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In vivo and in silico anti-inflammatory properties of the sesquiterpene valencene

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ABSTRACT

Valencene (VLN) is a sesquiterpene found in juices and essential oils of citrus species such as *Cyperus rotundus*. Considering the evidence that this species has anti-inflammatory effects, the present study aims to evaluate the anti-inflammatory activity of VLN in vivo and in silico. Swiss mice (n = 6) were orally treated according to their treatment groups as follows: VLN (10, 100 or 300 mg/kg), negative control (0.9% saline), and positive controls (indomethacin 25 mg/kg or promethazine 6 mg/kg). The anti-inflammatory activity was evaluated in murine models of acute and chronic inflammation. The inhibition of acute inflammation was evaluated in models of paw edema induced by different inflammatory agents (carrageenan, dextran, histamine, and arachidonic acid (AA)) and carrageenan-induced pleurisy and peritonitis. The modulation of chronic inflammation was evaluated in a granuloma model induced cotton pellets implantation. The interaction with inflammatory targets was evaluated in silico using molecular docking analysis. The administration of VLN to challenged mice significantly inhibited paw edema formation with no significant difference between the administered doses. The compound also reduced albumin extravasation, leukocyte recruitment, and the production of myeloperoxidase (MPO), IL-1β, and TNF-α in both pleural and peritoneal lavages. According to the mathematical-statistical model observed in silico analysis, this compound has favorable energy to interact with the cyclooxygenase enzyme (COX-2) and the histamine 1 (H1) receptor. Finally, animals treated with the sesquiterpene showed a reduction in both granuloma weight and concentration of total proteins in a chronic inflammation model. Given these findings, it is concluded that NLV presents promising pharmacological activity in murine models of acute and chronic inflammation.

1. Introduction

Terpenes are among the largest classes of natural compounds derived from the secondary metabolism of plants. This class of secondary metabolites is composed of compounds classified as hemiterpenes (C5), monoterpenes (C10), sesquiterpenes (C15), diterpenes (C20),

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sesterterpenes (C25), triterpenes (C30), and tetraterpenes (C40), according to their number of isoprene units [1,2]. Terpenes are commonly found in essential oils of aromatic plants, contributing to their biological properties, pleasant smell, and spicy flavor, which stimulate their use in the production of cosmetics, as well as food adjuvants.

The pharmacological properties of terpenes have been widely studied. It has been shown that due to their great structural diversity, terpenes present several biological activities, such as antiparasitic, antimicrobial, antitumor, antioxidant, antinociceptive and anti-inflammatory, encouraging the development of research with medicinal plants and isolated compounds for the development of new medicines [3]. In this context, previous research has shown that monoterpenes and sesquiterpenes have promising anti-inflammatory effects that result from the inhibition of mechanisms crucially associated with the development and perpetuation of the inflammatory response, including: production of inflammatory mediators (such as eicosanoids, vasoactive amines, cytokines, and chemokines), recruitment and activation of leukocytes to the inflammatory site, and activation of signaling pathways associated with pattern recognition receptors (PRRs), among others [4–7].

Valencene (VNL) is a sesquiterpene identified as major constituent of *Cyperus rotundus,* a plant species with anti-inflammatory, anti-allergic, antiviral, and antioxidant properties [8–12]. This compound has also been identified in juices and essential oils of citrus fruits such as orange and tangerine, potentially contributing to their medicinal properties [13,14]. Previous studies have shown that the topical use of VLN has antiseptic and anti-allergic properties associated with the reduction of dermal inflammatory lesions, highlighting the potential of this compound for the development of topical formulations [15,16].

Previous studies have shown that the biological activities (especially the anti-inflammatory and healing activities) of *Cyperus rotundus* essential oil, depend, at least partially, on the presence of aromatic compounds such as VLN and nootkatone (NTK). NTK is a bicyclic enone generated from the oxidation of VLN. This enone was found to inhibit the activity of enzymes such as superoxide dismutase (SOD), glutathione S-transferase (GST), cyclooxygenase 2 (COX-2), and nitric oxide synthase (iNOS), in addition to inhibiting the production of cytokines and chemokines, and therefore exerts promising anti-inflammatory effects [17,18].

