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Anxiolytic and antinociceptive-like effects of cinnamic alcohol by possible GABAergic pathway modulation: *In vivo* and *in silico* studies

Efeitos ansiolítico e antinociceptivo do álcool cinâmico por possível modulação da via GABAérgica: Estudos *in vivo* e *in sílico*

DOI:10.34117/ bjdv6n7-690

Recebimento dos originais: 27/06/2020
Aceitação para publicação: 27/07/2020

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ABSTRACT
Introduction: Cinnamic alcohol (CA) is a phenylpropanoid found in the bark of *Cinnamomum verum* J.Presl (cinnamon) an ancient spice. Our main objective was to evaluate the central effects of CA in anxiety and pain models using *in vivo* and *in silico* studies. **Methods:** initially, Swiss male mice (*Mus musculus*) were treated intraperitoneally with CA at 6.25, 12.5 and 25 mg/kg, and underwent rota rod, elevated plus-maze, and formalin induced nociception tests. **Results**: in the rota rod test, there was no change in the performance of the animals treated with CA (6.25, 12.5, 25 mg/kg), not indicating a myo-relaxant or sedative effect. In the elevated plus-maze test, CA (6.25, 12.5, 25 mg/kg), an increased the number of entries and the length of stay of the animals in the open arms was observed. In the formalin test, the CA-treated animals (6.25, 12.5, 25 mg/kg) presented reduced paw licking behavior in the first and second phase of the test. Finally, the *in silico* studies (docking and molecular dynamics) indicated a positive interaction between CA and the $GABA_A$ receptor. **Limitations:** This is a non-clinical study, so all data are preliminary. **Conclusions:** thus, the results suggest that CA has anxiolytic and antinociceptive-like effects in mice, probably due to GABAergic system modulation.

Keywords: Cinnamon, Docking studies, Natural product, Phenylpropanoids.

RESUMO
Introdução: o álcool cinâmico (CA) é um fenilpropanóide presente nas cascas da *Cinnamomum verum* J.Presl, conhecida como canela, uma das mais antigas especiarias. O objetivo principal foi avaliar os efeitos centrais do CA em modelos de ansiedade e dor utilizando estudos *in vivo* e *in sílico*. **Métodos**: inicialmente, camundongos machos (Mus musculus - Swiss) foram tratados, via intraperitoneal, com o CA 6.25, 12.5 e 25 mg/kg e submetidos aos testes do rota rod, labirinto em cruz elevado e nocicepção induzida por formalina. **Resultados:** no teste do rota rod, não houve alteração no desempenho dos animais tratados com o CA (6.25, 12.5, 25 mg/kg) que indicasse efeito miorrelaxante ou sedativo. No teste do labirinto em cruz elevado, o CA (6.25, 12.5, 25 mg/kg)

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aumentou o número de entradas e o tempo de permanência dos animais nos braços abertos. No teste da formalina, os animais tratados com o CA (6.25, 12.5, 25 mg/kg) reduziram o comportamento de lamber a pata na primeira e segunda fase do teste. Por fim, os estudos in silico (docking e dinâmica molecular) indicaram haver interação positiva entre o CA e o receptor GABAA. **Limitações**: este é um estudo não clínico, portanto todos os dados são preliminares. **Conclusão:** os resultados obtidos sugerem, portanto, que o CA apresenta efeitos ansiolítico e antinociceptivo-símiles, em camundongos, por provável modulação do sistema GABAérgico.

Palavras-chave: Canela, Docking, Fenilpropanóide, Produtos naturais.

1 INTRODUCTION

Anxiety disorders (AD) are the most prevalent mental disorders and a principal cause of psychosocial dysfunction in the world (American Psychiatric Association, 2013). The World Health Organization (WHO) states that 3.6% of the world's population suffers from some form of AD. A multicenter study conducted in six European countries has also assessed anxiety disorders and highlighted a new case prevalence of 13.6% (BRENTINI et al., 2018).

Pain is another CNS-associated behavioral condition of worldwide importance. According to the International Association for the Study of Pain (IASP), pain refers to an unpleasant sensory and emotional experience that may be related to a tissue injury or cognitive and emotional variables (LOESER; TREEDE, 2008; SIDDALL; TAYLOR; COUSINS, 1997). Chronic pain is estimated to affect 11-40% of the world population (Dahlhamer et al. 2018). Unfortunately, the anxiolytic (UZUN et al., 2010) and analgesic (PORRECA; OSSIPOV, 2009) drugs commonly used in clinical practice have adverse effects which both limit treatment efficacy and patient acceptance.

