid CB1 receptor antagonist, ameliorates deficits in the T-maze, object recognition and Social Recognition Tasks in rodents. *Neurobiology of Learning and Memory*, vol. 93, no. 4, pp. 522-531. http://dx.doi.org/10.1016/j.nlm.2010.01.010. PMid:20132903.

BRUNELLO, N., BLIER, P., JUDD, L.L., MENDLEWICZ, J., NELSON, C.J., SOUERY, D., ZOHAR, J. and RACAGNI, G., 2003. Noradrenaline in mood and anxiety disorders: basic and clinical studies. *International Clinical Psychopharmacology*, vol. 18, no. 4, pp. 191-202. http://dx.doi.org/10.1097/01.yic.0000073880.93678.68. PMid:12817153.

CASARRUBEA, M., FAULISI, F., PENSABENE, M., MENDOLA, C., DELL'UTRI, R., CARDACI, M., SANTANGELO, A. and CRESCIMANNO, G., 2017. Effects of the benzodiazepine inverse agonist FG7142 on the structure of anxiety-related behavior of male Wistar rats tested in hole board. *Psychopharmacology*, vol. 234, no. 3, pp. 381-391. http://dx.doi.org/10.1007/s00213-016-4474-8. PMid:27838748.

CASARRUBEA, M., SORBERA, F. and CRESCIMANNO, G., 2009. Structure of rat behavior in hole-board: I) multivariate analysis of response to anxiety. *Physiology & Behavior*, vol. 96, no. 1, pp. 174-179. http://dx.doi.org/10.1016/j.physbeh.2008.09.025. PMid:18948127.

CHAKRABORTY, S., SINGH, O.P., DASGUPTA, A., MANDAL, N. and DAS, H.N., 2009. Correlation between lipid peroxidation-induced TBARS level and disease severity in obsessive–compulsive disorder. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, vol. 33, no. 2, pp. 363-366. http://dx.doi.org/10.1016/j.pnpbp.2009.01.001. PMid:19272303.

CHEN, J., WERSINGER, C. and SIDHU, A., 2003. Chronic stimulation of D1 dopamine receptors in human SK-N-MC neuroblastoma cells induces nitric-oxide synthase activation and cytotoxicity. *The Journal of Biological Chemistry*, vol. 278, no. 30, pp. 28089-28100. http://dx.doi.org/10.1074/jbc.M303094200. PMid:12738794.

CHENG, L.L., CHEN, X.N., WANG, Y., YU, L., KUANG, X., WANG, L.L., YANG, W. and DU, J.R., 2011. Z-ligustilide isolated from Radix Angelicae sinensis ameliorates the memory impairment induced by scopolamine in mice. *Fitoterapia*, vol. 82, no. 7, pp. 1128-1132. http://dx.doi.org/10.1016/j.fitote.2011.07.011. PMid:21807074.

CHINO, A., HONDA, S., MORITA, M., YONEZAWA, K., HAMAGUCHI, W., AMANO, Y., MORIGUCHI, H., YAMAZAKI, M., AOTA, M., TOMISHIMA, M. and MASUDA, N., 2019. Synthesis, SAR study, and biological evaluation of novel 2, 3-dihydro-1H-imidazo [1, 2-a] benzimidazole derivatives as phosphodiesterase 10A inhibitors. *Bioorganic & Medicinal Chemistry*, vol. 27, no. 16, pp. 3692-3706. http://dx.doi.org/10.1016/j.bmc.2019.07.010. PMid:31301949.

CHIOCA, L.R., FERRO, M.M., BARETTA, I.P., OLIVEIRA, S.M., SILVA, C.R., FERREIRA, J., LOSSO, E.M. and ANDREATINI, R., 2013. Anxiolytic-like effect of lavender essential oil inhalation in mice: participation of serotonergic but not GABAA/benzodiazepine neurotransmission. *Journal of Ethnopharmacology*, vol. 147, no. 2, pp. 412-418. http://dx.doi.org/10.1016/j.jep.2013.03.028. PMid:23524167.

CLARK, D.A. and BECK, A.T., 2010. Cognitive theory and therapy of anxiety and depression: convergence with neurobiological findings. *Trends in Cognitive Sciences*, vol. 14, no. 9, pp. 418-424. http://dx.doi.org/10.1016/j.tics.2010.06.007. PMid:20655801.

CORTÉS, R., GUEYE, B., PAZOS, A., PROBST, A. and PALACIOS, J.M., 1989. Dopamine receptors in human brain: autoradiographic distribution of D1 sites. *Neuroscience*, vol. 28, no. 2, pp. 263-273. http://dx.doi.org/10.1016/0306-4522(89)90178-4. PMid:2522166.

CRASKE, M.G., RAUCH, S.L., URSANO, R., PRENOVEAU, J., PINE, D.S. and ZINBARG, R.E., 2011. What is an anxiety disorder? *Focus*, vol. 9, no. 3, pp. 369-388. https://doi.org/10.1176/foc.9.3.foc369.

CRAWLEY, J.N., 1985. Exploratory behavior models of anxiety in mice. *Neuroscience and Biobehavioral Reviews*, vol. 9, no. 1, pp. 37-44. http://dx.doi.org/10.1016/0149-7634(85)90030-2. PMid:2858080.

