Combination of the essential oil constituents \(\alpha\)-pinene and \(\beta\)-caryophyllene as a potentiator of trypanocidal action on \textit{Trypanosoma evansi}

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**Abstract**

The effect of the combination of \(\alpha\)-pinene and \(\beta\)-caryophyllene on the proliferation of the \textit{Trypanosoma evansi} was analyzed. These compounds were added individually and in combination at different concentrations to \textit{T. evansi} cultures, and the in vivo study used mice of 1.0 and 1.5 mL kg\(^{-1}\) of \(\alpha\)-pinene and \(\beta\)-caryophyllene individually and in combination against \textit{T. evansi}. In order to evaluate the in vitro safety, lymphocytes and VERO cells were assessed using tetrazolium salt (MTT) assay. The trypanocidal effect of \(\alpha\)-pinene and \(\beta\)-caryophyllene was observed in vitro when applied independently or combined at 0.5, 1 and 2% concentrations. The combination effect showed a faster trypanocidal effect when compared to chemotherapy (diminazene aceturate – D.A.). Concerning the in vivo evaluation, \(\alpha\)-pinene + \(\beta\)-caryophyllene at 1.0 mL kg\(^{-1}\) was able to extend animal longevity and showed 83.33% curative efficacy, a superior result when compared to D.A. (16.66%). The MTT assay showed that the assessed compounds do not present toxicity to used cells. Also, in vivo safety evaluation of \(\alpha\)-pinene + \(\beta\)-caryophyllene at 1.0 mL kg\(^{-1}\) not altered the aminotransferases levels in serum. Combination between \(\alpha\)-pinene and \(\beta\)-caryophyllene shows in vitro and in vivo trypanocidal activity against \textit{T. evansi}, in a safe dose.

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Introduction

The genus Achyrocline belongs to the Asteraceae family and comprises approximately 40 species including Achyrocline satureioides, popularly known as “macela” or “marcela” (Retta et al., 2012). This species is native to southeastern South America – especially found in Brazil, Uruguay and Argentina – and grows in sandy or stony soils, on hilly or plain terrain (De Souza et al., 2007). It is widely used in Brazil due to its digestive and anti-inflammatory action (Kadarian et al., 2002; Alaniz et al., 2010). Furthermore, A. satureioides is considered an antiprotozoal agent, since it counteracts Trypanosoma evansi (Baldissera et al., 2014). Studies about A. satureioides report its antiinflammatory, antifungal, antimicrobial, antioxidant and trypanocidal activity (Rojas de Arias et al., 1995; Carney et al., 2002; Arredondo et al., 2004; Vogt et al., 2010; Joray et al., 2011). Recently, we focused on the chemical composition of the essential oil extracted from A. satureioides and its potential activity against the trypanomastigote form of T. evansi (Baldissera et al., 2014). The gas chromatography–mass spectrometry (GC–MS) analysis detected 22 compounds, where α-pinene (36.19%) and β-caryophyllene (25.65%) were the major ones. The identification of plant-derived compounds for use as trypanocidal drugs has been widely investigated (Rodrigues et al., 2015). Although several studies have successfully identified plant extracts and/or purified compounds with trypanocidal activity, an effective alternative therapeutics for the treatment of T. evansi infection has not yet been developed.

In the absence of vaccines, chemotherapy plays an important role controlling trypanosomosis (surra) (Andrews et al., 2014). However, the chemotherapy currently available for the treatment of this disease is far from satisfactory. The treatment of surra relies on one drug mainly: diminazene aceturate (D.A.), which was introduced into clinical therapy over four decades ago. Nevertheless, this drug is ineffective for many animals (Peregrine and Mamman, 1993). Most of the drugs used for treating “surra” do not provide total control of the infection and are combined with recurrence and mortality rates (Da Silva et al., 2008). This situation usually happens when the trypanosomes pass through the blood–brain barrier. The central nervous system becomes a refuge area for T. evansi during the residual period of the drug in the circulation. D.A. does not cross the blood–brain barrier in sufficient quantity to kill all the parasites (Masocha et al., 2007). In many cases, the D.A. treatment may not be effective, inducing toxic effects on kidney and liver and the need of prolonged treatment (Spinosa et al., 1999). Given the limitations, there is an urge for new therapies to treat trypanosomosis.