In this context, it is observed that foods rich in bioactive compounds could be a safer therapeutic alternative and in some cases more effective in preventing or treating anxiety and pain (SILVA et al., 2020; DHAWAN et al., 2001; NASCIMENTO-SILVA, 2019). In particular, essential oils and their chemical components have shown promising results in studies using animal models of anxiety and pain that reinforce the great neuro-pharmacological potential of these natural products (DOBETSBERGER; BUCHBAUER, 2011; LA ROCCA et al., 2017; SOBREIRA et al., 2019).

Cinnamic alcohol (CA) is one of the major phenylpropanoids (AMORIM; PESTANA; MENDES, 2017; GROSS; BOLKART; ZENK, 1968) found in the essential oil of the bark of *Cinnamomum verum* J.Presl (Family - Lauraceae Juss.) (ARCHER, 1988; KOKETSU et al., 1997), popularly known as cinnamon; one of the oldest known spices. However, CA can also be found naturally in other plants, and is most commonly used in cooking and folk medicine, as examples we have the *Ficus carica* Linnaeus (Fig tree) (GIBERNAU et al., 1997; VALLEJO; MARÍN; TOMÁS-

BARBERÁN, 2012), *Centella asiatica* Linnaeus (Asian sparkle) (BRINKHAUS et al., 2000) and *Rhodiola rosea* Linnaeus (Golden root) (VAN DIERMEN et al., 2009). Studies have demonstrated CA's antiepileptic, sedative, antidepressant (SAKINA; DANDIYA, 1990) anti-inflammatory (GUNAWARDENA et al., 2015; LIAO et al., 2012) and neuro-inflammation inhibiting effects (HO; CHANG; CHANG, 2013). The present study aimed to evaluate the central effects of CA in anxiety and pain models *in vivo* and through *in silico* studies.

2 MATERIAL AND METHODS

2.1 ANIMALS

Swiss male mice (*Mus musculus*) weighting of 25–30 g were housed in polypropylene cages under controlled temperature conditions (22 ± 2° C) and a 12h light/dark cycle, with free access to water and food. The mice were allowed to adapt to the laboratory conditions for at least 24h before testing. All of the experimental procedures were previously approved by the CEAR - Committee of Ethics in Animal Research at Federal University of Paraíba-Brazil, under certificate CEAR nº 3226070818.

2.2 DRUGS

All drugs were diluted/dissolved in saline and injected intraperitoneally (i.p.) in a total volume of 0.1 mL/10 g. Cinnamic alcohol (CA) was emulsified with Tween 80 (5% in saline solution). The control group received the vehicle (Tween 80 - 5% in saline solution). CA, diazepam, and morphine were purchased from Merck - Sigma Chemical Co. (USA).

2.3 IN VIVO TESTS

2.3.1 Rota rod test

In this test, the animals were pre-selected in a training session, 24 hours previous to the test, based on their capacity of staying on the rotating bar (at 10 rpm) for 1 minute. Groups of the pre-selected animals (n = 8) were treated (i.p.) with vehicle (control group: Tween 80 - 5%), diazepam (4 mg/kg), or CA (6.25, 12.5, and 25 mg/kg); and 60, 90, and 120 minutes later the animals were placed on the rotating bar to evaluate the time spent. For each animal, the time spent up to 3 minutes was registered (DUNHAM; MIYA, 1957; CARLINI, 1979).

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2.3.2 Elevated plus-maze test

Groups of mice (n = 8) were injected intraperitoneally (i.p.) with vehicle (control group: Tween 80 - 5%), diazepam (1 mg/kg), or CA (6.25, 12.5, and 25 mg/kg). After 1 hour, each animal was placed individually on the central platform of the apparatus, with the head turned to one of the closed arms. The behavioral parameters observed were: the number of entries and the time spent (in seconds) in the open arms (PELLOW et al., 1985).

2.3.3 Formalin test

Groups of animals (n = 8) received intraperitoneally (i.p.), vehicle (control group: Tween 80 - 5%), morphine (6 mg/kg), or CA (6.25, 12.5, and 25 mg/kg). After 1 hour each mouse received 20μL of 2.5% formalin diluted in distilled water in the subplantar region. The animals were then immediately placed in a glass box to record paw licking times. The nociceptive behavior was recorded in two phases after administration of formalin: phase 1 (0-5 min), and phase 2 (15-30 min.) (HUNSKAAR; FASMER; HOLE, 1985).