Our research group recently demonstrated that NTK had anti-edematogenic effects and inhibited leukocyte recruitment through mechanisms involving decreased vascular permeability and inhibition of the production of MPO, IL-1β and TNF-α. Moreover, *in silico* analysis suggested that this compound could interfere with COX-2 activity and histamine H_1 receptor activation [19].

Therefore, considering the evidence that VLN may be an active principle of anti-inflammatory and healing species, the present study aims to evaluate the anti-inflammatory activity of this sesquiterpene in murine models of acute and chronic inflammation, as well as simulate its interaction with inflammatory targets *in silico*.

2. Materials and methods

2.1. Drugs and reagents

Valencene, all inflammatory agents (carrageenan, dextran, histamine, and arachidonic acid (AA)), ELISA kits and o-opnisidine (myeloperoxidase assay) were acquired from Sigma-Aldrich (NewYork, NY, USA); the kits for the quantification of albumin and total proteins were provided by Labtest (Lagoa Santa, MG, Brazil); ketamine was acquired from VETNIL (São Paulo, SP, Brazil), while xylazine was purchased from CEVA (São Paulo, SP, Brazil).

2.2. Animals

Swiss mice *(Mus musculus)* of both sexes weighing between 20 g and 30 g were randomly assigned into groups and kept in polypropylene cages in a room at 22 ± 3 °C, under a 12 h light/dark cycle, with free access to water and food (Labina, Purina®). The research was conducted according to the guidelines for animal testing (Nih Guide for the Care and Use of Laboratory Animals, Nih - National Institute of Health -USA, 1996; Federal Law No. 11,794/2008 and the National Council for Control of Animal Experimentation (CONCEA)). The protocols used in this study were approved by the review board of the animal experimentation ethics committee (CEUA) of the Regional University of Cariri (protocol number 100/2019.2).

2.3. Evaluation of anti-inflammatory activity

The effects of VLN on acute inflammation were evaluated using the following experimental models in mice: carrageenan-induced peritonitis, carrageenan-induced pleurisy, and paw edema induced by carrageenan, dextran, histamine, or AA. In each protocol, the mice received a single oral treatment 1 h before the challenge. These paw edema models were used to evaluate the development of a local inflammatory response, while the pleurisy and peritonitis models were used for the analysis of systemic inflammatory parameters. In the chronic inflammation protocol, the animals were treated daily for ten consecutive days, after receiving surgically a persistent stimulus (cotton pellets). This protocol induces the formation of granuloma, which has been widely used as a model for the evaluation of new drug candidates for chronic inflammation.

2.3.1. Assessment of the antiedematogenic activity

The animals (n = 6 per group) were randomly assigned into groups and treated orally with VLN (10, 100, or 300 mg/Kg), vehicle (0.9% saline, negative control) or control drugs (promethazine 10 mg/Kg or indomethacin 25 mg/Kg) 1 h before the challenge. Each challenge was performed though the injection of 20 μL of a 1% (w/v) solution containing an inflammatory agent (carrageenan, dextran, histamine, or AA) into the right hind paw. Equal volume of saline was administered into the left hind paw. The volume of both paws was measured at different time-points using a plethysmometer and the difference between the hind and left paws was determined. The results were expressed as a percentage of edema, considering the value of the untreated challenged group as 100% [20–22].

2.3.2. Carrageenan-induced peritonitis

The animals (n = 6) were treated orally with the vehicle or VLN (10 mg/Kg) 1 h before receiving an intraperitoneal injection (1 mL) of 1% carrageenan (challenge). Four hours after the challenge, these animals were euthanized by CO_2, the peritoneal cavity was washed with 3 mL of heparinized PBS (10 IU/mL), and the peritoneal lavage was collected for the quantification of total leukocytes, MPO, and total proteins [20]. Total leukocyte counts were performed using an automatic counter (SDH-20).

