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Original Article

Evaluation of the antinociceptive effect generated by citronellal monoterpene isomers

Avaliação do efeito antinociceptivo gerado por isômeros do monoterpeno citronelal

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Abstract

Due to the complex nature of pain and the participation of physical, cognitive, psychological and behavioral aspects, pain management has several approaches. The use of medicinal plants in developing countries is quite expressive. Seeking new options for the treatment of emerging or debilitating diseases. Therefore, the present study seeks to elucidate the effects of the monoterpene, citronellal, differentiating its activity by isomers (R)-(+) and (S)-(-) citronellal. The study used several methods to evaluate the effects of citronellal isomers on motor coordination, nociceptive response, and the involvement of opioid, glutamatergic, and transient receptor pathways. The methods included rota-rod, hot-plate, and formalin tests, as well as the use of specific inhibitors and agonists. Data were analyzed using inferential statistics with a 95% confidence level. Both isomers did not significantly affect the motor coordination of the studied animals. The isomer (S)-(-) citronellal showed better results in relation to its structural counterpart, managing to have an antinociceptive effect in the formalin and hot plate tests with a lower concentration (100 mg/kg) and presenting fewer side effects, however, the this study was not able to elucidate the mechanism of action of this isomer despite having activity in studies with substances that act on specific targets such as glutamate and capsaicin, its activity was not reversed with the use of antagonists for pathways related to nociception. While the (R)-(+) citronellal isomer, despite showing total activity only at a concentration of 150 mg/kg, was able to determine its mechanism of action related to the opioid pathway by reversing its activity by the antagonist naloxone, being this is a pathway already correlated with nociception control treatments, however, it is also related to some unwanted side effects. In this way, new studies are sought to elucidate the mechanism related to the isomer (S)-(-) citronellal and a possibility of use in other areas related to the treatment of pain or inflammation.

Keywords: citronellal, monoterpene, isomer, nociception.

Resumo

Devido à natureza complexa da dor e a sua participação de aspectos físicos, cognitivos, psicológicos e comportamentais, o manejo da dor possui diversas abordagens. O uso de plantas medicinais em países em desenvolvimento é bastante expressivo. Buscando novas opções para o tratamento de denças emergentes ou debilitantes. Portanto, o presente estudo busca elucidar os efeitos do monoterpeno, citronelal, diferenciando sua atividade pelos isômeros (R)-(+) e (S)-(-) citronelal. O estudo utilizou diversos métodos para avaliar os efeitos dos isômeros de citronelal na coordenação motora, resposta nociceptiva e o envolvimento de vias opioides, glutamatérgicas e de receptores transitórios. Os métodos incluíram testes de rota-rod, placa quente e formalina, além do uso de inibidores e agonistas específicos. Os dados foram analisados estatisticamente com um intervalo de confiança de 95%. Ambos os isomeros não afetaram significativamente a coordenação motora dos animais em estudo. O isômero (S)-(-) citronelal apresentou melhores resultados em relação ao seu homólogo estrutural, conseguindo ter um efeito antinociceptivo nos testes de formalina e placa quente com menor concentração (100 mg/kg) e apresentando menos efeitos colaterais, entretanto, o presente estudo não foi capaz de elucidar o mecanismo de ação deste isomero apesar de ter atividadade em estudos com substancias que agem em alvos específicos como glutamato e capsaicina, sua atividade não foi revertida com a utilização de antagonistas para as vias relacionadas à nocicepção. Enquanto o isômero (R)-(+) citronelal, apesar de apresentar de apresentar total atividade somente na concentração de 150 mg/kg, foi capaz de determinar seu mecanismo de ação relacionado à via opióide pela reversão da sua atividade pelo antagonista naloxona, sendo esta uma via já correlacionada com os tratamentos de controle da a nocicepção, no entanto, também está relacionada a alguns efeitos colaterais indesejados.

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Desta forma busca-se novos estudos para elucidação do mecanismo relacionados ao isomero (S)-(-) citronellal e uma possibilidade de utilização em outras areas relacionadas ao tratamento da dor ou inflamação.

Palavras-chave: citronelal, monoterpeno, isômero, nocicepção.

1. Introduction

Pain had your concept declared for the first time in 1986 by the International Association for the Study of Pain (IASP); being defined as a response of the Central Nervous System to a tissue injury or an emotional change described in terms of this lesion, classifiable as acute or chronic, adaptive or non-adaptive and as physiological or pathological. The neural process of coding and processing noxious stimuli is called nociception (Klaumann et al., 2008; Naidu and Pham, 2015; Raja et al., 2020).

Due to the complex nature of pain with the participation of physical, cognitive, psychological and behavioral aspects, pain management has several approaches, involving physical and psychological rehabilitation, pharmacological treatment, lifestyle changes and surgical interventions (Dale and Stacey, 2016).