2.4 IN SILICO TESTS

2.4.1 Docking studies

The structure of cinnamic alcohol was used as input data for Marvin 14.9.1.0, 2014, ChemAxon (http://www .chemaxon.com). We used Standardizer Software [JChem 14.9.1.0, 2014; ChemAxon (http://www.chemaxon.com)] to canonize the structure, add hydrogens, perform aromatic conversions, clean the molecular graphing in three dimensions, and save the compounds in sdf format (IMRE et al., 2003).

For molecular docking studies we selected the $GABA_A$ receptor α subunit, under the PDB ID 4COF code. Initially, all water molecules and cofactors were removed from the crystal structure and the mean square root deviation (RMSD) of poses, indicating the degree of docking reliability was calculated. RMSD predicts the binding mode close to the experimental structure and is considered successful if the value is below 2.0 Å. A prediction value of binding affinity superior to the crystallographic ligand (PDB BEN - benzamidine) (MILLER; ARICESCU, 2014) is considered satisfactory. Docking was performed in the Molegro Virtual Docker v6.0 (MVD) program with the parameters predefined by the software. For the (ligand - protein) coupling procedure a GRID of 15 Å radius and 0.30 resolution covering the binding site was used as defined from a known ligand for each protein. A model was generated to perform matching with the characteristics expected between the ligand and the protein, using a heuristic search algorithm that combines differential evolution

with the crystallographic ligand (as a template). The cavity prediction algorithm (Moldock) and the Moldock scoring function were selected to obtain the results (THOMSEN; CHRISTENSEN, 2006). The results were visualized through the MVD software.

2.4.2 Molecular dynamics simulations

Molecular dynamics simulations were performed to estimate the flexibility of protein-ligand interactions using GROMACS 5.0software (ABRAHAM et al., 2015; BERENDSEN; VAN DER SPOEL; VAN DRUNEN, 1995). The ligand topology was prepared using the PRODRG topology generator (http://davapc1.bioch.dundee.ac.uk/cgi-bin/prodrg/submit.html) (SCHÜTTELKOPF; VAN AALTEN, 2004), applying the GROMOS43a1 force field. The protein topology was also prepared using GROMOS43a1 force field in GROMACS. Molecular dynamic simulations were performed using the SPC water model of an extended point charge in a dodecahedral box (BONDI, 1964). The system was neutralized by the addition of ions (Cl^- and Na^+), and minimized to remove bad contacts between complex molecules and solvent.

The system was also balanced at 300K using the 100ps V-rescale algorithm represented by NVT (constant number of particles, volume, and temperature), followed by equilibrium at 1 atm. pressure using the Parrinello-Rahman algorithm as NPT (numerical particles constant pressure and temperature) up to 100 ps. MD simulations were performed in 500.000 steps at 10 ns. To determine the flexibility of the structure and whether the complex was stable when near the experimental structure, the mean square root displacement (RMSD) of all alpha carbon atoms (Cα) was calculated relative to the starting structures. Residual fluctuations (RMSF) were also analyzed to understand the role of residues when near the receptor binding site. RMSD and RMSF plots were generated using Grace software (http://plasma-gate.weizmann.ac.il/Grace/); protein and ligands were visualized using UCSF Chimera (PETTERSEN et al., 2004).

2.5 STATISTICAL ANALYSIS

The *in vivo* data were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's test or unpaired *t* testing for comparison between the means. Data were expressed as means ± S.E.M. (standard error of the mean) and values were considered significant when presenting a level of significance (p) of less than 0.05.

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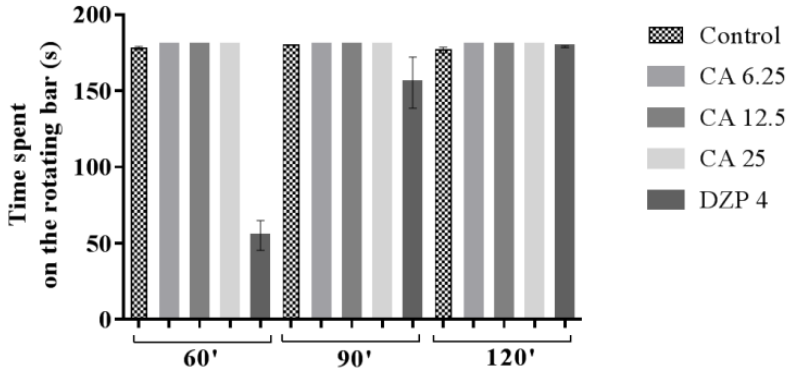
3 RESULTS

3.1 IN VIVO ANALYSIS

3.1.1 Rota rod

All CA-treated groups showed no significant change in the time spent on the rota rod as compared to the control animals (Figure 1).