CROUZIER, L., GILABERT, D., ROSSEL, M., TROUSSE, F. and MAURICE, T., 2018. Topographical memory analyzed in mice using the Hamlet test, a novel complex maze. *Neurobiology of Learning and Memory*, vol. 149, pp. 118-134. http://dx.doi.org/10.1016/j.nlm.2018.02.014. PMid:29458097.

CUMMINGS, C.M., CAPORINO, N.E. and KENDALL, P.C., 2014. Comorbidity of anxiety and depression in children and adolescents: 20 years after. *Psychological Bulletin*, vol. 140, no. 3, pp. 816-845. http://dx.doi.org/10.1037/a0034733. PMid:24219155.

DELLO RUSSO, C., BOULLERNE, A.I., GAVRILYUK, V. and FEINSTEIN, D.L., 2004. Inhibition of microglial inflammatory responses by norepinephrine: effects on nitric oxide and interleukin-1β production. *Journal of Neuroinflammation*, vol. 1, no. 1, p. 9. http://dx.doi.org/10.1186/1742-2094-1-9. PMid:15285793.

DOSSAT, A.M., JOURDI, H., WRIGHT, K.N., STRONG, C.E., SARKAR, A. and KABBAJ, M., 2017. Viral-mediated Zif268 expression in the prefrontal cortex protects against gonadectomy-induced working memory, long-term memory, and social interaction deficits in male rats. *Neuroscience*, vol. 340, pp. 243-257. http://dx.doi.org/10.1016/j.neuroscience.2016.10.062. PMid:27816701.

DRAPER, H.H. and HADLEY, M., 1990. Malondialdehyde determination as index of lipid Peroxidation. *Methods in Enzymology*, vol. 186, pp. 421-431. http://dx.doi.org/10.1016/0076-6879(90)86135-I. PMid:2233309.

DUAN, D., CHEN, L., YANG, X., TU, Y. and JIAO, S., 2015. Antidepressant-like effect of essential oil isolated from Toona ciliata Roem. var. yunnanensis. *Journal of Natural Medicines*, vol. 69, no. 2, pp. 191-197. http://dx.doi.org/10.1007/s11418-014-0878-0. PMid:25465853.

EDUVIERE, A.T., UMUKORO, S., ADERIBIGBE, A.O., AJAYI, A.M. and ADEWOLE, F.A., 2015. Methyl jasmonate enhances memory performance through inhibition of oxidative stress and acetylcholinesterase activity in mice. *Life Sciences*, vol. 132, pp. 20-26. http://dx.doi.org/10.1016/j.lfs.2015.04.007. PMid:25921767.

FONSÊCA, D.V., SALGADO, P.R., ARAGÃO NETO, H.C., GOLZIO, A.M.F.O., CALDAS FILHO, M.R.D., MELO, C.G.F., LEITE, F.C., PIUVEZAM, M.R., PORDEUS, L.C., BARBOSA FILHO, J.M. and ALMEIDA, R.N., 2016. Ortho-eugenol exhibits anti-nociceptive and anti-inflammatory activities. *International Immunopharmacology*, vol. 38, no. 1, pp. 402-408. http://dx.doi.org/10.1016/j.intimp.2016.06.005. PMid:27355133.

GILMOUR, G., PORCELLI, S., BERTAINA-ANGLADE, V., ARCE, E., DUKART, J., HAYEN, A., LOBO, A., LOPEZ-ANTON, R., PICH, E.M., PEMBERTON, D.J., HAVENITH, M.N., GLENNON, J.C., HAREL, B.T., DAWSON, G., MARSTON, H., KOZAK, R. and SERRETTI, A., 2019. Relating constructs of attention and working memory to social withdrawal in Alzheimer's disease and schizophrenia: issues regarding paradigm selection. *Neuroscience and Biobehavioral Reviews*, vol. 97, pp. 47-69. http://dx.doi.org/10.1016/j.neubiorev.2018.09.025. PMid:30399355.

GOODWIN, G.M., 2015. The overlap between anxiety, depression, and obsessive-compulsive disorder. *Dialogues in Clinical Neuroscience*, vol. 17, no. 3, pp. 249-260. http://dx.doi.org/10.31887/DCNS.2015.17.3/ggoodwin. PMid:26487806.

GOUVEIA, K. and HURST, J.L., 2017. Optimising reliability of mouse performance in behavioural testing: the major role of non-aversive handling. *Scientific Reports*, vol. 7, no. 1, p. 44999. http://dx.doi.org/10.1038/srep44999. PMid:28322308.

GOUVEIA, K. and HURST, J.L., 2019. Improving the practicality of using non-aversive handling methods to reduce background stress and anxiety in laboratory mice. *Scientific Reports*, vol. 9, no. 1, p. 20305. http://dx.doi.org/10.1038/s41598-019-56860-7. PMid:31889107.

GREEN, L.C., TANNENBAUM, S.R. and GOLDMAN, P., 1981. Nitrate synthesis in the germfree and conventional rat. *Science*, vol. 212, no. 4490, pp. 56-58. http://dx.doi.org/10.1126/science.6451927. PMid:6451927.