Besides the development of therapeutic agents for the treatment of pain and inflammation has been a challenging process. These treatments have different limitations due to the number of side effects and it is common for patients with acute intoxication by these drugs (Simon and Prince, 2017).

The use of medicinal plants in developing countries is a significant practice, resulting in the identification of biologically active natural products, including molecules that can be isolated and studied for their potential medicinal uses. These molecules may ultimately lead to the discovery of new drugs. Thus, medicinal plants serve as a natural source of biologically active compounds, while molecules isolated from plants can be studied for their medicinal properties and potentially developed into new drugs. Both medicinal plants and molecules isolated from plants are important sources for the development of new drugs (Newman and Cragg, 2020)

Several pharmacological studies of natural compounds have documented activity like the anticancer, antimicrobial, antifungal, antiviral, antihyperglycemic, analgesic, anti-inflammatory and antiparasitic properties, most of them by secondary metabolites, specially the terpene class (Guimarães et al., 2014).

Among studies carried out with monoterpenes, major compounds of essential oils present in aromatic plants, which have good activity and are always present in PRE-clinical innovations, some are isomeric and others in absolute forms, and these forms may or may not influence in your mechanism. We mention in particular the citronellal compound, which already has a good basis in the literature for its antinociceptive activity, among other activities, such as antioxidant and antimicrobial (Melo et al., 2010; Quintans-Júnior et al., 2011).

Melo et al., in 2010, demonstrated the activity of citronellal in hypnosis (Hypnotic and sedative) and nociception models, evaluating that at doses from 50 mg/kg the phytoconstituent guaranteed an increase in sleep time of the animals and decreased the nociceptive effect induced by a study model using acetic acid. Some tests are performed with citronellal associated with β-cyclodextrin, having an antihyperalgesic effect on chronic non-inflammatory muscle pain in mice, this effect greater than the form without association, and in this same study the *in silico* interaction of the citronellal compound with glutamatergic receptors, concluding that it is a possible mechanism of action to be studied for this nociception inhibition (Assis et al., 2020).

Therefore, the present study seeks to elucidate the effects of the monoterpene, citronellal, by differentiating its activity by (R)-(+) and (S)-(-) citronellal isomers, thus verifying his antinociceptive capacity and comparing these effects to determine the spatial positioning that presents better activity, as well as to determine if, as an isolated molecule, it could present a superior or inferior effect, thus bringing relevant data to the research of new drugs derived from natural products.

2. Materials and Methods

2.1. Animals

In all of our experiments, male Swiss mice over 4 weeks of age were used (26–32 g). The animals were kept in a temperature-controlled room at 25 ± 2 °C with a 12/12 h light/dark cycle (the light was turned on at 6:00), with food and water provided ad libitum. They were acclimatized to the laboratory conditions for at least 1 h before. These experiments were performed from 09:00 to 13:30 in a quiet room in which the conditions described above were maintained. Each animal was used only once. The number of animals and the intensity of the noxious stimuli were the minimum necessary to demonstrate consistent effects of the drug treatments.

The experimental protocol was approved by the Ethics Committee for Animal Research of the Federal University of Paraíba, under protocol CEUA nº 9586090819. The experiments were carried out in accordance with current guidelines for the care of laboratory animals, as well as ethical guidelines for investigations of experimental pain in animals (Damy et al., 2010).

2.2. Drugs

All of the substances used in this study, including citronellal isomers, were purchased from Sigma–Aldrich (St. Louis, MO, USA), with the exception of morphine and naloxone wich were donated by the Brazilian pharmaceutical laboratory, Cristália-Produtos Químicos Farmacêuticos, LTDA (Itapira, SP, BR). All substances were diluted in saline and intraperitoneally administered (i.p.) at a total volume of 0.1 mL/10 g. Citronellal isomers was initially emulsified with Tween 80 (0.5%) in 0.9% saline. The control group received the vehicle (Tween 80-0.5% in 0.9% saline),

2.3. In vivo tests

2.3.1. Evaluation of motor coordination (rota-rod test)

The rota-rod methodology assesses motor impairment in animals after administration of substances with potential CNS activity. The animals were exposed to the apparatus 24 h before the test, and the animals managing to stay on the rotating bar (at 10 rpm) for a period of 1 minute were selected for testing. These preselected animals (n = 6) were treated (i.p.) with vehicle (control group: Tween 80-0.5%), diazepam (4 mg/kg), administered 30 min before the start of the test, or (R)-(+) and (S)-(-) citronellal (50, 100, and 150 mg/kg each). At 30, 60, and 120 minutes, the animals were placed on the rotating bar to assess time performance. For each animal, the time spent on the rotating bar was recorded for 3 minutes (Dunham and Miya, 1957; Mattei and França, 2006). The doses of citronellal isomers were defined from previous studies (Quintans Junior et al., 2011; Melo et al., 2010).

