Research, Society and Development, v. 10, n. 11, e191101119571, 2021
(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v10i11.19571

Alpha-terpineol: evaluation and pharmacological screening as an antidepressant agent

Alfa-terpineol: avaliação e triagem farmacológica como agente antidepressivo

Alfa-terpineol: evaluación y cribado farmacológico como agente antidepresivo

Received: 08/19/2021 | Reviewed: 08/23/2021 | Accept: 08/26/2021 | Published: 08/28/2021

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Abstract

Objective: To evaluate the possible antidepressant effects of alpha-terpineol in rodents. Material and Methods: Depression levels were analyzed by comparing the total immobility time presented by the animals of the experimental groups in the test session, using the Forced Swimming Test and the Tail Suspension Test. The parameters of locomotion (central, peripheral and total) and motor coordination were evaluated in the Open Field Test and in the Rota Rod Test, respectively. In the second stage, the involvement of the noradrenergic system in the antidepressant action of alpha-terpineol in Forced Swimming Test was investigated. Results and Discussion: After performing the experimental tests, it was observed that the animals that received alpha-terpineol had reduced immobility time in Forced Swimming Test and Tail Suspension Test, compared to the other groups. In the Open Field Test and Rota-rod, the mice showed, respectively, good exploratory activity and motor coordination during the tests. In addition, the

study of the Noradrenergic System proved to be a promising mechanism used during its antidepressant action. Conclusion: In view of the results of the experimental tests, alpha-terpineol presented similar responses to those found in other monoterpenes investigated in the literature. Thus, it is shown as a promising antidepressant to be used clinically in humans, with less side effects and low production cost.
Keywords: Terpenes; Antidepressive agents; Norepinephrine.

Resumo

Objetivo: Avaliar os possíveis efeitos antidepressivos do alfa-terpineol em roedores. Material e Métodos: Foram analisados níveis de depressão por meio da comparação do tempo total de imobilidade apresentado pelos animais dos grupos experimentais na sessão de teste, através do Teste Nado Forçado e Teste Suspensão pela Cauda. Os parâmetros de locomoção (central, periférica e total) e coordenação motora foram avaliados no Teste de Campo Aberto e no Teste do Rota Rod, respetivamente. Na segunda etapa, foi investigado o envolvimento do sistema noradrenérgico na ação antidepressiva do alfa-terpineol no Teste Nado Forçado. Resultados e Discussão: Após a realização dos testes experimentais, observou-se que os animais que receberam alfa-terpineol tiverem tempo reduzido de imobilidade no Teste Nado Forçado e Teste Suspensão pela Cauda, comparados aos outros grupos. No Teste Campo Aberto e Rota-rod, os camundongos, apresentaram, respectivamente, boa atividade exploratória e coordenação motora durante os ensaios. Além disso, o estudo do Sistema Noradrenérgico se mostrou uma via promissora de mecanismo utilizada durante sua ação antidepressiva. Conclusão: Diante dos resultados dos testes experimentais, o alfa-terpineol apresentou respostas semelhantes às encontradas em outros monoterpenos investigados na literatura. Desse modo, mostra-se como promissor antidepressivo a ser utilizado clinicamente em humanos, com menos efeitos colaterais e baixo custo de produção.
Palavras-chave: Terpenos; Antidepressivos; Noradrenalina.

Resumen

Objetivo: Evaluar los posibles efectos antidepresivos del alfa-terpineol en roedores. Material y Métodos: Los niveles de depresión analizados comparando el tiempo total de inmovilidad que presenta el cabello animado son dos grupos experimentales en sesión, utilizando el Test de Natación Forzada y el Test de Cola Suspendida. Los parámetros de locomoción (central, periférica y total) y coordinación motora se evalúan en el Test de Campo Abierto y en el Test Rota-rod, respectivamente. En la segunda etapa, investigamos la participación del sistema noradrenérgico en la acción antidepresiva del alfa-terpineol sobre el Test de Natación Forzada. Resultados y Discusión: Después de realizar dos pruebas experimentales, noto que se le anima a recibir alfa-terpineol con un tiempo de inmovilidad más corto en Test de Natación Forzada y Test de Cola Suspendida, en comparación con los otros grupos. En el Test de Campo Abierto y Rota-rod, los ratones mostrarán buena actividad exploratoria y coordinación motora, respectivamente, durante las pruebas. Además, el estudio del sistema noradrenérgico ha demostrado ser un mecanismo prometedor utilizado durante su acción antidepresiva. Conclusión: Con dos resultados, dos problemas experimentales, o alfa-terpineol, presenta respuestas similares a las encontradas en otros monoterpenos investigados en la literatura. Por tanto, se convierte en un antidepresivo prometedor para uso clínico en humanos, con menos efectos secundarios y menores costes de producción.
Palabras clave: Terpenos; Antidepresivos; Noradrenalina.