GRIEBEL, G., BELZUNG, C., PERRAULT, G. and SANGER, D.J., 2000. Differences in anxiety-related behaviours and in sensitivity to diazepam in inbred and outbred strains of mice. *Psychopharmacology*, vol. 148, no. 2, pp. 164-170. http://dx.doi.org/10.1007/s002130050038. PMid:10663431.

GRIMA, G., BENZ, B., PARPURA, V., CUÉNOD, M. and DO, K.Q., 2003. Dopamine-induced oxidative stress in neurons with glutathione deficit: implication for schizophrenia. *Schizophrenia Research*, vol. 62, no. 3, pp. 213-224. http://dx.doi.org/10.1016/S0920-9964(02)00405-X. PMid:12837517.

HALDER, S., MEHTA, A.K., KAR, R., MUSTAFA, M., MEDIRATTA, P.K. and SHARMA, K.K., 2011. Clove oil reverses learning and memory deficits in scopolamine-treated mice. *Planta Medica*, vol. 77, no. 8, pp. 830-834. http://dx.doi.org/10.1055/s-0030-1250605. PMid:21157682.

HASEGAWA, Y., INOUE, T., KAWAMINAMI, S. and FUJITA, M., 2016. Effects of scallop shell extract on scopolamine-induced memory impairment and MK801-induced locomotor activity. *Asian Pacific Journal of Tropical Medicine*, vol. 9, no. 7, pp. 662-667. http://dx.doi.org/10.1016/j.apjtm.2016.05.019. PMid:27393094.

HASSAN, W., SILVA, C.E.B., MOHAMMADZAI, I.U., ROCHA, J.B.T. and LANDEIRA-FERNANDEZ, J., 2014. Association of oxidative stress to the genesis of anxiety: implications for possible therapeutic interventions. *Current Neuropharmacology*, vol. 12, no. 2, pp. 120-139. http://dx.doi.org/10.2174/1570159X11666131120232135. PMid:24669207.

HENDERSON, L.J., DANI, B., SERRANO, E.M.N., SMULDERS, T.V. and ROUGHAN, J.V., 2020. Benefits of tunnel handling persist after repeated restraint, injection and anaesthesia. *Scientific Reports*, vol. 10, no. 1, p. 14562. http://dx.doi.org/10.1038/s41598-020-71476-y. PMid:32884048.

HOVATTA, I., JUHILA, J. and DONNER, J., 2010. Oxidative stress in anxiety and comorbid disorders. *Neuroscience Research*, vol. 68, no. 4, pp. 261-275. http://dx.doi.org/10.1016/j.neures.2010.08.007. PMid:20804792.

HURD, Y.L., SUZUKI, M. and SEDVALL, G.C., 2001. D1 and D2 dopamine receptor mRNA expression in whole hemisphere sections of the human brain. *Journal of Chemical Neuroanatomy*, vol. 22, no. 1-2, pp. 127-137. http://dx.doi.org/10.1016/S0891-0618(01)00122-3. PMid:11470560.

HURST, J.L. and WEST, R.S., 2010. Taming anxiety in laboratory mice. *Nature Methods*, vol. 7, no. 10, pp. 825-826. http://dx.doi.org/10.1038/nmeth.1500. PMid:20835246.

IRIE, Y., ITOKAZU, N., ANJIKI, N., ISHIGE, A., WATANABE, K. and KEUNG, W.M., 2004. Eugenol exhibits antidepressant-like activity in mice and induces expression of metallothionein-III in the hippocampus. *Brain Research*, vol. 1011, no. 2, pp. 243-246. http://dx.doi.org/10.1016/j.brainres.2004.03.040. PMid:15157811.

IRIFUNE, M., SATO, T., NISHIKAWA, T., MASUYAMA, T., NOMOTO, M., FUKUDA, T. and KAWAHARA, M., 1997. Hyperlocomotion during recovery from isoflurane anesthesia is associated with increased dopamine turnover in the nucleus accumbens and striatum in mice. *Anesthesiology*, vol. 86, no. 2, pp. 464-475. http://dx.doi.org/10.1097/00000542-199702000-00022. PMid:9054265.

IRIFUNE, M., SHIMIZU, T. and NOMOTO, M., 1991. Ketamine-induced hyperlocomotion associated with alteration of presynaptic components of dopamine neurons in the nucleus accumbens of mice. *Pharmacology, Biochemistry, and Behavior*, vol. 40, no. 2, pp. 399-407. http://dx.doi.org/10.1016/0091-3057(91)90571-I. PMid:1805243.

IZQUIERDO, I., 2018. *Memória*. 3rd ed. Porto Alegre: Artmed.

JAYASHREE, T. and SUBRAMANYAM, C., 1999. Antiaflatoxigenic activity of eugenol is due to inhibition of lipid peroxidation. *Letters in Applied Microbiology*, vol. 28, no. 3, pp. 179-183. http://dx.doi.org/10.1046/j.1365-2672.1999.00512.x. PMid:10196764.

KABUTO, H., TADA, M. and KOHNO, M., 2007. Eugenol [2-methoxy-4-(2-propenyl) phenol] prevents 6-hydroxydopamine-induced dopamine depression and lipid peroxidation inductivity in mouse striatum. *Biological & Pharmaceutical Bulletin*, vol. 30, no. 3, pp. 423-427. http://dx.doi.org/10.1248/bpb.30.423. PMid:17329831.