Figure 1: Effect of CA on the time spent in the rota rod test after 60, 90 and 120 minutes. Mice were pretreated with vehicle (control), cinnamic alcohol (CA 6.25, 12.5, 25 mg/kg, i.p.), or diazepam (DZP 4 mg/kg, i.p.). Each column represents the mean ± S.E.M. (n = 8). Statistical analysis: one-way ANOVA, followed by Tukey's test. $p < 0.05$: (CA) vs. control, and (DZP) vs. control.



3.1.2 Elevated plus-maze

Intraperitoneal administration of CA 6.25 (4.8 ± 0.7), 12.5 (5.0 ± 0.6), and 25 mg/kg (7.0 ± 0.8) increased the number of open arm entrances as compared to the control group (2.3 ± 0.4) by 52.0%, 54.0%, and 67.1%, respectively (Figure 2a). CA 6.25 (41.1 ± 5.2), 12.5 (53.1 ± 9.6), and 25 mg/kg (75.8 ± 14.8) also increased the length of stay in the open arms as compared to the control group (18.3 ± 4.9) by 55.4%, 65.5%, and 75.8%, respectively (Figure 2b).

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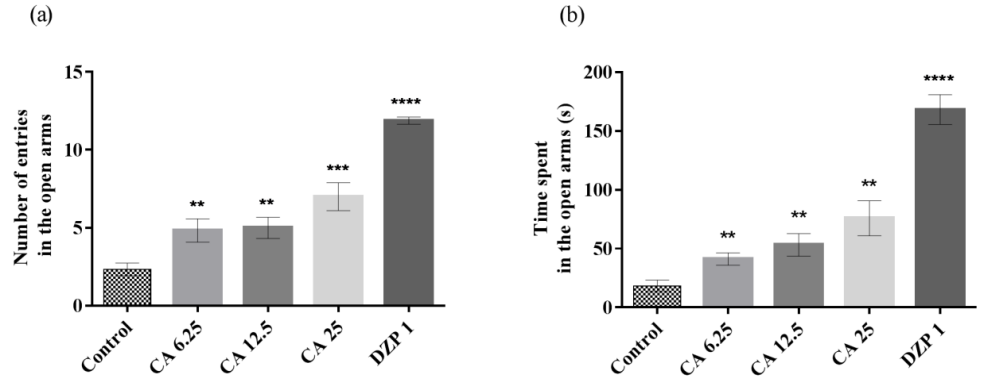
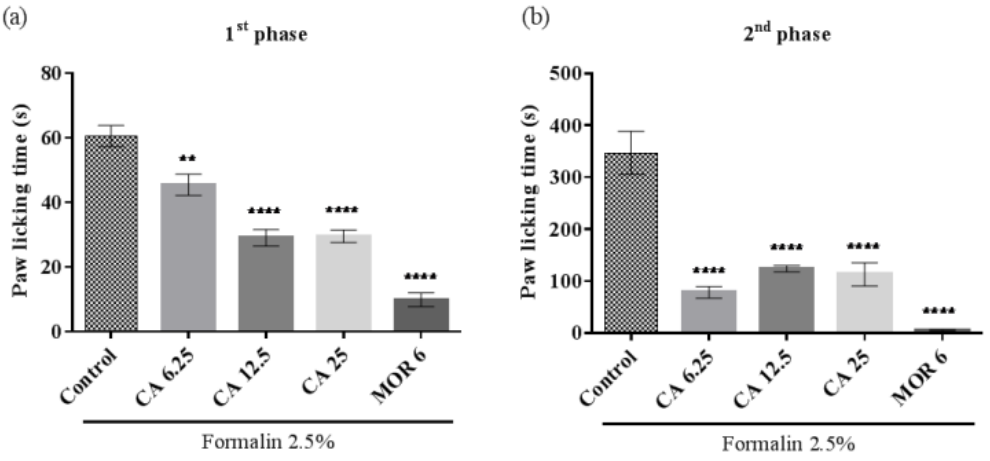
Figure 2. Effect of CA on the number of entries (a) and time spent (b) in the open arms. Mice were pretreated with vehicle (control), cinnamic alcohol (CA 6.25, 12.5, and 25 mg/kg, i.p.) or diazepam (DZP 1 mg/kg, i.p.). Each column represents the mean ± S.E.M. (n = 8). Statistical analysis: one-way ANOVA followed by Tukey's test. $p < 0.05$: (CA) vs. control, and (DZP) vs. control.