2.3.2. Hot-plate test

This test was used to measure response latencies according to the method previously described. Animals were placed on a hot plate maintained at 55 ± 1 °C. The time elapsed between placing the animal on the hot plate and the animal either licking its fore or hind paws or jumping on the surface was considered the response latency. Mice with baseline latencies of more than 15 s were excluded from the study. Response latency testing was measured prior to intraperitoneal administration (baseline) of (R)-(+) and (S)-(-) citronellal (50, 100, and 150 mg/kg each), vehicle (control) and morphine (10 mg/kg) and at 30, 60 and 120 min after each treatment. The cut-off time for the hot-plate test latency was set at 15 seg (Woolfe & Macdonald, 1944; Yamamoto et al., 2002).

2.3.3. Formalin test

Nociceptive response was evaluated according to a previously described model. The animals were injected with 20 mL of formalin 2.5% (0.92% formaldehyde diluted in saline) in the subplantar area of the right hind paw. The duration of paw licking was measured at 1—5 min (first phase) and 15—30 min (second phase) after the formalin injection. The amount of time spent licking the injected paw was considered as the nociceptive response. Animals were submitted to intraperitoneal administration of (R)-(+) and (S)-(-) citronellal (50, 100, and 150 mg/kg each), vehicle (control) and morphine (10 mg/kg), 30 min prior to the injection of formalin (Hunskaar and Hole, 1985, Almeida and Oliveira, 2006).

2.3.4. Opioid pathway

For the investigation of a possible involvement and its modifications in opioid receptors and in the antinociceptive response of (R)-(+) and (S)-(-) citronellal, naloxone will be used, which is a non-selective antagonist of opioid receptors (Bodnar, 2021).

The control group will be treated with vehicle. Other groups containing 6 mice each will be treated with doses of (R)-(+) and (S)-(-) citronellal, and positive control morphine 10 mg/kg, i.p., respectively. More groups will receive a pretreatment with naloxone (5 mg/kg, s.c.), 15 minutes before administration of (R)-(+) or (S)-(-) citronellal, or morphine. After 30 minutes of administration of morphine and (R)-(+) or (S)-(-) citronellal, the mice will be submitted to the formalin test.

2.3.5. Antinociceptive test with specific targets

For the following studies, the isomer with the highest activity and its effective concentration was selected in all studies, reaching the result of the isomer (S)-(-) citronellal (100 mg/kg).

Glutamatergic pathway- Initially, the nociception induction test by glutamate was performed. This experiment was described by Beirith et al. (2002), with modifications. Animals were submitted to intraperitoneal administration (S)-(-) citronellal (100 mg/kg), vehicle (control) and MK-801 (Dizocilpine) (10 mg/kg), (a specific glutamate receptor inhibitor), 30 min prior to the injection of glutamate. A volume of 20 µL of a glutamate solution (30 µM/paw) was used, which was injected intraplantarly into the ventral surface of the mouse's right hind paw. Then, the animals were observed for 15 minutes after the application of the glutamate.

Transient receptor pathway- Capsaicin-induced nociception was performed by subcutaneous injection of 20 µL (2.5 µg) of capsaicin, dissolved in ethanol, dimethyl sulfoxide and distilled water (1:1:8), intraplantarly into the ventral surface of the mouse's right hind paw. Animals were submitted to intraperitoneal administration (S)-(-) citronellal (100 mg/kg), vehicle (control) morphine (10 mg/kg), 30 min prior to the injection of capsaicin. The nociceptive behavior of paw licking was observed for 20 minutes (Tamaddonfard et al., 2015).

2.3.6. Antinociceptive tests with specific antagonists

The following tests were carried out to try to determine a possible mechanism of action of the studied compound, for this, known antagonists of each pathway involved, directly or not with nociception, were used to find out if this pathway inhibition would be able to reverse the activity of (S)-(-) citronellal. To define the nociceptive effect, the formalin test described above was used with a pre-treatment of antagonists.

Each group was pre-treated with the pathway antagonist 15 min before the administration of (S)-(-) citronellal, namely: glibenclamide (10 mg/ kg, s.c.) to evaluate the action on K+ATP channels, yohimbine (0.15 mg/kg, i.p.) to evaluate the action on α2-adrenergic receptors, sulpiride (20 mg/kg s.c.) for the dopaminergic pathway because it is a D2 receptor antagonist or caffeine (10 mg/kg s.c.) as an adenosinergic receptor antagonist. After 15 min of pretreatment, they were treated intraperitoneally with (S)-(-) citronellal (100 mg/kg) or vehicle (Control). After 30 min, the formalin test was performed (Lopes et al., 2012)

3. Results

3.1. In vivo tests

3.1.1. Rota rod test

After the administration of (R)-(+) and (S)-(-) citronellal on the concentration of 50, 100, and 150 mg/kg each, the animals are tested on rota rod and results are expressed in Figure 1, showing that has no interference by the tested substances on the motor control of the animals, compared to diazepam at 4 mg/kg, which has significant effect on the time of stay to the animals on rota rod bar.