Drug-resistant strains of the causative organisms have seriously impaired the therapeutic value of known drugs, increasing their risk of becoming obsolete (Mendoza-Martinez et al., 2015). In this context, intensive efforts have been made to find novel prototype compounds among natural and synthetic sources aiming the development of new drugs to treat this parasitic disease. Given this scenario, this study aims to investigate the in vitro and in vivo action of α-pinene and β-caryophyllene against T. evansi.

Material and methods

Compounds

α-Pinene and β-caryophyllene were supplied by Sigma-Aldrich Corporation (St. Louis, United States). These compounds presented purity of 99 and 98.5%, respectively.

Trypanosoma evansi isolate

This study was conducted in two consecutive experiments (in vitro and in vivo). The cryopreserved isolate of T. evansi used in these experiments was obtained from a naturally infected dog (Colpo et al., 2005). Two rats (Rb and Rb) were infected intraperitoneally with blood contaminated by trypomastigotes, which was kept cryopreserved in liquid nitrogen. This process was performed in order to obtain a large amount of viable parasites for in vitro tests (Rb), and to infect the mice in the experimental groups (Rb).

In vitro test

The culture medium for T. evansi was adapted from Baltz et al. (1985) as previously published by Baldissera et al. (2013). The protozoans were acquired from the rat infected with the T. evansi isolate (Rb). At 3 days post-infection, the rat showed high parasitemia (6.0 x 10⁶ trypomastigotes/µL) and it was anesthetized with isoflurane for blood collection by cardiac puncture. The blood was stored in tubes containing EDTA (ethylenediamine tetraacetic acid). For trypomastigomes separation, each 200 µL of blood was diluted in complete culture medium (DMEM) 1:1 (v/v), added to microcentrifuge tubes and centrifuged at 400 x g for 10 min at 25°C. The supernatant was removed and resuspended in culture medium and the number of parasites was counted in a Neubauer chamber.

The culture medium containing the parasites was distributed in microtiter plates (270 µL/well), and 25 µL of each compound were added (diluted in culture medium). For this assessment as well as for the evaluation of the combination effect, the α-pinene, the β-caryophyllene and the α-pinene + β-caryophyllene were used at concentrations of 0.5, 1.0 and 2.0%. A positive control (0.5% D.A.) was also adopted at the same volume (25 µL). 20 µL of each well was used to the count the number of parasites at 1, 3, 6, 9 and 12 hours after the onset of the experiment in the Neubauer chambers. Percentage of compounds was corrected with purification of standard.

In vivo test

Animal model

Fifty-six 60-day-old heterogenic conventional female outbred strain mice, weighing an average of 26.5 ± 0.9 g were used as the experimental model. The animals were maintained under controlled light and environment (12:12 h light-dark cycle, 23 ± 1°C, 70% relative humidity) with free access to commercial food and water. Experiments were carried out between 8 am and 5 pm. All animals were subjected to a period of 15 days for adaptation. All efforts were made to minimize animal suffering and to reduce the number of animals used in the experiments.
Experimental design and parasitemia estimation
The mice were assigned to nine groups (A–I), with six animals each. Group A consisted of uninfected and untreated animals (negative control); Group B: infected and untreated mice (positive control); Group C: animals infected and treated with α-pinene 1.0 mL kg⁻¹; Group D: animals infected and treated with α-pinene 1.5 mL kg⁻¹; Group E: animals infected and treated with β-caryophyllene 1.0 mL kg⁻¹; Group F: animals infected and treated with β-caryophyllene 1.5 mL kg⁻¹; Group G: animals infected and treated with α-pinene and β-caryophyllene 1.0 mL kg⁻¹ (0.5 mL kg⁻¹ for each compound); Group H: animals infected and treated with α-pinene and β-caryophyllene 1.5 mL kg⁻¹ (0.750 mL kg⁻¹ for each compound); Group I: animals infected and treated with D.A. The infected animal groups were inoculated intramuscularly with 0.02 mL of blood from Rb containing 2.0 × 10⁵ trypanosomes.

The D.A. was intramuscularly injected in a single dose of 3.5 mg kg⁻¹. At 1 h post-infection, α-pinene and β-caryophyllene were orally administered. A daily supply of α-pinene and β-caryophyllene was maintained for 5 days.