3.1.3 Formalin test

In the first phase of the test (Figure 3a) it was found that the animals treated with CA 6.25 (45.4 ± 3.2), 12.5 (29.0 ± 2.5), and 25 mg/kg (29.5 ± 1.9) significantly decreased paw licking time in the control group (60.5 ± 3.2) by 24.9%, 52.0%, and 51.2%, respectively. Decreased paw licking behavior was also observed in the second phase (Figure 3b) in animals treated with CA 6.25 (78.6 ± 11.2), 12.5 (124.3 ± 5.9), and 25 mg/kg (113.0 ± 22.4) compared to the control group (347.0 ± 41.5) at 77.3%, 64.1%, and 67.4% respectively.

Figure 3. Effect of CA on the paw licking time in the 1st phase (a) and the 2nd phase (b). Mice were pretreated with vehicle (control), cinnamic alcohol (CA 6.25, 12.5, and 25 mg/kg, i.p.) or morphine (MOR 6 mg/kg, i.p.) one hour before subplantar formalin administration (2.5%, 20 µL/paw). Each column represents the mean ± S.E.M. (n = 8). Statistical analysis: one-way ANOVA followed by Tukey's test. $p < 0.05$: (CA) vs. control and (MOR) vs. control.



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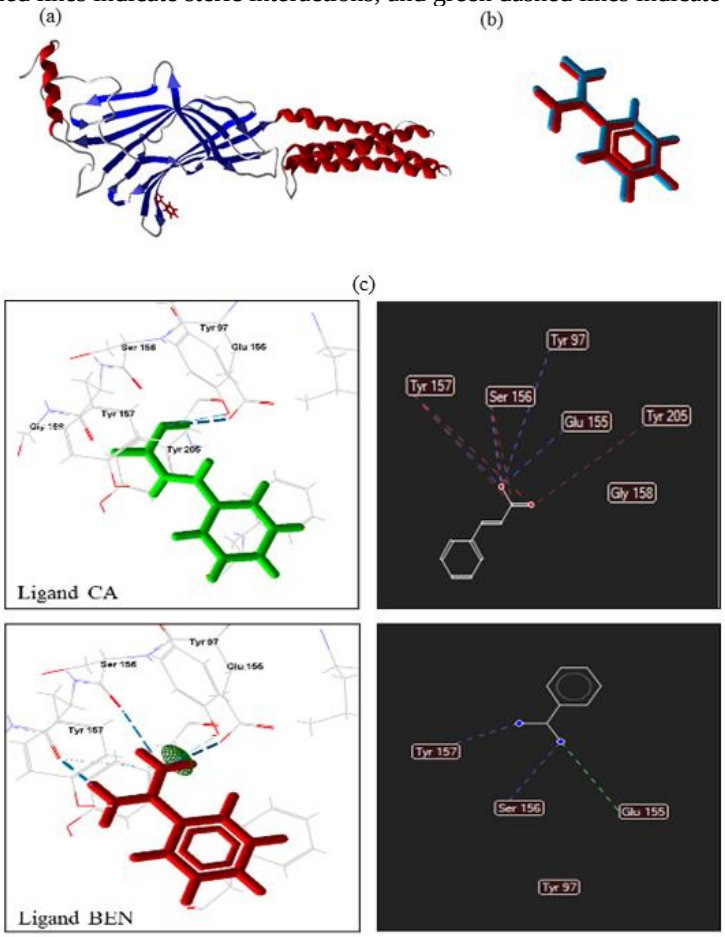
3.2 IN SILICO ANALYSIS

3.2.1 Cinnamic alcohol in docking studies

The results showed that the PDB BEN (benzamidine) ligand pose presented an RMSD of 0.15 Å, which indicates docking reliability. Figure 4 (a,b) shows the overlap of the redocking pose and the PDB ligand.

Figure 4. Protein-ligand complex (a), Redocking (b). RMSD of the BEN crystallographic ligand complexed to the GABAA receptor α subunit. Protein is complexed to its respective binder (blue and red), and overlaps of the best redocking pose (blue) and crystallographic binder (red). RMSD = root mean square deviation, (c). 3D and 2D interactions for the CA and BEN ligands at the GABAA receptor α-subunit active site. Blue dashed lines indicate hydrogen bonds, red dashed lines indicate steric interactions, and green dashed lines indicate electrostatic interactions.