3.1.2. Hot plate test

The results expressed in Figure 2 demonstrate the effect according to the time after the administration of the (S)-(-) citronellal isomers in the concentration of 50, 100, and 150 mg/kg, in the permanence of the animals in the hot plate, while whereas Figure 3 shows the results of the (R)-(+) citronellal isomer at the same concentrations. In both tests, morphine at a concentration of 10 mg/kg is also used for comparison. The isomer (S)-(-) citronellal at 100 mg/kg (7.6 sec ±1.7) showed significant activity, in relation to the control group (4.9 sec ±0.1), and similar effect of morphine (9.2 sec ±2.0) at 60 and 120 min after administration of the compounds.

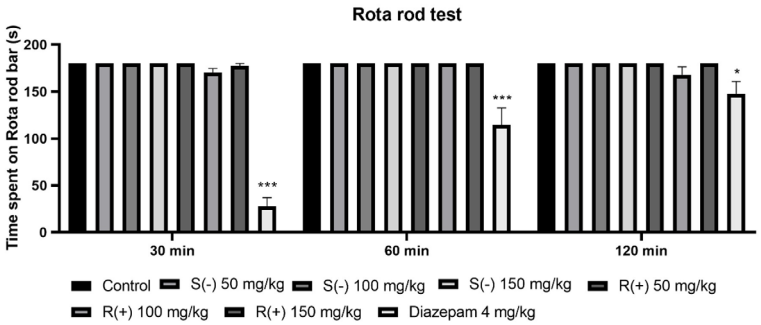

Figure 1. Effect of citronellal isomers (mg/kg, i.p.), the time of stay (s) of the animals on the rota rod. The column represents the average ± S.E.M. ($n = 6$). Statistical analysis: ANOVA followed by the Dunnet test, $^{*}p < 0.05$, $^{***}p < 0.0005$: Citronellal isomers = vs.control and Diazepam = vs.control.

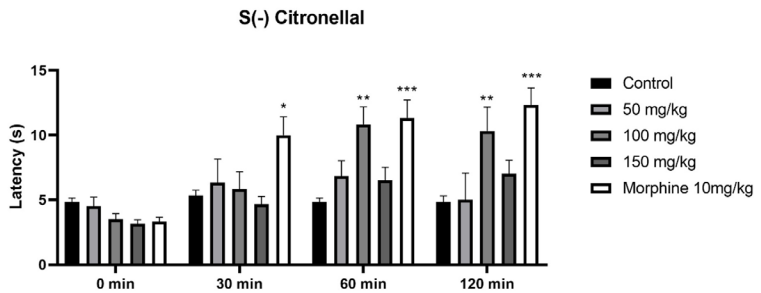

Figure 2. Effect of (S)-(-)-citronellal (mg/kg, i.p.), the time of latency (s) of the animals on the hot-plate. The column represents the average ± S.E.M. ($n = 6$). Statistical analysis: ANOVA followed by the Dunnet test, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.005$: (S)-(-)-citronellal= vs.control and Morphine = vs.control.

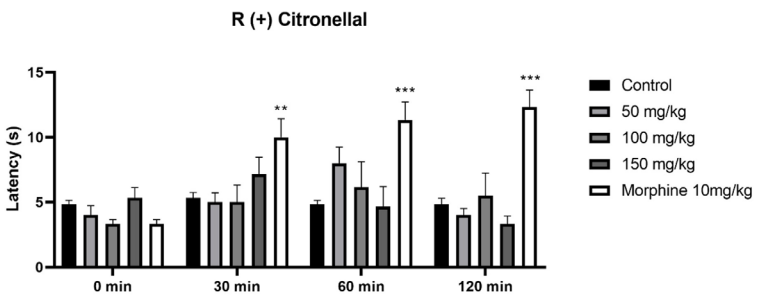

Figure 3. Effect of (R)-(+)-citronellal (mg/kg, i.p.), the time of latency (s) of the animals on the hot-plate. The column represents the average ± S.E.M. ($n = 6$). Statistical analysis: ANOVA followed by the Dunnet test, $^{**}p < 0.01$, $^{***}p < 0.005$: (R)-(+)-citronellal= vs.control and Morphine = vs.control.

The isomer (R)-(+) citronellal can't significantly increase the time of latency to the mice on the hot plate in all tested concentrations. Morphine was the only capable to show significant results on all the times of the test.