The peripheral blood from the tail of the rats was examined daily for scoring the degree of parasitemia. Each slide was prepared with fresh blood collected from the tail coccgeal vein, stained by the Romanowski method, and visualized at a magnification of 1000 × according to the method described by Da Silva et al. (2006). The mice were observed for up to 60 days.

Treatment efficacy
The number of mice that did not show clinical signs of T. evansi infection and did not die after treatment determined the treatment efficacy. Prepatent period, longevity and animal mortality were also observed.

Sample collections and molecular diagnosis
To prove that animals were free of T. evansi infection, on day 60 of the experiment, survival animals (groups A, C and I) were anesthetized with isoflurane in an anesthetic chamber for bleeding by cardiac puncture. The blood collected was placed in tubes with anticoagulant (EDTA). One animal was infected with T. evansi (same conditions such cited in experimental design), and was used as positive control for polymerase chain reaction (PCR) validation. Thereafter, animals were euthanized by decapitation following recommendations of the Ethics Committee. Therefore, the DNA extraction of samples was performed using the Purelink Genomic DNA (Invitrogen®) according recommendations of the manufacturer.

Conventional PCR was performed using primers specific for T. evansi: 21/22 mer with product size of 227 pb (fwd 5′ TGGAGAC-GACCTGAGCCTGACT 3′, rev 5′ TCTCTGAAAGCTCTTGCTCGCTT 3′) in a concentration of 0.25 μM each primer, 2.0 U of homed Taq DNA polymerase, 2 mm of MgCl₂, 200 μM of each DNTP. Reactions were submitted to initial denaturation at 94 °C (7 min), followed by 30 cycles composed of 3 steps: 94 °C (1 min), 58 °C (1 min) and 72 °C (1 min) with a final extension at 72 °C (5 min) according to Wufts et al. (1995). Amplified samples were analyzed by 1.5% agarose gel electrophoresis at 80 V, stained with GelRed™ (Biotium, Hayward, CA), and photographed in UV light.

Cytotoxicity assay
Lymphocytes culture
Samples of peripheral blood were supplied by the Clinical Analysis Laboratory of the Franciscan University Center. The project was approved by the Research Ethics Committee of the Franciscan University Center, CAAE: 31211214.4.0000.5306. Peripheral blood samples were collected from three apparently healthy volunteers (22–25 years old), who did not smoke, drink alcoholic beverages more than two times a week, or take prescription drugs. The samples were collected after 12 h of overnight fasting by venipuncture using a top Vacutainer® (BD Diagnostics, Plymouth, UK) and heparin tubes. Lymphocyte cells were separated by Histopaque-1077 (Sigma–Aldrich Co. (St. Louis, USA)) density gradient centrifugation using 4 mL of blood samples. After further centrifugation for 15 min at 2500 × g, cells were transferred to culture media containing 5 mL of RPMI 1640 supplemented with 10% fetal bovine serum, 1% penicillin and streptomycin. The cells were cultured in a 96-well microplate at an initial density of 2 × 10⁵ for 24, 48 and 72 h at 37 °C in a 5% humidified CO₂ atmosphere (Wilms et al., 2005).

VERO cell culture
Green monkey kidney (VERO) cells (Adolpho Lutz Institute, São Paulo, Brazil) (20–30 passages) were cultured in 96-well plates (Corning, USA) with E-MEM supplemented with 10% (v/v) fetal bovine serum (Gibco BRL), 1% (v/v) l-glutamine (200 mmol L⁻¹), 10 mg mL⁻¹ ciprofloxacin (Baytril, Bayer), and 0.025 g mL⁻¹ amphotericin B (Gibco BRL). The cells were maintained at 37 °C in a humidified incubator with a 5% CO₂ atmosphere.

Cell viability
The cytotoxicity of the α-pinene and β-caryophyllene against lymphocytes (non-adherent) and VERO cells (adherent) was evaluated as described by Sagrillo et al. (2015), using the tetrazolium salt MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide colorimetric method based on the cleavage of the reagent by dehydrogenases in viable cells (Mosman, 1983). In the microplates, cells were incubated at 37 °C in a humid atmosphere with 5% CO₂ for 24, 48 and 72 h. At the end of incubation, 50 μL of MTT solution was added to each well of the microplate and the cells were incubated for three more hours. The supernatant was removed and 200 μL of dimethyl sulfoxide (DMSO) were added to each well. The microplate was analyzed using an ELISA reader at a wavelength of 560 nm. As a control, cells were grown in a medium lacking the constituents. The experiment was performed in triplicate. Finally, we determined the cell viability, which was expressed as a percentage of the control value.