KANAZAWA, L.K., VECCHIA, D.D., WENDLER, E.M., HOCAYEN, P.A., BEIRÃO JUNIOR, P.S., MÉLO, M.L., LÍVERO, F.A.R., CORSO, C.R., STIPP, M.C., ACCO, A. and ANDREATINI, R., 2017. Effects of acute and chronic quercetin administration on methylphenidate-induced hyperlocomotion and oxidative stress. *Life Sciences*, vol. 171, pp. 1-8. http://dx.doi.org/10.1016/j.lfs.2017.01.007. PMid:28104366.

KAUR, S. and SINGH, R., 2017. Role of different neurotransmitters in anxiety: a systematic review. *International Journal of Pharmaceutical Sciences and Research*, vol. 8, no. 2, p. 411. http://dx.doi.org/10.25258/ijcprr.v8i01.9088.

KIM, H.K., ANDREAZZA, A.C., YEUNG, P.Y., ISAACS-TREPANIER, C. and YOUNG, L.T., 2014. Oxidation and nitration in dopaminergic areas of the prefrontal cortex from patients with bipolar disorder and schizophrenia. *Journal of Psychiatry & Neuroscience*, vol. 39, no. 4, pp. 276-285. http://dx.doi.org/10.1503/jpn.130155. PMid:24485387.

KRAEUTER, A.-K., GUEST, P.C. and SARNYAI, Z., 2019. The Y-maze for assessment of spatial working and reference memory in mice. In: P.C. GUEST, ed. *Pre-clinical models: techniques and protocols*. New York: Humana Press, pp. 105-111. http://dx.doi.org/10.1007/978-1-4939-8994-2_10.

KUMAR, A., SIDDIQI, N.J., ALRASHOOD, S.T., KHAN, H.A., DUBEY, A. and SHARMA, B., 2021. Protective effect of eugenol on hepatic inflammation and oxidative stress induced by cadmium in male rats. *Biomedicine and Pharmacotherapy*, vol. 139, p. 111588. http://dx.doi.org/10.1016/j.biopha.2021.111588. PMid:33862491.

LAINIOLA, M., PROCACCINI, C. and LINDEN, A.M., 2014. mGluR3 knockout mice show a working memory defect and an enhanced response to MK-801 in the T-and Y-maze cognitive tests. *Behavioural Brain Research*, vol. 266, pp. 94-103. http://dx.doi.org/10.1016/j.bbr.2014.03.008. PMid:24631392.

LALONDE, R., 2002. The neurobiological basis of spontaneous alternation. *Neuroscience and Biobehavioral Reviews*, vol. 26, no. 1, pp. 91-104. http://dx.doi.org/10.1016/S0149-7634(01)00041-0. PMid:11835987.

LEDOUX, J.E. and PINE, D.S., 2016. Using neuroscience to help understand fear and anxiety: a two system framework. *The American Journal of Psychiatry*, vol. 173, no. 11, pp. 1083-1093. http://dx.doi.org/10.1176/appi.ajp.2016.16030353. PMid:27609244.

LEPICARD, E.M., JOUBERT, C., HAGNEAU, I., PEREZ-DIAZ, F. and CHAPOUTHIER, G., 2000. Differences in anxiety-related behavior and response to diazepam in BALB/cByJ and C57BL/6J strains of

mice. *Pharmacology, Biochemistry, and Behavior*, vol. 67, no. 4, pp. 739-748. http://dx.doi.org/10.1016/S0091-3057(00)00419-6. PMid:11166064.

LI, B.M., MAO, Z.M., WANG, M. and MEI, Z.T., 1999. Alpha-2 adrenergic modulation of prefrontal cortical neuronal activity related to spatial working memory in monkeys. *Neuropsychopharmacology*, vol. 21, no. 5, pp. 601-610. http://dx.doi.org/10.1016/S0893-133X(99)00070-6. PMid:10516956.

MACÊDO, D.S., LUCENA, D.F., QUEIROZ, A.I., CORDEIRO, R.C., ARAÚJO, M.M., SOUSA, F.C., VASCONCELOS, S.M., HYPHANTIS, T.N., QUEVEDO, J., MCINTYRE, R.S. and CARVALHO, A.F., 2013. Effects of lithium on oxidative stress and behavioral alterations induced by lisdexamfetamine dimesylate: relevance as an animal model of mania. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, vol. 43, pp. 230-237. http://dx.doi.org/10.1016/j.pnpbp.2013.01.007. PMid:23333378.

MACHADO, B.F.M.T. and FERNANDES JUNIOR, A., 2011. Óleos essenciais: aspectos gerais e usos em terapias naturais. *Cadernos Acadêmicos*, vol. 3, no. 2, pp. 105-127.

MARCOTTE, M., BERNARDO, A., LINGA, N., PÉREZ-ROMERO, C.A., GUILLOU, J.L., SIBILLE, E. and PREVOT, T.D., 2021. Handling techniques to reduce stress in mice. *Journal of Visualized Experiments*, no. 175, p. e62593. PMid:34633376.