The docking results showed that CA presents a predicted binding energy of -35.60 kcal/mol, and that the PDB BEN binder presents a predicted binding energy of -48.31 kcal/mol. Figure 4(c) presents the 3D and 2D interactions of the α-subunit of the $GABA_A$ receptor complexed with the ligands. CA was able to form four hydrogen bonds (Tyr97, Glu155, Ser156, and Tyr157) that stabilize the interaction with the active site. Steric interactions were also observed for the amino

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acids Ser156, Tyr157 and Tyr205. BEN formed hydrogen bonds with amino acids Ser156 and Tyr157, and an electrostatic interaction with amino acid Glu155.

3.2.2 Molecular dynamics

RMSD analysis for the $GABA_A$ receptor showed that within 10ns, the protein reached conformations ranging from 0.3 to 0.8nm in size. The results also show that the protein conformation changes drastically. This high conformational instability can be observed for both the non-complexed protein and for the BEN ligand complexed protein (Figure 5A). On the other hand, CA presented stability throughout the simulation (Figure 5B). However, when analyzed through the graphics programs, it was noted that CA loses interactions with the protein's active site after 150ps (Figure 6D). Figures 6 A, B, and C show the presence of the ligand in the active site for up to 100ps. The compound then begins to distance and yet remain within the system outside of the protein 10ns (Figures 6D, E and F). The BEN crystallographic ligand only reached stability after 4ns of simulation.

Figure 5(A). RMSD of GABAA receptor alpha carbon (Cα) atoms and (GABAA-CA and GABAA-BEN) complexes, (B). RMSD of CA and BEN ligand alpha (Cα) carbon atoms, (C). RMSF from GABAA receptor alpha carbon (Cα) atoms complexed to CA and BEN ligands.

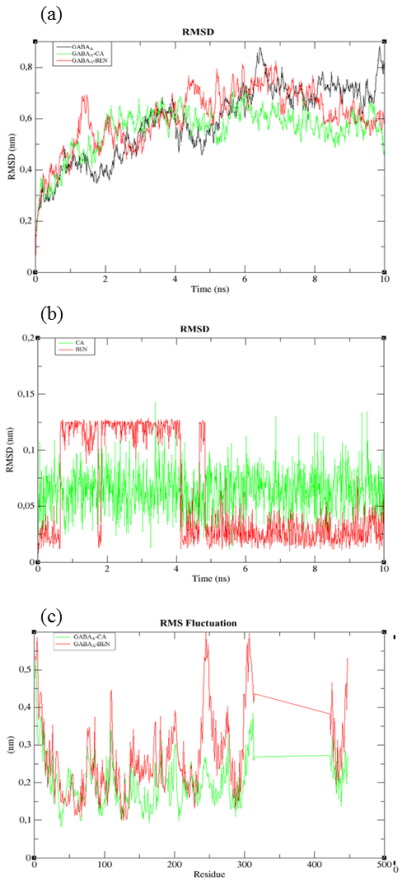

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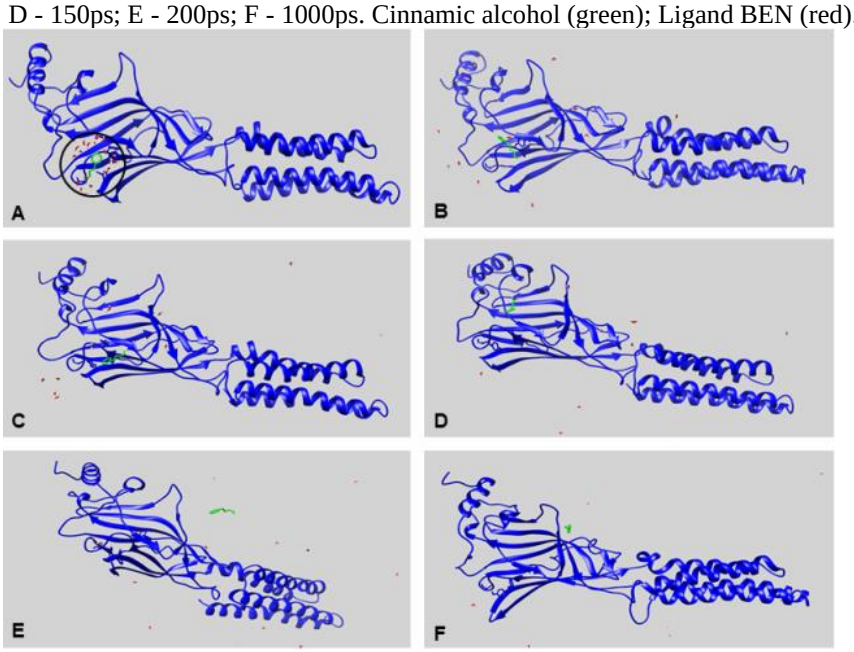
Figure 6. Molecular dynamic simulations lasting 1000ps. A - structure of the GABAA-CA complex and its active site; B - 50ps; C - 100ps; D - 150ps; E - 200ps; F - 1000ps. Cinnamic alcohol (green); Ligand BEN (red).