Safety evaluation of treatment with α-pinene + β-caryophyllene at 1.0 mL kg⁻¹
Twelve female mice were divided into two groups, with 6 animals each. Group A (untreated) and group B (treated with α-pinene + β-caryophyllene at dose of 1.0 mL kg⁻¹). The animals were maintained at the same laboratorial conditions described for in vivo test, and the group B was treated as described for in vivo test (treatment via oral during 5 days at dose of 0.500 mL kg⁻¹ for each compound). At day 6 after the beginning of experiment, the
mice were euthanized with isoﬂurane inside an anesthetic chamber to perform collection of blood samples (1 mL by intracardiac puncture). Total blood was collected in tubes without anticoagulant, centrifuged at 3000 × g during 10 min to obtain serum. The seric levels of ALT and AST were evaluated in a semi-automatic analyzer (TP Analyzes Plus, Thermoplate, China) using commercial kits (Labtest Diagnóstica S.A., Lagoa Santa, MG, Brazil). All tests were carried out in duplicate.

Statistical analysis

Data are expressed as mean and standard deviation. The bilateral two-way analysis of variance (ANOVA) or Student’s test (when appropriated) followed by the Bonferroni post hoc test was used at the significance level 2α = 0.05.

Results

In vitro test

The trypanocidal effect of α-pinene and β-caryophyllene on T. evansi was directly proportional to the concentration used, and better trypanocidal action was observed when compounds were combined (Fig. 1). A reduction of live trypomastigotes was observed at all concentrations when compared to the control.

![Graphs showing trypanocidal activity](image)

Fig. 1 – In vitro trypanocidal activity in 0.5, 1.0 and 2.0% of α-pinene (A), β-caryophyllene (B) and combination of α-pinene and β-caryophyllene (C) against Trypanosoma evansi when compared to control (untreated) and diminazene aceturate (trypanocidal drug). The results within a circle are not statistically different (p > 0.05), at the same time (h) according to the Bonferroni post hoc test.
group. After 6 h, there were no living trypomastigotes in 2% concentration. After 9 h of assay, there were also no living trypomastigotes in 0.5 and 1% concentrations as well as on the D.A. treatment (Fig. 1A).

The β-caryophyllene results are shown in Fig. 1B. Similarly, a reduction of live trypomastigotes was observed at all concentrations when compared to the control group. After 6 h, there were no living trypomastigotes in 2% concentration. After 9 h of assay, there were also no living trypomastigotes in 0.5 and 1% concentrations as well as on the D.A. treatment.

The results of the combination of α-pinene and β-caryophyllene are shown in Fig. 1C. The increase in trypanocidal effect is clearly observed since 1 and 2% α-pinene and β-caryophyllene completely eliminated the trypomastigotes forms after 6 h and 3 h of assay, respectively. This result was not observed when the compounds were not combined. After 9 h of assay, there were no living trypomastigotes in 0.5% concentration as well as on the D.A. treatment. A contrary, in control samples the parasites were all alive, what validates our experiment.

In vivo test

There were no differences between groups regarding the prepatent period (Table 1). Longevity of the group A (negative control) was exactly represented by the days that the experiment lasted (60 days). Mice from groups G and I lived for 51.2 and 30.2 days, respectively \( (p < 0.05) \). The combination of α-pinene + β-caryophyllene (group G) showed a better trypanocidal effect, increasing the longevity of the animals when compared to positive control (group B), and showed 83.33% of curative efficacy when compared to chemotherapy (D.A.), which showed 16.66% of curative efficacy \( (p < 0.05) \).

Molecular diagnosis

The molecular analysis was performed to confirm the effectiveness of the therapeutic protocol used, since parasitemia was not detected in blood smear the survivor animals in groups G and I. But in conventional PCR assays from blood in the survivor animals were positive for the presence of \( T. evansi \) (Fig. 2).