1. Introduction

Depression is a common disorder worldwide, and it is estimated that more than 300 million people suffer from it. The condition is different from the usual fluctuations in mood and short-term emotional responses to the challenges of everyday life Pan American Health Organization - PAHO (2018). In addition, it is associated with anxiety, decreases the patient's quality of life, and may present degrees of suffering ranging from sadness to thoughts of death (Pimenta, Seixas, Gregório, & Santos, 2016).

Although intense research on depression is being carried out, the etiology and pathogenesis of this disease are still unclear. There are preclinical and clinical studies suggesting that monoaminergic neurotransmitters such as serotonin (5-HT), norepinephrine (NA) and dopamine (DA) play an important role in the pathophysiology of the central nervous system (CNS) (Możdżeń, Wąsik, Romańska, Michaluk, & Antkiewicz-Michaluk, 2017).

According to Tundo, de Filippis and Proietti (2015), about 30% of depressed patients evaluated in their research do not respond adequately to initial drug therapy. As found in the study by Ibanez (2014), which reports the difficulty of these

individuals adhering to drug treatment, and a considerable portion of the population has a low level of knowledge in relation to the dose and frequency of administration.

In this perspective, the use of plant species for therapeutic purposes, for the treatment, cure and prevention of diseases is one of the oldest means practiced by medicine. The production of drugs of plant origin involves ethnopharmacological surveys, phytochemical and pharmacological studies. In addition, biological investigations are essential and animal models of anxiety and depression have played relevant roles in the research process for new drugs (Lima *et al*., 2019).

Alpha-terpineol, a volatile monoterpenoid alcohol, is the main component of essential oils from various species of aromatic plants, such as *Origanium vulgare* L. and *Ocimum canum* Sims, widely used for medicinal purposes. Studies indicate that it can also be isolated from a variety of sources, such as cajuput, pine and petitgrai oils, as well as having a wide range of biological properties, including cardiovascular and antihypertensive effects; antioxidant, antinociceptive, antiulcerative, anticonvulsant and sedative activity (Khaleel, Tabanca, & Buchbauer, 2018).

Faced with so many therapeutic possibilities presented by this monoterpene, this work has the general objective of elucidating the possible antidepressant action exerted by alpha-terpineol.

2. Material and Methods

2.1 Nature of the Study

This study corresponds to an experimental research with a quantitative and qualitative approach. It is classified as explanatory in terms of objectives, as it proposes to investigate the cause and effect relationships of data collected in the laboratory (Pereira, Shitsuka, Parreira & Shitsuka, 2018).

According to Yin (2015), quantitative and qualitative studies can be complementary in order to clarify the phenomenon studied.

2.2 Study Scenario

The research was carried out at the Biotechnology and Biodiversity Research Center (NPBio, as per its Portuguese acronym) at the State University of Piaui (UESPI, as per its Portuguese acronym) and at the Pharmaceutical Technology Center (NTF, as per its Portuguese acronym) at the Federal University of Piaui (UFPI, as per its Portuguese acronym).