Considering that the amino acids with fluctuations greater than 0.3nm contribute to the flexibility of the protein structure, we found that of the 433 amino acids of the $GABA_A$ receptor α subunit, only 29 amino acids contributed to the conformational change of the $GABA_A$-CA complex, while 67 amino acids contributed to the structural change of the $GABA_A$-BEN complex. The main residues involved in protein structure flexibility were: 1-11, 13, 14, 21, 110, 277 and 307 (Figure 5c).

4 DISCUSSION

In the present study, no significant changes in the animals' residence times on the rota rod test were detected in relation to the control group; this indicates that CA did not promote myorelaxant or sedative effects. Then, specific anxiety and pain animal models were performed to assess whether CA presents anxiolytic and antinociceptive-like effects.

The elevated plus-maze is a widely used model for study of anxiety behavior in rats and mice (LEITE; SIQUEIRA, 2006) (SCHRADER et al., 2018). The test is based on the natural aversion that rodents have for open and elevated areas (WALF; FRYE, 2007). Therefore, substances that present anxiolytic activity in the animals tend also to increase both the number of entries and residence time in the open arms of the elevated plus-maze apparatus (PELLOW et al., 1985). Thus, animals treated with diazepam, enter the open arms more often and stay longer (CRAWLEY, 1981; SCHRADER et al., 2018). From our results, CA increased the number of entries and residence times

of mice in the open arms of the maze. Benzodiazepine-class drugs are known to have various pharmacological properties (DRUMMER, 2002). However, they also present adverse effects such as ataxia (LONGO; JOHNSON, 2000). Therefore, based on the respective results of the elevated plus-maze and rota rod tests, one may suggest that CA presents potential anxiolytic-like effects at low doses, with the advantage of presenting no adverse effects on the motor coordination of the animals.

Subsequently, to analyze the possible antinociceptive-like activity of CA, the formalin test was performed, a methodology widely used as a model of persistent pain (HUNSKAAR; HOLE, 1987; TJØLSEN et al., 1992). The parameter recorded in this test consists of intense licking activity for the paw that receives the noxious stimulus; in two distinct periods. The first phase, lasting about 5 minutes, begins immediately after formalin administration. The second phase occurs from 15 to 30 minutes after the administration.

These test phases have distinct nociceptive mechanisms, the first is characterized by a neurogenic or acute response, and the second phase involves inflammatory responses[43]. Thus, the behavioral states follow a biphasic pattern. The initial phase is characterized by acute pain related to direct stimulation of nociceptors (usually C fibers), mainly by glutamate, histamine, substance P, and nitric oxide. During the second phase the animal exhibits tonic pain behavior due to central sensitization of the dorsal horn of the medulla, accompanied by an inflammatory response mediated by cellular release of proinflammatory agents (TNFα, interleukins, bradykinin, prostaglandins, serotonin) (HUNSKAAR; HOLE, 1987).

Opioid analgesics are effective in both phases, while non-steroidal anti-inflammatory drugs with analgesic profiles suppress only the second phase (MALMBERG; YAKSH, 1992; SHIBATA et al., 1989). Depending on the response in each phase, observation indicates whether the test substance has central or peripheral antinociceptive effects or both. The results indicated that CA reduced the paw licking time of the animals in both phases. The results therefore suggest that CA presents antinociceptive-like effect through both central and anti-inflammatory activity. This effect has been confirmed by studies demonstrating that CA is capable of inhibiting the production of important proinflammatory factors which mediate central and peripheral nociceptive responses, such as nitric oxide, interleukins, tumor necrosis factor alpha (TNFα), and prostaglandin E2 (PGE2) effects associated with suppression of iNOS, COX-2, and NFKB expression (GUNAWARDENA et al., 2015; LEE, 2009; LIAO et al., 2012).