Cytotoxicity assay

In order to determine whether α-pinene and β-caryophyllene, individually or in combination, led to an increased cellular toxicity, the MTT assay for cell viability was used. As shown in Table 2, α-pinene and β-caryophyllene did not cause cytotoxicity at the concentrations and conditions tested 24, 48 and 72 h after incubation in lymphocyte and VERO cell cultures \( (p > 0.05) \).

Safety evaluation of treatment with α-pinene + β-caryophyllene at 1.0 mL kg\(^{-1}\)

In order to determine the in vivo safety of α-pinene + β-caryophyllene at 1.0 mL kg\(^{-1}\) dose, seric levels of ALT and AST were evaluated. As shown in Fig. 3, ALT and AST levels did not differ between groups \( (p > 0.05) \), demonstrating the security of treatment for liver tissue on evaluated parameters.

<table>
<thead>
<tr>
<th>Groups (n = 6)</th>
<th>Treatment</th>
<th>Prepatent period (day)</th>
<th>Longevity (day)</th>
<th>Mortality (n)</th>
<th>Therapeutic success (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Negative control</td>
<td>–</td>
<td>60.0 (±0.0)</td>
<td>0/6</td>
<td>–</td>
</tr>
<tr>
<td>B</td>
<td>Positive control</td>
<td>1.0 (±0.0)</td>
<td>3.5 (±0.5)</td>
<td>6/6</td>
<td>0</td>
</tr>
<tr>
<td>C</td>
<td>α-Pinene 1.0 mL kg(^{-1})</td>
<td>1.5 (±0.5)</td>
<td>4.3 (±0.5)</td>
<td>6/6</td>
<td>0</td>
</tr>
<tr>
<td>D</td>
<td>α-Pinene 1.5 mL kg(^{-1})</td>
<td>1.7 (±0.5)</td>
<td>4.0 (±0.6)</td>
<td>6/6</td>
<td>0</td>
</tr>
<tr>
<td>E</td>
<td>β-Caryophyllene 1.0 mL kg(^{-1})</td>
<td>1.7 (±0.5)</td>
<td>4.8 (±0.9)</td>
<td>6/6</td>
<td>0</td>
</tr>
<tr>
<td>F</td>
<td>β-Caryophyllene 1.5 mL kg(^{-1})</td>
<td>1.7 (±0.6)</td>
<td>5.3 (±0.5)</td>
<td>6/6</td>
<td>0</td>
</tr>
<tr>
<td>G</td>
<td>α-Pinene 1.0 mL kg(^{-1}) + β-caryophyllene 1.0 mL kg(^{-1})</td>
<td>1.9 (±0.2)</td>
<td>51.2 (±8.5)</td>
<td>1/6</td>
<td>83.33</td>
</tr>
<tr>
<td>H</td>
<td>α-Pinene 1.5 mL kg(^{-1}) + β-caryophyllene 1.5 mL kg(^{-1})</td>
<td>1.5 (±0.5)</td>
<td>4.5 (±0.8)</td>
<td>6/6</td>
<td>0</td>
</tr>
<tr>
<td>I</td>
<td>D.A. (3.5 mg kg(^{-1}))</td>
<td>10.9 (±2.7)</td>
<td>30.2 (±3.0)</td>
<td>5/6</td>
<td>16.66</td>
</tr>
</tbody>
</table>

Statistically significant as compared with the positive control. The experiment lasted 60 days after infection.
pentostatin exhibited superior activity against effects. For instance, a combination of cordycepin and shown that many drug combinations raised the trypanocidal development of drug resistance are the main advantages of new compounds with anti-infection presents serious limitations. The need to identify Fig. 3 promising approaches to develop new anti-leishmanial and
Discussion

Drug combination currently constitutes one of the most promising approaches to develop new anti-leishmanial and trypanocidal formulations since the chemotherapy of T. evansi infection presents serious limitations. The need to identify new compounds with anti-Trypanosomal properties that are more effective and less toxic compared to conventional drugs motivated this study. Synergistic effect and decreased development of drug resistance are the main advantages of combination therapy (Pink et al., 2005). Several studies have shown that many drug combinations raise the trypanocidal effects. For instance, a combination of cordycepin and pentostatin exhibited superior activity against T. evansi in animal model when compared to the use of individual drugs (Dalla Rosa et al., 2013).