MAURICE, T., HIRAMATSU, M., ITOH, J., KAMEYAMA, T., HASEGAWA, T. and NABESHIMA, T., 1994. Behavioral evidence for a modulating role of σ ligands in memory processes. I. Attenuation of dizocilpine (MK-801)-induced amnesia. *Brain Research*, vol. 647, no. 1, pp. 44-56. http://dx.doi.org/10.1016/0006-8993(94)91397-8. PMid:8069704.

MAURICE, T., JUNIEN, J.L. and PRIVAT, A., 1997. Dehydroepiandrosterone sulfate attenuates dizocilpine-induced learning impairment in mice via σ1-receptors. *Behavioural Brain Research*, vol. 83, no. 1-2, pp. 159-164. http://dx.doi.org/10.1016/S0166-4328(97)86061-5. PMid:9062676.

MERAM, T.D., CHOWDURY, A., EASTER, P., ATTISHA, T., KALLABAT, E., HANNA, G.L., ARNOLD, P., ROSENBERG, D.R. and DIWADKAR, V.A., 2021. Evoking network profiles of the dorsal anterior cingulate in youth with Obsessive-Compulsive Disorder during motor control and working memory. *Journal of Psychiatric Research*, vol. 132, pp. 72-83. http://dx.doi.org/10.1016/j.jpsychires.2020.09.028. PMid:33068817.

MIKI, R., HONDA, I., HAMASAKI, R., KAWAHARA, R., SOEDA, F., SHIRASAKI, T., MISUMI, S. and TAKAHAMA, K., 2019. Effects of tipepidine on MK-801-induced cognitive impairment in mice. *Brain Research*, vol. 1710, pp. 230-236. http://dx.doi.org/10.1016/j.brainres.2018.12.032. PMid:30584927.

MILLAN, M.J., AGID, Y., BRÜNE, M., BULLMORE, E.T., CARTER, C.S., CLAYTON, N.S., CONNOR, R., DAVIS, S., DEAKIN, B., DERUBEIS, R.J., DUBOIS, B., GEYER, M.A., GOODWIN, G.M., GORWOOD, P., JAY, T.M., JOËLS, M., MANSUY, I.M., MEYER-LINDENBERG, A., MURPHY, D., ROLLS, E., SALETU, B., SPEDDING, M., SWEENEY, J., WHITTINGTON, M. and YOUNG, L.J., 2012. Cognitive dysfunction in psychiatric disorders: characteristics, causes and the quest for improved therapy. *Nature Reviews. Drug Discovery*, vol. 11, no. 2, pp. 141-168. http://dx.doi.org/10.1038/nrd3628. PMid:22293568.

MISHRA, A., SINGH, S. and SHUKLA, S., 2018. Physiological and functional basis of dopamine receptors and their role in neurogenesis: possible implication for Parkinson's disease. *Journal of Experimental Neuroscience*, vol. 12, p. 1179069518779829. http://dx.doi.org/10.1177/1179069518779829. PMid:29899667.

MISSALE, C., NASH, S.R., ROBINSON, S.W., JABER, M. and CARON, M.G., 1998. Dopamine receptors: from structure to function. *Physiological Reviews*, vol. 78, no. 1, pp. 189-225. http://dx.doi.org/10.1152/physrev.1998.78.1.189. PMid:9457173.

MIZOGUCHI, K., TANAKA, Y. and TABIRA, T., 2010. Anxiolytic effect of a herbal medicine, yokukansan, in aged rats: involvement of serotonergic and dopaminergic transmissions in the prefrontal cortex. *Journal of Ethnopharmacology*, vol. 127, no. 1, pp. 70-76. http://dx.doi.org/10.1016/j.jep.2009.09.048. PMid:19799980.

MORILAK, D.A. and FRAZER, A., 2004. Antidepressants and brain monoaminergic systems: a dimensional approach to understanding their behavioural effects in depression and anxiety disorders. *The International Journal of Neuropsychopharmacology*, vol. 7, no. 2, pp. 193-218. http://dx.doi.org/10.1017/S1461145704004080. PMid:15003145.

MULY III, E.C., SZIGETI, K. and GOLDMAN-RAKIC, P.S., 1998. D1 receptor in interneurons of macaque prefrontal cortex: distribution and subcellular localization. *The Journal of Neuroscience*, vol. 18, no. 24, pp. 10553-10565. http://dx.doi.org/10.1523/JNEUROSCI.18-24-10553.1998. PMid:9852592.

MURAKAMI, Y., SHOJI, M., HANAZAWA, S., TANAKA, S. and FUJISAWA, S., 2003. Preventive effect of bis-eugenol, a eugenol ortho dimer, on lipopolysaccharide-stimulated nuclear factor kappa B activation and inflammatory cytokine expression in macrophages. *Biochemical Pharmacology*, vol. 66, no. 6, pp. 1061-1066. http://dx.doi.org/10.1016/S0006-2952(03)00419-2. PMid:12963494.

MURROUGH, J.W., YAQUBI, S., SAYED, S. and CHARNEY, D.S., 2015. Emerging drugs for the treatment of anxiety. *Expert Opinion on Emerging Drugs*, vol. 20, no. 3, pp. 393-406. http://dx.doi.org/10.1517/14728214.2015.1049996. PMid:26012843.