Based on the results obtained in the *in vivo* behavioral studies, we sought to perform *in silico* analyses in order to investigate possible interactions of CA with $GABA_A$ receptors that would allow

us to propose, preliminarily, a mechanism of action for the anxiolytic and antinociceptive-like effects observed.

GABAA receptor is the most important promoter of biological inhibitory effects in the CNS mediated by GABAergic system (BURT; KAMATCHI, 1991; OLSEN, 2018). The $GABA_A$ receptor is a hetero-pentameric protein complex (with two α, two β, and one γ or δ subunit), which forms an aqueous central channel (ionophore) that allows selective passage of chloride (Cl-) anions; a result of binding-dependent activation of the GABA neurotransmitter (γ-aminobutyric acid) (NAKAMURA et al., 2015; OLSEN, 2018).

Docking is widely used to predict the most stable conformation between a ligand and a protein, contributing to the discovery of potentially therapeutic drugs. According to our results, CA has a predicted binding energy (-35.60 kcal/mol), close to that of the $GABA_A$ benzamidine receptor ligand (PDB BEN)(MILLER; ARICESCU, 2014). In this study, the docking results were validated by crystallographic ligand redocking and the mean square deviation (RMSD) of the poses obtained was calculated. Redocking consists of positioning and predicting the binding affinity of the crystallographic ligand in the active site region of the protein. RMSD compares and calculates the mean square root deviation of the poses obtained during redocking with the experimentally obtained ligand structure. Thus, for docking to be considered reliable, the RMSD value must be less than or equal to 2.0 Å. The results showed that the PDB BEN ligand pose has an RMSD of 0.15 Å, which indicates *docking* reliability.

To characterize the interactions between the $GABA_A$ receptor, and CA and the complexed crystallographic ligand (BEN); molecular dynamic (MD) simulations were performed. First, in order to study the flexibility and conformational changes in the complexes during MD simulation, the RMSD was calculated for the α-subunit atoms of the protein and separately for the structures of each ligand. In accordance with these analyses, it is suggested that CA presents biological activity in the GABAergic pathway, however in a very short time period.

Finally, to understand the flexibility of the residues and amino acids which contribute to $GABA_A$ receptor conformational change, the root mean square fluctuations (RMSF) of each amino acid of the receptor were also calculated. Most of the observed amino acids are part of the protein's alpha-helical secondary structure, protruding from the helical skeleton. This $GABA_A$ receptor region is rich in neutral, polar, and nonpolar amino acids such as Gln, Ser, Asn, Asp, Pro, Lys, Leu, and Val. Residues with high RMSF values suggest more flexibility; those with lower RMSF values reflect less flexibility. As compared to the $GABA_A$-BEN complex (of 67 amino acids), only 29 amino acids contribute to the conformational change in the $GABA_A$-CA complex. However, none

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of these amino acids make up part of the critical residues at the active site which contribute to binding affinity maintenance between complexes.

Substances which activate the $GABA_A$ receptor are able to counterbalance neuronal excitability in diverse processes (CZUCZWAR; PATSALOS, 2001; OLSEN, 2018). Inhibiting neuronal activity in cortical areas, the GABAergic system is known to be involved in suppression of both anxiety (BABAEV; PILETTI CHATAIN; KRUEGER-BURG, 2018; RUDOLPH; CRESTANI; MÖHLER, 2001) and pain perception (MALCANGIO; BOWERY, 1996). Studies in various animal models have shown that the use of GABA receptor agonists is antinociceptive. Increased GABAergic neurotransmission may promote analgesic effects by downward inhibition of spinal nociceptive neurons (JASMIN et al., 2003). In addition, peripheral administration of GABAergic agents or GABA transaminase inhibitors enhances the antinociceptive effect of opioid agents (BUCKETT, 1980). Thus, based on the *in silico* results, one may suggest that the anxiolytic and antinociceptive-like effects of CA, observed *in vivo*, are partly due to a possible interaction with $GABA_A$ receptors.

5 CONCLUSION

The present study demonstrated that cinnamic alcohol presents important activity such as anxiolytic and antinociceptive-like effect at the central nervous system level, and possibly through positive modulation of the GABAergic system. The findings suggest the medicinal value of cinnamic alcohol-rich foods such as cinnamon, which may well be a complementary therapeutic alternative for treatment of anxiety and pain.

LIMITATIONS

This is a non-clinical study, so all data are preliminary.

ACKNOWLEDGMENTS

This study was supported by funds from the Coordination for the Improvement of Higher Education Personnel (CAPES) and National Council for Scientific and Technological Development (CNPq).

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