On the in vitro assessment, the trypanocidal activity against T. evansi was verified to be proportional to the dose applied for both tested compounds, individually or combined. Previous studies suggest that \( \alpha \)-pinene shows trypanocidal activity against Trypanosoma cruzi epimastigotes (IC\(_{50}\) 2.74 \( \mu \)g/mL) and amastigotes (IC\(_{50}\) 1.92 \( \mu \)g/mL) (Leal et al., 2013). Besides, \( \alpha \)-pinene presents leishmanicidal activity against Leishmania amazonensis promastigote (IC\(_{50}\) 19.7 \( \mu \)g/mL) and amastigote (IC\(_{50}\) 15.6 \( \mu \)g/mL) (Rodrigues et al., 2015). Moreover, researchers verified the trypanocidal action of \( \beta \)-caryophyllene against epimastigotes (IC\(_{50}\) 78.4 \( \mu \)M), trypomastigote (IC\(_{50}\) 1593 \( \mu \)M) and amastigote (IC\(_{50}\) 63.7 \( \mu \)M) of T. cruzi in vitro (Izumi et al., 2012). The combination between components was able to eliminate all parasites after 3 h post-incubation for 6 h for the isolated compounds. A similar result was reported by Azeredo and Soares (2013) using the combination of citral, eugenol and thymol against Crithidia fasciculata and T. cruzi in vitro.

Although the therapeutic protocol used with \( \alpha \)-pinene or \( \beta \)-caryophyllene did not present curative efficacy, an increase in longevity of animals treated with the combination of \( \alpha \)-pinene + \( \beta \)-caryophyllene was observed. In addition, this combination showed a higher curative efficacy (83.33%) when compared to chemotherapy with DA (16.66%). A study conducted by Izumi et al. (2012) described the synergism effect observed when \( \beta \)-caryophyllene and copalic acid were combined, resulting in an EC\(_{50}\) reduction of 25 and 11 times when compared to their individual usage, respectively. The mechanism of action of \( \beta \)-caryophyllene against T. cruzi is related to lipid peroxidation, leading to changes on cell membrane permeability and on mitochondrial potential (Izumi et al., 2012). According to Kolodziej and Kiderlen (2005), the leishmanicidal activity of \( \alpha \)-pinene is due to macrophages activation. The macrophages modify nitric oxide levels and cytokine levels. An important pathway that may be involved in the mechanism of anti-Leishmania activity could be the stimulation of NO production in the macrophages, which was considered for a long time to be the most effective mechanism involved (Gantt et al., 2001). Therefore, we suggest that this may be the mechanism.

![Fig. 3](image)

Fig. 3 – Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in serum of untreated mice (group A) and treated mice with of \( \alpha \)-pinene + \( \beta \)-caryophyllene at 1.0 mL kg \(^{-1}\) dose (group B) at day 6 after beginning of the treatment. *Statistically significant as compared with the control by Student t test.
responsible for the trypanocidal action and healing of animals infected by T. evansi.

In order to investigate the safety of α-pinene and β-caryophyllene on the mammalian cells, we evaluated the cytotoxicity of the compounds in human lymphocytes and VERO cells. α-pinene and β-caryophyllene exhibited a good safety profile for both cell types. Rodrigues et al. (2015) evaluated the cytotoxicity of α-pinene using the MTT assay against macrophages and red blood cells and reported that such compound does not cause cytotoxicity, as also observed in this study. Izumi et al. (2012) showed that β-caryophyllene does not cause cytotoxicity in vitro against LLCMK2 cells (monkey kidney cells) but is toxic for red blood cells. However, the β-caryophyllene is 30 times more toxic to the T. cruzi than to host cells. Also, we evaluated the in vivo security of treatment with trypanocidal action (group G). The seric aminotransferase have long been considered as sensitive indicators of hepatic damage (Molander et al., 1995). In necrosis or membrane damage, the enzymes are released into circulation and can be measured in plasma (Raza, 2011). Thus, elevated levels of seric enzymes are indicative of cellular leakage and loss of functional integrity of cellular damage. The AST and ALT levels in this study indicating the nontoxic effects of treatment on liver tissue, corroborating the in vitro results.

We can conclude that the combination between α-pinene and β-caryophyllene presents in vitro and in vivo potential in the treatment of trypanosomosis, due to their trypanocidal activity against T. evansi, while being safe for mammalian cells used in this study.

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