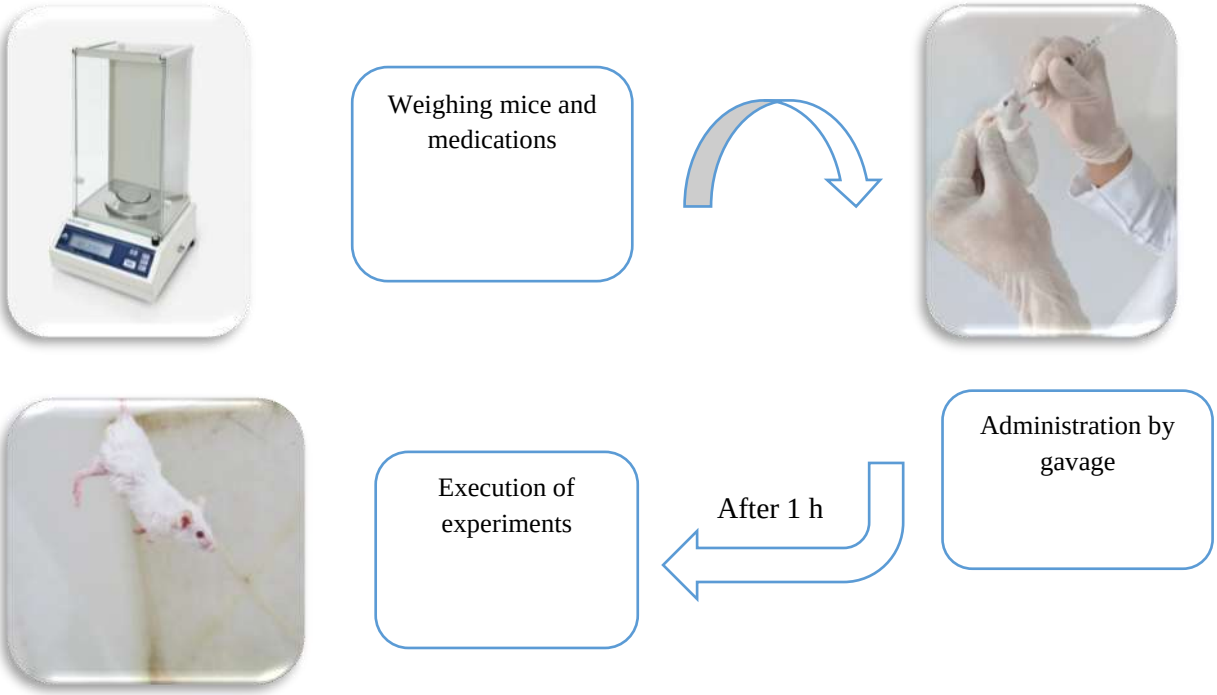
2.3 Animals

Albino mice (*Mus musculus*), Swiss variety, adult males, weighing between 25 and 30 g, were placed in acrylic cages and kept at 22 ± 2 ° C with water and food ad libitum, kept in a light / dark cycle 12:12 hours (7: 00-19: 00 h). The animals came from the NPBio Vivarium and, after the experimental procedures, euthanized with sodium Pentobarbital overdose, 150mg / kg, intraperitoneally (i.p.) associated with 10 mg/kg of lidocaine.

2.4 Treatment of animals

The animals received the treatments orally 1 hour before each test: Group I - distilled water as a Negative Control (NC); Group II - Fluoxetine (FLX) /kg as a positive control; Group III - Terpineol 50 mg/kg (TEP 50); Group IV - Terpineol 100 mg/kg (TEP 100) (Figure 1).

Figure 1. Schematic representation of the mouse treatment protocol.



Source: Personal archive (2021).

2.5 Behavioral tests

2.5.1 Forced Swim Test (FST)

This test was based on the model by Porsolt, Le Pichon and Jalfre (1977). The animals were immobilized for a certain period (duration of six minutes) keeping a record of their behavior. A tank of 22 cm in diameter and 40 cm in height containing fresh water at 26 ± 1ºC up to half was used, so that when immersed, the mouse's tail did not touch the bottom of the container. The animals, divided into 4 groups of 6 mice (n = 6 / group) and treated as previously described, were placed, one at a time, in the tank. The immobility time was recorded in seconds for 5 minutes and the animal was considered immobile when it remained floating in the water, making only the smooth movements necessary to keep its head on the surface (Figure 2).

Figure 2. Forced Swim Behavioral Testing.

Source: Personal archive (2021).

2.5.2 Tail Suspension Test (TST)

The test protocol was performed based on the work of Steru, Chermat, Thierry and Simon (1985). In this experiment, the mice were isolated on a platform, being suspended by the tail at a height of 50 cm from the ground, using an adhesive tape placed approximately 1 cm from the tip of the tail (Figure 3). Thus, the total immobility time of the animal in the last 4 minutes of a total of 6 minutes of testing was analyzed. It is proposed that substances with antidepressant activity decrease the animals' immobility time in this test, without altering their locomotor activity.

Figure 3. Tail Suspension Behavioral Testing.

Source: Personal archive (2021).

2.5.3 Open Field Testing (OFT)

This test was initially proposed by Archer (1973). It was used a transparent glass box with a black background and dimensions 30x30x15cm divided into nine equal quadrants (Figure 4). Each animal was placed in the center of the box before the test, allowing them to explore it for five minutes, with only the number of squares crossed by the animal (used as a parameter of spontaneous locomotor activity and the number of surveys performed) being recorded. Between each experiment, the box was sanitized with 5% ethanol to remove feces and urine from the animals.

Figure 4. Open Field Behavioral Testing.

Source: Personal archive (2021).

2.5.4 Rota-rod test

The adapted test by Gonçalves *et al*. (2008) by Montenegro, Sena, Barbosa Filho and Almeida (2010) and Nogueira Neto *et al*. (2012) measures the effect of muscle relaxation or impaired motor coordination produced by compounds in animals.

The mice were placed (with four legs) on a bar 2.5 cm in diameter, raised 25 cm from the floor, in a rotation of 16 rpm, for a period of three minutes (Figure 5). Thus, the time spent on the rotating bar in seconds was considered and the number of falls was counted, with a maximum of three renewals, following the protocol of previous studies.

Figure 5. Rota-rod behavior test.

Source: Personal archive (2021).