NAGABABU, E. and LAKSHMAIAH, N., 1992. Inhibitory effect of eugenol on non-enzymatic lipid peroxidation in rat liver mitochondria. *Biochemical Pharmacology*, vol. 43, no. 11, pp. 2393-2400. http://dx.doi.org/10.1016/0006-2952(92)90318-D. PMid:1319160.

NAMGYAL, D., ALI, S., HUSSAIN, M.D., KAZI, M., AHMAD, A. and SARWAT, M., 2021. Curcumin ameliorates the Cd-induced anxiety-like behavior in mice by regulating oxidative stress and neuro-inflammatory proteins in the prefrontal cortex region of the brain. *Antioxidants*, vol. 10, no. 11, p. 1710. http://dx.doi.org/10.3390/antiox10111710. PMid:34829581.

NIKOLIN, S., TAN, Y.Y., SCHWAAB, A., MOFFA, A., LOO, C.K. and MARTIN, D., 2021. An investigation of working memory deficits in depression using the n-back task: a systematic review and meta-analysis. *Journal of Affective Disorders*, vol. 284, pp. 1-8. http://dx.doi.org/10.1016/j.jad.2021.01.084. PMid:33581489.

NORTE, M.C.B., COSENTINO, R.M. and LAZARINI, C.A., 2005. Effects of methyl-eugenol administration on behavioral models related to depression and anxiety, in rats. *Phytomedicine*, vol. 12, no. 4, pp. 294-298. http://dx.doi.org/10.1016/j.phymed.2003.12.007. PMid:15898707.

OADES, R.D., TAGHZOUTI, K., RIVET, J.M., SIMON, H. and LE MOAL, M., 1986. Locomotor activity in relation to dopamine and noradrenaline in the nucleus accumbens, septal and frontal areas: a 6-hydroxydopamine study. *Neuropsychobiology*, vol. 16, no. 1, pp. 37-42. http://dx.doi.org/10.1159/000118294. PMid:3106851.

OGATA, M., HOSHI, M., URANO, S. and ENDO, T., 2000. Antioxidant activity of eugenol and related monomeric and dimeric compounds. *Chemical & Pharmaceutical Bulletin*, vol. 48, no. 10, pp. 1467-1469. http://dx.doi.org/10.1248/cpb.48.1467. PMid:11045452.

OHNO, M. and WATANABE, S., 1995. Intrahippocampal administration of (+)-SKF 10,047, a σ ligand, reverses MK-801-

induced impairment of working memory in rats. *Brain Research*, vol. 684, no. 2, pp. 237-242. http://dx.doi.org/10.1016/0006-8993(95)00489-D. PMid:7583231.

OTTO, M.W., EASTMAN, A., LO, S., HEARON, B.A., BICKEL, W.K., ZVOLENSKY, M., SMITS, J.A. and DOAN, S.N., 2016. Anxiety sensitivity and working memory capacity: risk factors and targets for health behavior promotion. *Clinical Psychology Review*, vol. 49, pp. 67-78. http://dx.doi.org/10.1016/j.cpr.2016.07.003. PMid:27611632.

OZYURT, B., OZYURT, H., AKPOLAT, N., ERDOGAN, H. and SARSILMAZ, M., 2007a. Oxidative stress in prefrontal cortex of rat exposed to MK-801 and protective effects of CAPE. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, vol. 31, no. 4, pp. 832-838. http://dx.doi.org/10.1016/j.pnpbp.2007.01.029. PMid:17374554.

OZYURT, B., SARSILMAZ, M., AKPOLAT, N., OZYURT, H., AKYOL, O., HERKEN, H. and KUS, I., 2007b. The protective effects of omega− 3 fatty acids against MK-801-induced neurotoxicity in prefrontal cortex of rat. *Neurochemistry International*, vol. 50, no. 1, pp. 196-202. http://dx.doi.org/10.1016/j.neuint.2006.08.002. PMid:16971021.

PARK, S.H., SIM, Y.B., LEE, J.K., KIM, S.M., KANG, Y.J., JUNG, J.S. and SUH, H.W., 2011. The analgesic effects and mechanisms of orally administered eugenol. *Archives of Pharmacal Research*, vol. 34, no. 3, pp. 501-507. http://dx.doi.org/10.1007/s12272-011-0320-z. PMid:21547684.

PATRI, M. and SINGH, A., 2019. Protective effects of noradrenaline on benzo [a] pyrene-induced oxidative stress responses in brain tumor cell lines. *In Vitro Cellular & Developmental Biology. Animal*, vol. 55, no. 8, pp. 665-675. http://dx.doi.org/10.1007/s11626-019-00378-9. PMid:31292939.

PELLOW, S., CHOPIN, P., FILE, S.E. and BRILEY, M., 1985. Validation of open: closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. *Journal of Neuroscience Methods*, vol. 14, no. 3, pp. 149-167. http://dx.doi.org/10.1016/0165-0270(85)90031-7. PMid:2864480.

PRASAD, S.N. and MURALIDHARA, 2013. Neuroprotective efficacy of eugenol and isoeugenol in acrylamide-induced neuropathy in rats: behavioral and biochemical evidence. *Neurochemical Research*, vol. 38, no. 2, pp. 330-345. http://dx.doi.org/10.1007/s11064-012-0924-9. PMid:23161090.