2.3.3. Carrageenan-induced pleurisy

The mice (n = 6) were pretreated orally as described previously and 1 h later, challenged through an intrathoracic injection of carrageenan (1%, i.p., 0.25 mL). Four hours following the carrageenan injection, the animals were anesthetized with ketamine (8 mg/kg) and xylazine (8 mg/kg) and euthanized by cervical displacement. The pleural cavity was washed with 1 mL of saline (0.9% containing Ethylenediamine tetra acetic acid - EDTA, Sigma, NY, USA). The pleural samples were centrifuged (5000 rpm, 5 min, at room temperature), and the supernatants were stored at − 80 °C for further analysis. The precipitate was resuspended with 1 mL of PBS, and total leukocytes were counted under optical microscopy after diluting pleural wash samples in Turk fluid (2% acetic acid) [20].

2.3.4. Quantification of MPO and total proteins

Samples of the peritoneal lavage were centrifuged at 6000 rpm for 2 min and the concentration of albumin in the supernatants was determined using a colorimetric test according to the manufacturer's instructions (Labtest, Lagoa Santa, Brazil). In this method, the absorption of bromocresol green, which specifically binds albumin, is proportional to the concentration of protein in the sample. An increase in the albumin levels was used as a parameter of protein extravasation due to increased vascular permeability. The readings were performed using a spectrophotometer with absorbance adjusted between 600 and 640 nm.

The concentrations of MPO in the supernatants of peritoneal lavages were determined as follows: An aliquot of 40 μL of the peritoneal wash was added to a test tube containing 1960 μL of the o-dianiside regent and H_2O_2 in PBS (PH = 6.0). The readings were performed using a spectrophotometer at a 450 nm wavelength.

2.3.5. Cytokine quantification

The concentrations of TNF-α and IL-1β in the supernatants of pleural lavages were analyzed using ELISA kits in accordance with the manufacturer's instructions (Invitrogen®). The readings were performed at 450 nm using a microplate reader (ASYS®, NY, USA) and data were obtained by interpolation from a standard curve and expressed in pg/mL.

2.3.6. Cotton pellet-induced granuloma

After being anesthetized with ketamine (80 mg/kg) and xylazine (20 mg/kg), mice (n = 6) had four cotton pellets (0.01 g each) implanted in the back through a small incision. Twenty-four hours later, the animals were treated orally with vehicle or VLN (10 mg/Kg) for ten consecutive days. On day 11th, the animals were euthanized, and the pellets, as well as the surrounding tissue, were removed, dried at 37 °C for 24 h, and weighed. The results were expressed as the difference between the final weight and the initial weight [23].

For total protein quantification, the pellets were placed in test tubes and homogenized with 1 mL of 0.9% saline solution. The concentration of total proteins was determined using a colorimetric kit (Labtest, Lagoa Santa, Brazil) based on the reaction of copper ions in alkaline medium, creating a violet-colored complex whose absorbance is proportional to the concentration of proteins in the sample. The readings were performed at 550 nm using a spectrophotometer.

2.4. In silico analysis of COX-2 inhibition and H_1 receptor antagonism

This study performed anchorage simulations for protein-ligands complexes, obtained at the protein data bank (PDB, ID. 1PXX and 3RZE). The complex structure was adjusted using a protein preparation tool provided by the Chimera package, the 3D ligand structures were obtained using the Corina structure generator® 3D, and energy minimization was achieved using the UCSF Chimera structure building module. The binding region was defined by a 10 × 10 × 10 box adjusted in the centroid of the co-crystallized ligand in the crystalline complex to explore a larger region binding structure. The docking analysis was performed using the UCSF Chimera and AutoDock Vina softwares, based on the global optimizer of local search iterate. Proteins and ligands were kept flexible during the coupling process. The selection of protein flexible residues was based on the active site at 4.0 Å of the co-crystallized ligands. The most favorable binding free energy was represented by the grouping of the RMSD positional results with no more than 1.0 Å. The final coupled complexes were analyzed using the Discovery Studio viewer 3.1 [24].