2.6 Assessment of mechanisms of action

2.6.1 Involvement of the noradrenergic system

All compounds were dissolved in saline (NaCl 0.9%) and administered i.p., except alpha-terpineol, fluoxetine and distilled water, which were administered orally (gavage). In order to investigate the participation of the noradrenergic system in the antidepressant effect of alpha-terpineol, the animals in Group 1 were pre-treated with 1mg/kg yohimbine, ip, α_2-adrenergic receptor antagonist) and after thirty minutes received an effective dose of alpha-terpineol. Group 2 animals were treated with propranolol (2 mg/kg, i.p., β-adrenergic receptor antagonist) and after thirty minutes received an effective dose of alpha-terpineol. Both groups (1 and 2), after 1 hour, were submitted to FST.

The animals in groups 4, 5 were treated, respectively, with yohimbine (1mg/kg, ip, α_2-adrenergic receptor antagonist) and propranolol (2 mg/kg, ip, β-adrenergic receptor antagonist) and after 30 minutes, submitted to FST.

During the last stage, the mice in group 6 received only the effective dose of alpha-terpineol (by gavage); those in the positive control group received an effective dose of fluoxetine (20mg/kg, v.o.) and those in the negative control group were treated with an appropriate vehicle of distilled water (0.1 mL/10g of weight, v.o.). After 60 minutes, the animals were submitted to FST.

2.7 Statistical analysis of the data

The data were analyzed using the GraphPad Prism 7.0 program using One-Way ANOVA followed by the Tukey test. For all tests, a significance level of 5% was considered. The results were expressed as means and standard deviation and considered positive when there was a statistically significant increase ($p < 0.05$).

2.8 Ethical and legal aspects

This work was developed following the ethical principles of animal experimentation, in accordance with criteria proposed by Law No. 11,794 / 2008: animal experimentation. The research project was submitted for consideration by the Ethics Committee on the Use of Animals (CEUA, as per its Portuguese acronym) of the State University of Piauí (UESPI, as per its Portuguese acronym) and approved under protocol number 297/2019.

This work was also registered in the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SisGen, as per its Portuguese acronym) with the number A06A748.

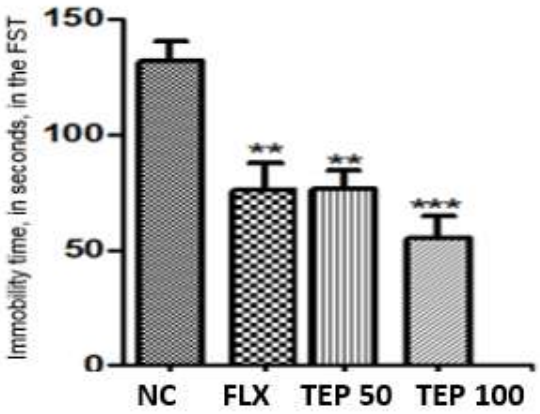
3. Results and Discussion

3.1 Behavioral tests

3.1.1 Forced Swimming

The FST results shown in Figure 6 and Table 1 demonstrate that there was a significant difference between the groups Fluoxetine ($p < 0.01$), TEP 50 mg/kg ($p < 0.01$) and TEP 100 mg/kg ($p < 0.001$) in relation to the negative control. That is, the animals reduced the immobility time after receiving the doses of this monoterpene, showing a probable antidepressant action.

Figure 6. Animal immobility time, in seconds, in the Forced Swimming Test.



Subtitle:

NC: Negative Control

FLX: Fluoxetine 20 mg/kg

TEP 50: Alpha-Terpineol 50 mg/kg

TEP100: Alpha-Terpineol 100 mg/kg

The results were expressed as mean ± standard deviation of the immobility times of the animals in the forced swimming test by groups. ***p<0.001 and **p<0.01 when compared to the negative control group. Statistical analysis was performed using the ANOVA variance test, followed by the Tukey test.
Source: Personal archive (2021).

Table 1. Mean values of the animals' immobility time, in seconds, in the Forced Swim Test.

GROUPS	AVERAGE ± STANDARD DEVIATION
Negative Control	131.80 ± 19.72
Fluoxetine	76.00 ± 25.91
Alpha-Terpineol 50	70.50 ± 11.96
Alpha-Terpineol 100	55.25 ± 19.38

Source: Personal archive (2021).

Thus, preclinical experimental studies using mice and behavioral testing protocols are of paramount importance to assess the effectiveness of substances with potential antidepressants. In one of these trials, there was a reduction in the immobility time of mice in FST, when they received *Proposis cineria* at a dose of 200 mg/kg compared to the control group, demonstrating significant antidepressant activity and relaxation of skeletal muscle (George, Joseph, & Sharma, 2012). The same effect was verified by Mendonça-Netto *et al*. (2008) when using a fraction of total tertiary alkaloids 12 mg/kg of *Cissampelos sympodialis* Eichler (Menispermaceae).