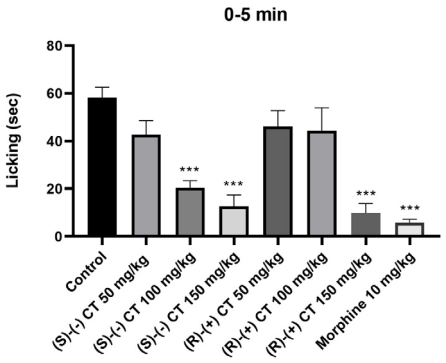

Figure 4. Effect of CT (citronellal) isomers (mg/kg, i.p.), the time of licking (s) of the animals in formalin test. The graph shows the first phase (Neurogenic phase) of formalin test, 0 – 5 min. The column represents the average ± S.E.M. ($n = 6$). Statistical analysis: ANOVA followed by the Dunnet test, $^{***}p < 0.005$: citronellal isomers = vs.control and Morphine = vs.control.

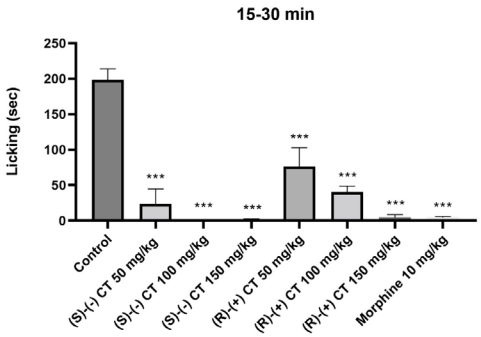

Figure 5. Effect of CT (citronellal) isomers (mg/kg, i.p.), the time of licking (s) of the animals in formalin test. The graph shows the second phase (Inflammatory phase) of formalin test, 15 – 30 min. The column represents the average ± S.E.M. ($n = 6$). Statistical analysis: ANOVA followed by the Dunnet test, < 0.01, $^{***}p < 0.005$: citronellal isomers = vs.control and Morphine = vs.control .

3.1.3. Formalin test

The results shown below are related to the formalin test, performed to verify the licking time of the animals in response to a painful stimulus from the injection of formalin in their paw sole. The test comprises two distinct phases where the first phase called neurogenic phase, measures the licking time that the animal presents in the first 5 minutes of exposure to the nociceptive stimulus. While the second phase, demonstrates the mediation of nociception by the inflammatory signaling generated by the animal in response to the stimulus of formalin to combat its effect. (Almeida and Oliveira, 2006).

In the first phase (Figure 4), the compounds (S)-(-) citronellal at concentrations of 50 (42.7 sec ±5.9) mg/kg and (R)-(+) citronellal at concentrations of 50 (46.1 sec ±6.6) and 100 (44.3 sec ±9.6) mg/kg were not able to significantly reverse the painful stimulus related by exposure formalin. While the (S)-(-) citronellal at concentrations of 100 (20.3 sec ±3.1) and 150 (12.7 sec ±4.8) mg/kg, (R)-(+) citronellal at 150 (9.8 sec ±3.9) mg/kg and morphine at 10 (5.7 sec ±1.4) mg/kg had a significant reduction to the time of licking by the animals of the control group (58.2 sec ± 4.4).

In the Figure 5 its show the results of second phase, understood to 15 – 30 min of formalin application, all concentrations of citronellal isomers: (S)-(-) citronellal 50 (23.7 sec ±20.9), 100 (0 sec ±0.0) and 150 (1.3 sec ±1.3) mg/kg, (R)-(+) citronellal 50 (76.3 sec ±26.3), 100 (40.3 sec ±8.2) and 150 (4.7 sec ±3.7) mg/kg and morphine 10 (4.4 sec ±1.5) mg/kg; were able to significantly reverse the inflammatory effect of control group (198.6 sec ±15.4) generated by the stimulus of formalin injection.

3.1.4. Opioid pathway

To evaluate if the citronellal isomers had their effect related to the opioid pathway, the formalin test was performed using naloxone, a known inhibitor of this pathway. Achieving the results shown in Figure 6.

The animals that were exposed to the isomer (S)-(-) citronellal, in the neurogenic phase, had their effects (31 sec ± 6.7 sec) maintained even in the presence of naloxone (26 sec ± 10.7), while the isomer of (R)-(+) citronellal had a reversal of its effect (12 sec ± 3.2) to a non-significant effect (51 sec ± 11.1) compared to control (65.3 sec ± 4.8).