RABIEE, A., VASAGHI-GHARAMALEKI, B., SAMADI, S.A., AMIRI-SHAVAKI, Y., ALAGHBAND-RAD, J., SEYEDIND, S. and HOSSEINI, S., 2018. Impaired nonverbal working memory in high-functioning autism spectrum disorder. *Medical Journal of the Islamic Republic of Iran*, vol. 32, no. 1, pp. 623-629. http://dx.doi.org/10.14196/mjiri.32.107. PMid:30815402.

RADENOVIC, L. and SELAKOVIC, V., 2005. Differential effects of NMDA and AMPA/kainate receptor antagonists on nitric oxide production in rat brain following intrahippocampal injection. *Brain Research Bulletin*, vol. 67, no. 1-2, pp. 133-141. http://dx.doi.org/10.1016/j.brainresbull.2005.06.019. PMid:16140172.

ROBERTS, B.M., HOLDEN, D.E., SHAFFER, C.L., SEYMOUR, P.A., MENNITI, F.S., SCHMIDT, C.J., WILLIAMS, G.V. and CASTNER, S.A., 2010. Prevention of ketamine-induced working memory impairments by AMPA potentiators in a nonhuman primate model of cognitive dysfunction. *Behavioural Brain Research*, vol. 212, no. 1, pp. 41-48. http://dx.doi.org/10.1016/j.bbr.2010.03.039. PMid:20347881.

ROSENBROCK, H., GIOVANNINI, R., SCHÄNZLE, G., KOROS, E., RUNGE, F., FUCHS, H., MARTI, A., REYMANN, K.G., SCHRÖDER, U.H., FEDELE, E. and DORNER-CIOSSEK, C., 2019. The novel phosphodiesterase 9A inhibitor BI 409306 increases cyclic guanosine monophosphate levels in the brain, promotes synaptic plasticity, and enhances memory function in rodents. *The Journal of Pharmacology and Experimental Therapeutics*, vol. 371, no. 3, pp. 633-641. http://dx.doi.org/10.1124/jpet.119.260059. PMid:31578258.

RYCHTYK, J., PARTYKA, A., GDULA-ARGASIŃSKA, J., MYSŁOWSKA, K., WILCZYŃSKA, N., JASTRZĘBSKA-WIĘSEK, M. and WESOŁOWSKA, A., 2019. 5-HT6 receptor agonist and antagonist improve memory impairments and hippocampal BDNF signaling alterations induced by MK-801. *Brain Research*, vol. 1722, p. 146375. http://dx.doi.org/10.1016/j.brainres.2019.146375. PMid:31412259.

SALIM, S., ASGHAR, M., TANEJA, M., HOVATTA, I., CHUGH, G., VOLLERT, C. and VU, A., 2011. Potential contribution of oxidative stress and inflammation to anxiety and hypertension. *Brain Research*, vol. 1404, pp. 63-71. http://dx.doi.org/10.1016/j.brainres.2011.06.024. PMid:21704983.

SHIRAISHI, E., SUZUKI, K., HARADA, A., SUZUKI, N. and KIMURA, H., 2016. The phosphodiesterase 10A selective inhibitor TAK-063 improves cognitive functions associated with schizophrenia in rodent models. *The Journal of Pharmacology and Experimental Therapeutics*, vol. 356, no. 3, pp. 587-595. http://dx.doi.org/10.1124/jpet.115.230482. PMid:26675680.

SIYAL, F.J., SIDDIQUI, R.A., MEMON, Z., ASLAM, Z., NISAR, U., IMAD, R. and SHAH, M.R., 2023. Eugenol and its liposome-based nano carrier reduce anxiety by inhibiting glyoxylase-1 expression in mice. *Brazilian Journal of Biology = Revista Brasileira de Biologia*, vol. 83, p. e251219. http://dx.doi.org/10.1590/1519-6984.251219. PMid:34669914.

TAKEDA, H., TSUJI, M. and MATSUMIYA, T., 1998. Changes in head-dipping behavior in the hole-board test reflect the anxiogenic and/or anxiolytic state in mice. *European Journal of Pharmacology*, vol. 350, no. 1, pp. 21-29. http://dx.doi.org/10.1016/S0014-2999(98)00223-4. PMid:9683010.

TAO, G., IRIE, Y., LI, D.J. and KEUNG, W.M., 2005. Eugenol and its structural analogs inhibit monoamine oxidase A and exhibit antidepressant-like activity. *Bioorganic & Medicinal Chemistry*, vol. 13, no. 15, pp. 4777-4788. http://dx.doi.org/10.1016/j.bmc.2005.04.081. PMid:15936201.

TELLES-CORREIA, D., GUERREIRO, D.F., OLIVEIRA, S. and FIGUEIRA, M.L., 2007. Diferenças farmacodinâmicas e farmacocinéticas entre os SSRI: implicações na prática clínica. *Acta Medica Portuguesa*, vol. 20, no. 2, pp. 167-174. PMid:17868523.

TERLAND, O., FLATMARK, T., TANGERÅS, A. and GRØNBERG, M., 1997. Dopamine oxidation generates an oxidative stress mediated by dopamine semiquinone and unrelated to reactive oxygen species. *Journal of Molecular and Cellular Cardiology*, vol. 29, no. 6, pp. 1731-1738. http://dx.doi.org/10.1006/jmcc.1997.0412. PMid:9220358.