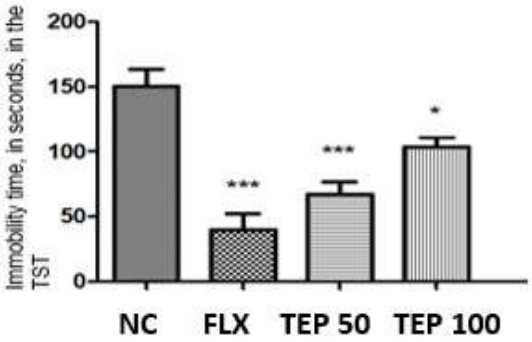
In addition, 1,8-cineole at doses of 4×10^{-4} and 4×10^{-2} mg / kg induced a significant decrease in the immobility time of mice by 44% and 39% in FST, respectively, in relation to the times in the control group (Dougnon & Ito, 2020). In another study, similar responses were found after treatment with oleuropein at doses of 16 and 32mg/kg, which caused a significant improvement comparable to treatment with fluoxetine ($p<0.05$). Oleuropein at 16 and 32 mg/kg caused a reduction of approximately 50% in immobility time compared to that of the control group ($p <0.001$) in FST (Badr, Attia, & Al-Rasheed, 2020).

The fractions and compounds isolated from the methanolic extract of *Castilleja tenuiflora* were tested in stressed mice. The chromatographic fractionation produced four fractions (FCt^{-1}, FCt^{-2}, CFt^{-3} and FCt^{-4}). In FST, the fractions FCt^{-2}, FCt^{-3} and FCt^{-4} significantly decreased the duration of immobility time of the mice compared to the control group (López-Rodríguez *et al*., 2019).

3.1.2 Tail suspension

In the TST, it can be observed that the groups that received terpineol in the doses 50 mg/kg ($p <0.01$) and 100 mg/kg ($p <0.1$) showed statistical difference in relation to the control group (Figure 7). During the suspension experiment, the mice showed greater mobility, with the probable antidepressant characteristic being noticeable without altering their locomotor activity (Table 2).

Figure 7. Animal immobility time, in seconds, in the Tail Suspension Test



Subtitle:

NC: Negative Control

FLX: Fluoxetine 20 mg/kg

TEP 50: Alpha-Terpineol 50 mg/kg

TEP100: Alpha-Terpineol 100 mg/kg

The results were expressed as mean ± standard deviation of the immobility times of the animals in the Tail Suspension test by groups. ***p<0.001 and *p<0.05 when compared to the negative control group. Statistical analysis was performed using the ANOVA variance test, followed by the Tukey test.
Source: Personal archive (2021).

Table 2. Mean values of the animals' immobility time, in seconds, in the Tail Suspension Test

GROUPS	AVERAGE ± STANDARD DEVIATION
Negative control	150.80 ± 29.30
Fluoxetine	39.80 ± 28.60
Alpha-Terpineol 50 mg/kg	67.00 ± 22.27
Alpha-Terpineol 100 mg/kg	103.60 ± 17.01

Source: Personal archive (2021).

In this perspective, the work of Dougnon and Ito (2020) proposed to investigate the substances 1,8- and 1,4-cineol, widely used for tracking antidepressant drugs in animal models, through the tests of forced swimming and suspension by tail. In these tests, the rats are forced to swim or are hung by the tail in an unavoidable situation; first they demonstrate vigorous activity trying to escape from the threatening environment and, finally, they remain immobile as a symptom of behavioral despair. Substances that decrease immobility time are known to demonstrate antidepressant properties in humans.

The TST results for 1,8- and 1,4-cineols confirmed the validity of the experiments, as they significantly reduced immobility times in mice by 62% and 55%, respectively, compared to the control group. Similar to the activities of these substances, the antidepressant action was previously demonstrated for terpinen-4-ol contained in *Origanum majorana* (Abbasi-Maleki, Kadkhoda, Taghizad-Farid, 2020); beta-pinene and linalool, main constituents of Litsea glaucescens (Guzmán-Gutiérrez, Gómez-Cansino, García-Zebadúa, Jiménez-Pérez, & Reyes-Chilpa, 2012), and or the essential oil of *Rosmarinus officinalis* (Machado *et* al., 2013).

Corroborating this line of reasoning, the studies by Tian *et al*. (2021) demonstrated that paeoniflorin, a monoterpenic glycoside compound derived from *Paeonia lactiflora*, improved behaviors similar to those of depression induced by reserpine, characterized by increased mobility time in the TST and FST, as well as the abnormal change in synaptic plasticity in the depressed hippocampus.