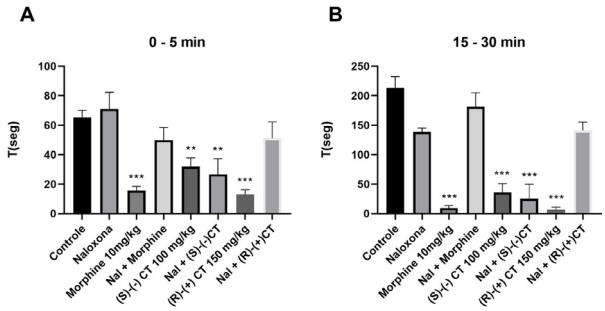

Figure 6. Effect of CT (citronellal) isomers (mg/kg, i.p.), the time of licking (s) of the animals in formalin test, with or without presence of naloxone (Nal). The graph shows (A)- the first phase (Neurogenic phase), by the elapsed time of 0-5 min., and (B)- second phase (Inflammatory phase) of formalin test, 15 – 30 min. The column represents the average ± S.E.M. ($n = 6$). Statistical analysis: ANOVA followed by the Dunnet test, $^{**}p < 0.01$, $^{***}p < 0.005$: citronellal isomers with and without naloxone = vs.control and Morphine with or without naloxone = vs.control.

This is more evident in the inflammatory phase, where the (R)-(+) citronellal isomer at a concentration of 150 mg/kg (6,7 sec ± 4), was not able to inhibit the nociceptive effect previously observed, in relation to the control in the presence of naloxone (5 mg/kg). kg) (141.2 sec ±14). This fact was observed by the isomer (S)-(-) citronellal 100 mg/kg (35.7 sec ± 15), which, even in the presence of naloxone 5 mg/kg , did not have its activity inhibited or reversed (25 sec ± 24).

3.1.5. Specific target nociceptive test

The animals are exposed to a nociceptive test on different pathways, now only testing the isomer (S)-(-) citronellal at 100 mg/kg concentration, which had present better activity in all the before tests, and this has a goal to evaluate the reaction of this exposure and take a glimpse to a possible mechanism of it antinociceptive action.

The glutamate test was performed and his results showed on Figure 7A, demonstrating that (S)-(-) citronellal (31.8 sec ±10.7) significantly reduces the time of licking to the animals in comparation to the control glutamate (62.7 sec ±12.2), the antagonist of NMDA receptor's to glutamate, MK (16.0 sec ±3.3), show how the antagonism of this receptor its capable to significantly reduce the glutamate mediated nociception. The association to MK + (S)-(-) citronellal (3.5 sec ±1.6) promoted the most significant reduction of the time licking in the animals.

Figure 7B demonstrastes a nocicicception test mediated by the capsaicin, a TRPV1 (Transient receptor potential vanilloid type 1) agonist, this activation was significantly reverted by the (S)-(-)-citronellal (66.6 sec ±20.7) and morphine (21.8 sec ±8.5), in comparation to the capsaicin control (228.8 sec ±24.1).

3.1.6. Antinociceptive tests with specific antagonists

The results expressed below are from tests performed using antagonists of pathways that may be related to nociception, thus leading to propose some antinociceptive mechanism for the (S)-(-) citronellal molecule, in case if there is a reversal of its activity.

The first phase of the test (Figure 8A) demonstrates the effect of formalin on the neurogenic phase of nociception, with (S)-(-) citronellal (20.3 sec ± 3.0) and morphine (5.7 sec ± 1.4) capable of significantly reversing this effect on licking time in relation to the control group (58.2 sec ±4.3). While the association of antagonists with (S)-(-) citronellal was not able to reverse its effect of significantly reducing the licking time of the animal, being the caffeine group + (S)-(-) citronellal (39.5 sec ± 4.0) which showed a lower degree of significance in relation to the control.

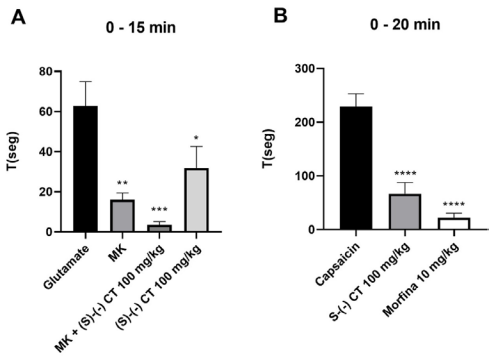

Figure 7. Effect of (S)-(-)-citronellal (100 mg/kg, i.p.) (Citro), the time of licking (s) of the animals on the specific targets nociceptive target. Glutamate test (A) and Capsaicin test (B). The column represents the average ± S.E.M. (n = 6). Statistical analysis: ANOVA followed by the Dunnet test, *p < 0.05, **p < 0.01, ***p < 0.005: (S)-(-)-citronellal= vs. Glutamate; MK = vs. Glutamate; (S)-(-)-citronellal = vs. Capsaicin and Morphine = vs. Capsaicin.