TROADEC, J.D., MARIEN, M., DARIOS, F., HARTMANN, A., RUBERG, M., COLPAERT, F. and MICHEL, P.P., 2001. Noradrenaline provides long-term protection to dopaminergic neurons by reducing oxidative stress. *Journal of Neurochemistry*, vol. 79, no. 1, pp. 200-210. http://dx.doi.org/10.1046/j.1471-4159.2001.00556.x. PMid:11595772.

UDAYABANU, M., KUMARAN, D., NAIR, R.U., SRINIVAS, P., BHAGAT, N., ANEJA, R. and KATYAL, A., 2008. Nitric oxide associated with iNOS expression inhibits acetylcholinesterase activity and induces memory impairment during acute hypobaric hypoxia. *Brain Research*, vol. 1230, pp. 138-149. http://dx.doi.org/10.1016/j.brainres.2008.06.081. PMid:18639532.

VASCONCELOS, C.F.M., FERREIRA, N.M.C., PONTES, N.H.L., REIS, T.D.S., SOUZA, R.B., CATUNDA JUNIOR, F.E.A., AGUIAR, L.M.V. and CUNHA, R.M.S., 2020. Eugenol and its association with levodopa in 6-hydroxydopamine-induced hemiparkinsonian

rats: behavioural and neurochemical alterations. *Basic & Clinical Pharmacology & Toxicology*, vol. 127, no. 4, pp. 287-302. http://dx.doi.org/10.1111/bcpt.13425. PMid:32353201.

WANG, M., DATTA, D., ENWRIGHT, J., GALVIN, V., YANG, S.T., PASPALAS, C., KOZAK, R., GRAY, D.L., LEWIS, D.A. and ARNSTEN, A.F.T., 2019. A novel dopamine D1 receptor agonist excites delay-dependent working memory-related neuronal firing in primate dorsolateral prefrontal cortex. *Neuropharmacology*, vol. 150, pp. 46-58. http://dx.doi.org/10.1016/j.neuropharm.2019.03.001. PMid:30858103.

WILLIS, C.L. and RAY, D.E., 2007. Antioxidants attenuate MK-801-induced cortical neurotoxicity in the rat. *Neurotoxicology*, vol. 28, no. 1, pp. 161-167. http://dx.doi.org/10.1016/j.neuro.2006.10.007. PMid:17141325.

WONG, P., HO, W.Y., YEN, Y.C., SANFORD, E. and LING, S.C., 2020. The vulnerability of motor and frontal cortex-dependent behaviors in mice expressing ALS-linked mutation in TDP-43. *Neurobiology of Aging*, vol. 92, pp. 43-60. http://dx.doi.org/10.1016/j.neurobiolaging.2020.03.019. PMid:32422502.

WORLD HEALTH ORGANIZATION, 2017. *Depression and other common mental disorders: global health estimates*. Geneva: World Health Organization.

YAMADA, K., KOMORI, Y., TANAKA, T., SENZAKI, K., NIKAI, T., SUGIHARA, H., KAMEYAMA, T. and NABESHIMA, T., 1999. Brain dysfunction associated with an induction of nitric oxide synthase following an intracerebral injection of lipopolysaccharide in rats. *Neuroscience*, vol. 88, no. 1, pp. 281-294. http://dx.doi.org/10.1016/S0306-4522(98)00237-1. PMid:10051207.

YAMAMOTO, B.K. and BANKSON, M.G., 2005. Amphetamine neurotoxicity: cause and consequence of oxidative stress. *Critical Reviews™ in Neurobiology*, vol. 17, no. 2, pp. 87-118. http://dx.doi.org/10.1615/CritRevNeurobiol.v17.i2.30. PMid:16808729.

YOSHIOKA, Y., NEGORO, R., KADOI, H., MOTEGI, T., SHIBAGAKI, F., YAMAMURO, A., ISHIMARU, Y. and MAEDA, S., 2021. Noradrenaline protects neurons against H2O2-induced death by increasing the supply of glutathione from astrocytes via β3-adrenoceptor stimulation. *Journal of Neuroscience Research*, vol. 99, no. 2, pp. 621-637. http://dx.doi.org/10.1002/jnr.24733. PMid:32954502.

ZEMANOVA, A., STANKOVA, A., LOBELLOVA, V., SVOBODA, J., VALES, K., VLCEK, K., KUBIK, S., FAJNEROVA, I. and STUCHLIK, A., 2013. Visuospatial working memory is impaired in an animal model of schizophrenia induced by acute MK-801: an effect of pretraining. *Pharmacology, Biochemistry, and Behavior*, vol. 106, pp. 117-123. http://dx.doi.org/10.1016/j.pbb.2013.03.014. PMid:23558085.

ZHANG, Y., LONG, Y., YU, S., LI, D., YANG, M., GUAN, Y., ZHANG, D., WAN, J., LIU, S., SHI, A., LI, N. and PENG, W., 2021. Natural volatile oils derived from herbal medicines: a promising therapy way for treating depressive disorder. *Pharmacological Research*, vol. 164, p. 105376. http://dx.doi.org/10.1016/j.phrs.2020.105376. PMid:33316383.