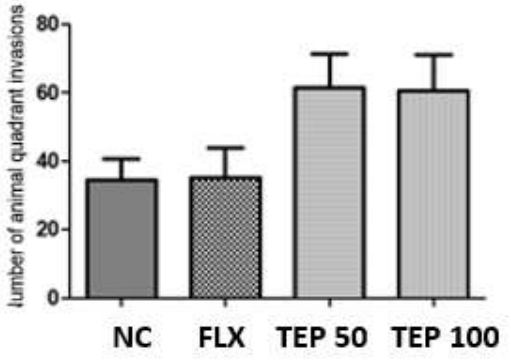
3.1.3 Open Field

Exploratory/locomotor activity and motor coordination were evaluated by the open field and Rota-rod tests, respectively. These tests are classic animal models to track the activity of the central nervous system and provide information on anxiety, depression, sedatives, seizures and myorelaxing activities (Diniz *et al*., 2019).

According to the information in Figure 8, the groups that received alpha-terpineol doses (50 mg/kg and 100 mg/kg) covered a greater number of quadrants in the time of 5 minutes (mean ± standard deviation 61.40 ± 22, 26 and 60.60 ± 23.37, respectively), in relation to the negative and positive control groups (Table 3). It is noticed that this monoterpene acted to improve the spontaneous locomotor activity of animals, similar to that observed in the action of conventional antidepressants. In addition, the means ± standard deviation of the number of withdrawals was also more significant in the groups treated with alpha-terpineol (Table 4).

In contrast, in the results by Abouhosseini Tabari, Hajizadeh Moghaddam, Maggi and Benelli (2018) the administration of *P. roseum* essential oil, diazepam and buspirone did not significantly change the general locomotor activity ($p > 0.05$), but the number of breeding and cleaning behavior was significantly reduced in diazepam, buspirone, *P. roseum* 20 mg, and mice treated with *P. roseum* 50 mg. That is, the exploratory activity of the rodents was not satisfactory, as identified with alpha-terpineol.

Figure 8. Number of animal quadrant invasions in the Open Field Test.



Subtitle:

NC: Negative Control

FLX: Fluoxetine 20 mg/kg

TEP 50: Alpha-Terpineol 50 mg/kg

TEP100: Alpha-Terpineol 100 mg/kg

The results were expressed as mean ± standard deviation of the number of invasions of animals in the Open Field test, by groups. In this protocol there was no statistical difference between the groups tested. Statistical analysis was performed using the ANOVA variance test, followed by the Tukey test
Source: Personal archive (2021).

Table 3. Average values of invasions per quadrant of animals in the Open Field Test.

GROUPS	AVERAGE ± STANDARD DEVIATION
Negative Control	34.60 ± 13.61
Fluoxetine	35.20 ± 19.24
Alpha-Terpineol 50 mg/kg	61.40 ± 22.26
Alpha-Terpineol 100 mg/kg	60.60 ± 23.37

Source: Personal archive (2021).

Table 4. Average values of surveys of animals in the Open Field Test.

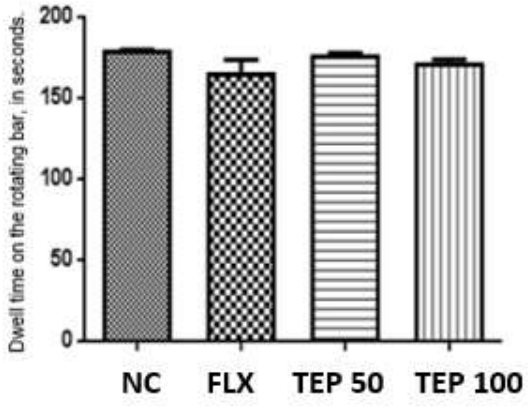
GROUPS	AVERAGE ± STANDARD DEVIATION
Negative Control	15.00 ± 6.68
Fluoxetine	24.00 ± 8.67
Alpha-Terpineol 50 mg/kg	40.00 ± 18.12
Alpha-Terpineol 100 mg/kg	29.00 ± 15.52

Source: Personal archive (2021).

3.1.4 Rota-rod

The findings found in the rota-rod test, shown in Figure 9 and Table 5, revealed that there was no statistical difference between the negative control, positive control (fluoxetine), TEP 50 mg/kg and TEP 100 mg/kg groups. Thus, the tested substance did not cause loss of performance and motor coordination of the animals. This response is of paramount importance in relation to the effectiveness of alpha-terpineol and the adverse effects that it could cause.

Figure 9. Animal dwell time, in seconds, on the rotating bar.



Subtitle:

NC: Negative Control

FLX: Fluoxetine 20 mg/kg

TEP 50: Alpha-Terpineol 50 mg/kg

TEP100: Alpha-Terpineol 100 mg/kg

Results were expressed as mean ± standard deviation of the time (s) on the rotating bar during the Rota-Rod test, by groups. In this protocol there was no statistical difference between the groups tested. Statistical analysis was performed using the ANOVA variance test, followed by the Tukey test
Source: Personal archive (2021).