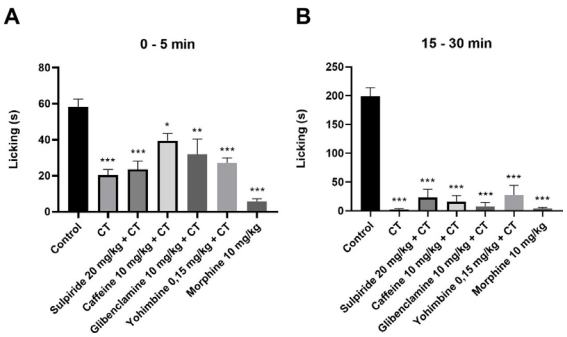

Figure 8. Effect of (S)-(-) citronellal (CT) (100 mg/kg, i.p.), in the time of licking to the animals in formalin test. The graph shows (A)- the first phase (Neurogenic phase), by the elapsed time of 0-5 min., and (B)- second phase (Inflammatory phase) of formalin test, understood between 15 – 30 min to the test. The column represents the average ± S.E.M. (n = 6). Statistical analysis: ANOVA followed by the Dunnet test, *p < 0.05, ¬¬**p < 0.01, ***p < 0.005: (S)-(-) citronellal = vs.control; (S)-(-) citronellal + antagonists = vs.control and Morphine = vs.control.

In Figure 8B we can see the second phase (inflammatory phase) of the formalin test applied to this methodology, resulting in a highly significant decrease in the licking time of all groups in relation to the control (198.6 sec ±15.4), reducing more than 80% of the effect in all groups tested, showing that associations with antagonists did not show reversal of effect.

4. Discussion

According to previous studies, citronellal monoterpene had antinociceptive and anti-inflammatory effects, as seen by orofacial nociception induction models and antioxidant capacity tests. In the present study, was possible to determine that the isomer (S)-(-) citronellal presents better results for antinociceptive activity in relation to its isomer (R)-(+) citronellal (Quintans-Júnior et al., 2011; Melo et al., 2010).

The Rota-rod test is a widely used method in research, as it can detect physical impairments caused by pharmacological agents, such as muscle relaxants and CNS depressants, strongly related to antinociceptive behavior. In the present study, they did not indicate interference in the motor coordination of these animals for the studied concentrations (50, 100 and 150 mg/kg) of both citronellal isomers. Following the structural similarity, Andrade et al. (2021) studied the effects of hydroxycitronellal on anxiolytic activity, reaching a similar result when comparing the motor effect of this substance. As observed in other studies, monoterpenes in general do not have a negative effect on the locomotor system even if they have anxiolytic effects, this is important for the formulation and discovery of new compounds, since their use is considered acceptable in terms of their motor activity and carrying out daily tasks (Souto-Maior et al., 2017; Matias Nascimento Maia et al., 2021).

For the initial nociception studies, the hot plate test was used, which allows evaluating the antinociceptive activity of the compound when exposed to a thermal stimulus to the animal. The hot-plate test showed a biphasic effect, in which only one concentration of the isomer (S)-(-) citronellal (100 mg/kg) showed a significant effect, this not being the highest concentration studied, thus leading to the thought that the substance might not follow the dose-response effect. Studies with the similar compound, citronellol, showed an effect at higher concentrations, however lower doses showed a higher significance level in the response in relation to higher doses, classifying it as a biphasic effect that may be related to several idiosyncratic factors of the substance or class studied (Melo et al., 2010; Brito et al., 2012). The biphasic effect of some compounds with antinociceptive action is reported by previous studies, especially in tests that consider nociception induced by thermal action, such as tail-flick and hot-plate. Increasing the dose concentration can induce responses mediated by the activation of nociceptive receptors, such as ion channels, which are involved in nociception induced by thermal stimuli. The results of this research suggest that new studies should be carried out to better understand the biphasic effect produced by (S)-(-) citronellal, especially in tests of thermal hyperalgesia, such as the tail-flick and the Hargreaves model. (Ogren and Berge, 1984; Wei et al., 1996).

Previous studies with citronellal already demonstrated its activity and in the present study its activity was observed in both phases of the formalin test, with special focus on the inflammatory phase, where all concentrations were capable of significantly inhibiting the nociceptive response mediated by inflammatory factors generated by the application of formalin. The best performance of the (S)-(-) citronellal isomer is clear, which in the first phase of the test was able to significantly inhibit, at two concentrations (100 and 150 mg/kg), the formalin-mediated nociceptive stimulus, while the isomer (R)-(+) citronellal, although also effective, was only able to significantly inhibit its highest concentration (150 mg/kg), as well as its result in the inflammatory phase, although significant in relation to the control group , showed a reduced effect at two concentrations (50 and 100 mg/kg), in relation to the use of morphine or the concentrations of its isomer (S)-(-) citronellal (Quintans-Junior et.al., 2010).