Table 5. Average values of residence time on the rotating bar of the Rota-Rod Test.

GROUPS	AVERAGE ± STANDARD DEVIATION
Negative Control	179.50 ± 0.76
Fluoxetine	165.80 ± 17.17
Alpha-Terpineol 50 mg/kg	176.80 ± 5.90
Alpha-Terpineol 100 mg/kg	172.00 ± 70.00

Source: Personal archive (2021).

In this perspective, the results of the research by Diniz *et al*. (2019) also demonstrated that during the Rota-rod test there was no change in motor coordination of animals treated with EO (Essential Oil) and EO-βCD (essential oil with inclusion complex in β-cyclodextrin), of the species *Annona vepretorum*, in all doses tested. In contrast, diazepam significantly decreased the time spent on the bar when compared to the negative control. Thus, no loss of performance and motor coordination in the rota-rod test was observed with any of the doses of essential oil and in the complex, suggesting that the

essential oil has anxiolytic and sedative effects without showing loss of reflex, a common side effect of benzodiazepines observed with diazepam in this experiment.

In addition, the absence of adverse drug combination effects is also suggested by the results of the rota-rod test, which is used as a standard test for estimating motor coordination. This worked showed that ketamine at a dose of 3 mg/kg, injected separately or in combination with LY341495 (0.3 mg/kg), did not significantly alter the motor coordination of rats (Podkowa, Pochwat, Brański, Pilc, & Pałucha-Poniewiera, 2016).

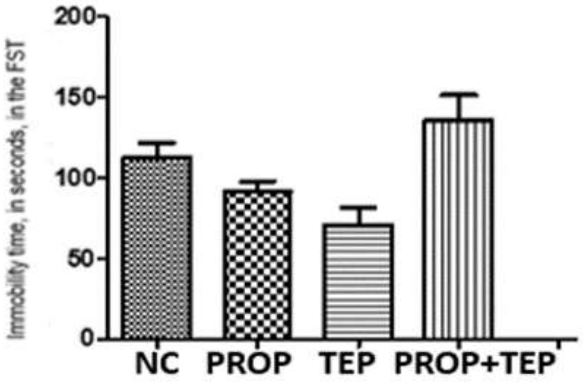
However, another study showed that the administration of *Nymphaea Lotus* extract at 180 mg/kg in mice increased the freezing time and reduced the total crossing, indicating that the substance may have sedative or muscle relaxant activities. When combined with the lack of motor coordination observed in the rota-rod test, this seems consistent (Fajemiroye, Adam, Zjawiony Jordan, Alves, & Aderoju, 2018).

3.2 Involvement of the Noradrenergic System

3.2.1 Propranolol 2 mg/kg

The results indicate that Propranolol, a beta-adrenergic blocker, when associated with alpha-terpineol 50 mg/kg increases the immobility time of animals in FST (Figure 10). It is argued that the drug acts by reducing the antidepressant activity in terpineol by blocking beta-adrenergic receptors, preventing the monoterpene from interacting with them, increasing the values of mean ± standard deviation, when compared to the other groups (Table 6).

Figure 10. Immobility time of animals, in seconds, in the Forced Swim Test treated with Propranolol 2 mg/kg.



Subtitle:

NC: Negative Control

PROP: Propranol 2 mg/kg

TEP: Alpha-Terpineol 50 mg/kg

The results were expressed as mean ± standard deviation of immobility time in the Forced Swimming test, by groups. In this protocol there was no statistical difference between the groups tested. Statistical analysis was performed using the ANOVA variance test, followed by the Tukey test.
Source: Personal archive (2021).

Table 6. Mean values of the animals' immobility time, in seconds, in the Forced Swim Test with Propranolol 2 mg/kg

GROUPS	AVERAGE ± STANDARD DEVIATION
Negative Control	112.40 ± 20.40
Propranolol 2 mg/kg	91.40 ± 14.60
Alpha-Terpineol 50 mg/kg	70.80 ± 24.00
Propranolol + Alpha-Terpineol 50 mg/kg	136.00 ± 34.60

Source: Personal archive (2021).

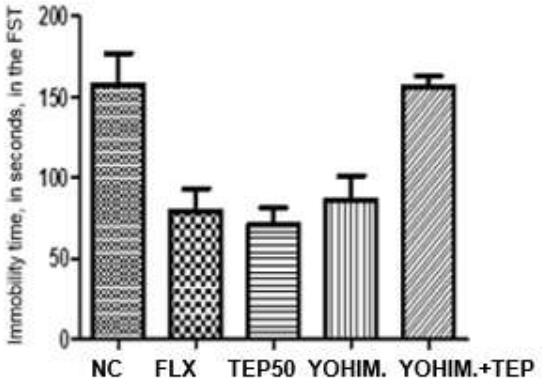
Propranolol or propranolol hydrochloride, chemically recognized as 1- isopropylamino-3- (nafityloxy) -2-propanol, is a non-selective β-adrenergic antagonist drug that interacts with β1 and β2 receptors with the same affinity (Godoy, Kummrow, & Pamplin, 2015).

Propranolol is a non-selective beta-adrenoreceptor antagonist, also classified as a class II antiarrhythmic agent. It exerts its response by competitively blocking beta-1 and beta-2 adrenergic stimulation in the heart, which is typically induced by epinephrine and norepinephrine (Rehsia & Dhalla, 2010).

3.2.2 Yohimbine 1 mg/kg

According to the results, there was no statistical difference between the groups that received Fluoxetine, Yohimbine 1mg/kg and Yohimbine combined with Terpineol 50 mg/kg compared to the control group (Figure 11). However, it was observed that this association increased the immobility time of mice treated with alpha-terpineol, suggesting that terpineol acts on alpha 2 adrenergic (α2-adrenergic) receptors. Thus, when yohimbine blocks these receptors, terpineol does not bind to them, ceasing to act as a possible antidepressant.

Figure 11. Time of immobility of the animals, in seconds, in the Forced Swim Test treated with Yohimbine 1mg/kg.



Subtitle:

NC: Negative Control

FLX: Fluoxetine 20 mg/kg

TEP 50: Alpha-Terpineol 50 mg/kg

YOHIM: Yohimbine 1mg/kg

The results were expressed as mean ± standard deviation of immobility time in the Forced Swimming test, by groups. In this protocol there was no statistical difference between the groups tested. Statistical analysis was performed using the ANOVA variance test, followed by the Tukey test.
Source: Personal archive (2021).

In this sense, the values of the mean ± standard deviation of the combination Yohimbine + Terpineol 50 mg/kg were similar to the negative control (Table 7). This demonstrates that the animals spent more time immobile and with depressive behavior during FST.

Table 7. Mean values of the immobility time of the animals, in seconds, in the Forced Swim Test treated with Yohimbine 1 mg / kg.

GROUPS	AVERAGE ± STANDARD DEVIATION
Negative Control	157.20 ± 44.70
Fluoxetine	79.20 ± 21.30
Alpha-Terpineol 50 mg/kg	70.80 ± 24.00
Yohimbine 1 mg/kg	86.20 ± 34.20
Yohimbine + Terpineol 50 mg/kg	156.20 ±15.70

Source: Personal archive (2021).

Thus, a similar response was observed in pretreatment with yohimbine (α_2 adrenoceptor antagonist), which blocked the effect of the extract of the species *Mangifera indica* (Anacardiaceae) of the antidepressant type, suggesting the involvement of the pre-synaptic α_2 autoreceptor activity in its effect (Ishola, Awodele, & Eluogu, 2016). This same effect was shown in the studies by Savegnago *et al*. (2007) and Posser *et al*. (2009), in which the antidepressant activity of some organocalcogens, such as diphenyl diselenide and ebselen, was blocked with previous an///tagonist treatment, such as prazosin and yohimbine.

Outside FST, researchers realized that the decrease in exploration in the Labyrinth Test and the increase in immobility in OFT of mice treated with yohimbine is characterized as reminiscent of the freezing/anxiety behavior. When associated with fear, it led them to remain without further exploration. In addition, yohimbine produced a clear anxiogenic effect in the open field, plate with orifice and chiaroscuro tests (Pitzer, La Porta, Treede, & Tappe-Theodor, 2019).

4. Conclusion

Thus, alpha-terpineol showed favorable responses as an antidepressant agent, this effect being similar to that of the reference antidepressants already used in clinical practice. Based on the investigation of the noradrenergic pathway, the responses presented by the testing of mechanisms with propranolol and yohimbine were promising. Thus, to exert its antidepressant effect, alpha-terpineol can act by increasing the levels of norepinephrine in the body.

In this sense, it is possible to perceive the benefits caused by this monoterpene during the screening of experimental protocols. In view of this and other actions already described in the literature, such as anti-inflammatory, anticonvulsant and antioxidant, alpha-terpineol proved to be an excellent pharmacological candidate.

Finally, we suggest the investigation of tests that verify the action of alpha-terpineol in the body, as well as other mechanisms of action related to its activity, in order to alleviate the limitations brought about by the work. Furthermore, the next steps in this study are: to evaluate the possible side effects caused by alpha terpineol, its best dosage and form of presentation; in order to provide security for a probable clinical use in humans.

Acknowledgments

We would like to thank the Biotechnology and Biodiversity Research Center (NPBio-UESPI) for providing the space, animals, equipment and supplies needed for research and the Pharmaceutical Technology Center (NTF-UFPI) for carrying out part of the analysis.

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