Regarding the studies of the opioid pathway, it was observed that the isomer (R)-(+) citronellal obtained a reversal of its effect by the presence of naloxone, corroborating with previous studies that already characterized this pathway as the one described in the mechanism of action of the monoterpene citronellal, however, in tests carried out with both isomers, it was possible to observe that the isomer (S)-(-) citronellal, even with proven nociceptive activity in previous tests, did not have its effect reversed by the presence of naloxone, which may suggest that this isomer has a different mechanism of action than its structural counterpart, and does not have the same side effects related to the opioid pathway (Quintans-Junior, et al, 2010)

A few authors demonstrate the divergent effect of monoterpene isomers, which may act on different receptors of the same family or show activity only on one of the isomers, which may lead to an improvement in activity by reducing adverse effects, thus demonstrating an important pathway of studies to be carried out both with natural products and with all for general use (Kang et al., 2015).

For subsequent tests, the (S)-(-) citronellal isomer at a concentration of 100 mg/kg was selected due to its greater efficiency in previous results and determined non-activity by the opioid pathway. Which, as demonstrated later, also had an effect on the results using specific mechanism pain agents, such as glutamate and capsaicin.

The glutamatergic pathway is of great importance for nerve impulse transmission of pain, thus being a focus in studies related to nociception. In the present study, the compound (S)-(-) citronellal at a concentration of 100 mg/kg was able to significantly inhibit the nociceptive effect triggered by the animal's exposure to glutamate, showing an effect almost similar to that of MK (inhibitor of receptors of glutamate of the NMDA type), this one presents an inhibition in greater order of significance in relation to the control for directly inhibiting the injected algic agent. Even so, the combination of the two compounds (S)-(-) citronellal + MK was able to almost completely inhibit the nociceptive effect generated by glutamate, demonstrating that a synergistic action of effects may occur, thus signaling a possible mechanism of (S))-(-) citronellal on a receptor other than glutamatergic NMDA.

Some previous studies have shown similar effects for citronellal and other monoterpenes, as well as computational docking studies indicate glutamate receptors as possible targets for citronellal and monoterpenes in general, showing that more specific studies are needed to determine the real interference of citronellal monoterpene in these receptors (Assis et al., 2020; Santos et al., 2016, Costa et al., 2020; Quintans-Junior et al., 2010).

The compound, (S)-(-) citronellal at a concentration of 100 mg/kg, was able to inhibit the nociceptive effect presented by capsaicin. This activity can be mediated by TRPV-type receptors or some associated mechanism, as well as by inhibiting a receptor of the same family of transient receptors. Similar activities have already been noted by previous studies and some monoterpenes have their activity elucidation closely related to receptors of this family, as well as menthol, myrcene, camphor, eucalyptol, cinnamaldehyde, among others. Even so, further studies are needed to determine a possible mechanism of action related to family receptors (Melo Júnior et al., 2017; Jansen et al., 2019; Oz et al., 2015).

In studies with selective antagonists for each pathway directly or indirectly related to nociception, it was observed that no antagonist was able to completely inhibit the significant antinociceptive effect of (S)-(-) citronellal in relation to the control. Other authors determine the antinociceptive effect of monoterpenes correlated to the opioid pathway, as well as the glutamatergic pathway in other cases, so in any case it is necessary to use more in-depth tools to determine the real mechanism of action with which these monoterpenes are capable to inhibit nociception. (La Rocca et al., 2017; Andrade Próspero et al., 2018; Ortiz et al., 2022).

Still on the effect of the antagonists, caffeine had an increase in the licking time of the animals in the neurogenic phase (Phase 1) but not in such a way as to significantly reverse the entire effect of (S)-(-) citronellal, and this effect may be related to caffeine activity itself, generating hyperactivity and alertness, which may have slightly increased the animal's nociceptive response to any stimulus (Baratloo et al., 2016).

Therefore, further research is needed to elucidate the mechanism of action underlying the antinociceptive activity of the isomer (S)-(-) citronellal and explore other potential activities associated with this isomer or its structural modifications that may enhance its efficacy. Molecular docking studies could be used to identify potential binding sites and interactions between the compound and its targets, shedding light on its mode of action. Additionally, these studies could reveal possible adverse effects and aid in the development of safer and more effective therapeutic strategies.

5. Conclusion

In short, the citronellal monoterpene was able to generate an antinociceptive activity through its two forms of optical isomers, not negatively affecting his motor coordination at the studied concentrations. The (S)-(-) citronellal isomer showed better results compared to its structural counterpart, managing to have an antinociceptive effect with a lower concentration and presenting fewer side effects, however, the present study was not able to elucidate the action mechanism on a fruitful way. While the (R)-(+) citronellal isomer, despite presenting a lower potency activity compared to that previously described, was able to determine its mechanism of action related to the opioid pathway, this being a pathway already correlated to the treatments of control of nociception, however, also related to some unwanted side effects.

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