2.5. Statistical analysis

Data were analyzed by One-way ANOVA followed by Tukey's post hoc test, or T test, using Graphpad prism software version 7.00 (GraphPad, San Diego, CA, USA, 2016). Values are expressed as means ± S.E.M. Statistical significance was considered when $p < 0.05$.

3. Results

3.1. VLN inhibits paw edema induced by different inflammatory agents

Fig. 1 shows the effects of VLN on the development of paw edema induced by carrageenan (Fig. 1A-B) and dextran (Fig. 1C and D). The treatment with VLN at 10, 100 or 300 mg/Kg significantly reduced carrageenan-induced paw edema at all time points in comparison with vehicle pretreatment (Fig. 1A). An analysis of the area under the curve (Fig. 1B), showed that the animals treated with VLN at 300, 100 and 10 mg/kg presented reductions of 55.78%, 48.21% and 45.83%, respectively.

A similar phenomenon was observed in dextran-induced paw edema. As can be seen in Fig. 1C, the sesquiterpene significantly inhibited ($p < 0.0001$) paw edema development during the peak of the response induced by dextran (60–120 min after the challenge). AUC analysis shows that VNL reduced edema formation by 37.0.5%, 21.17%, and 29.83% at doses of 300, 100, and 10 mg/Kg, respectively, demonstrating antiedematogenic activity in this model.

To investigate potential mechanisms associated with VLN antiedematogenic activity, this study evaluated the effect of this sesquiterpene on the development of histamine or AA-induced paw edema, since this vasoactive amine and AA metabolites, in particular PGE_2, are crucially involved in vascular and cellular changes associated with this phenomenon. To continue our study, we chose the dose of 10 mg/Kg, since it was the lowest dose tested and does not present significant differences from the highest doses. The results show that the treatment with VLN significantly reduced ($p < 0.0001$) the edema during the peak of the action of histamine, as observed at 30 and 60 min after the challenge, with an inhibition percentage of 52.87% and 47%, 37%, respectively, compared to the untreated control (Fig. 2A). This phenomenon corroborates the events observed in dextran-induced paw edema.

The administration of arachidonic acid to untreated mice induced the formation of edema, which reached its maximum effect in 15 and 30 min, decreasing after 45 min. The pretreatment with VLN reduced significantly ($p < 0.0001$) in these time intervals and maintained the inhibitory phenomenon until 60 min ($p < 0.01$). Considering the AUC, a reduction of 41.1%, 51.1%, 47.3%, and 50.4% is observed for the times of 15, 30, 45, and 60 min, respectively, in comparison with the untreated challenged mice (Fig. 2B).

3.2. VLN inhibits carrageenan-induced peritonitis

To assess the effects of VLN on acute inflammation at the systemic level, we used a model of carrageenan-induced peritonitis. As shown in Fig. 3, pretreatment with IND and VLN significantly reduced leukocyte recruitment (48.9%; $p < 0.01$, Fig. 3A), as well as the concentrations of MPO (66.9%; $p < 0.001$) and albumin (52%; $p < 0.0001$) in the peritoneal lavage of mice compared to the vehicle pretreatment.

3.3. VLN inhibits leukocyte recruitment and cytokine production in carrageenan-induced pleurisy

In order to address potential mechanisms associated with the vascular and cellular effects of VLN, we analyzed the production of TNF and IL1-β in carrageenan-induced pleurisy (Fig. 4). The administration of VLN (10 mg/kg) to carrageenan-challenged mice inhibited the recruitment of leukocytes ($p < 0,01$, Fig. 4A), and deceased the concentrations of IL1-β ($p < 0,05$, Fig. 4B) and TNF-α ($P < 0001$, Fig. 4C), corroborating the effects of VLN on acute inflammation.

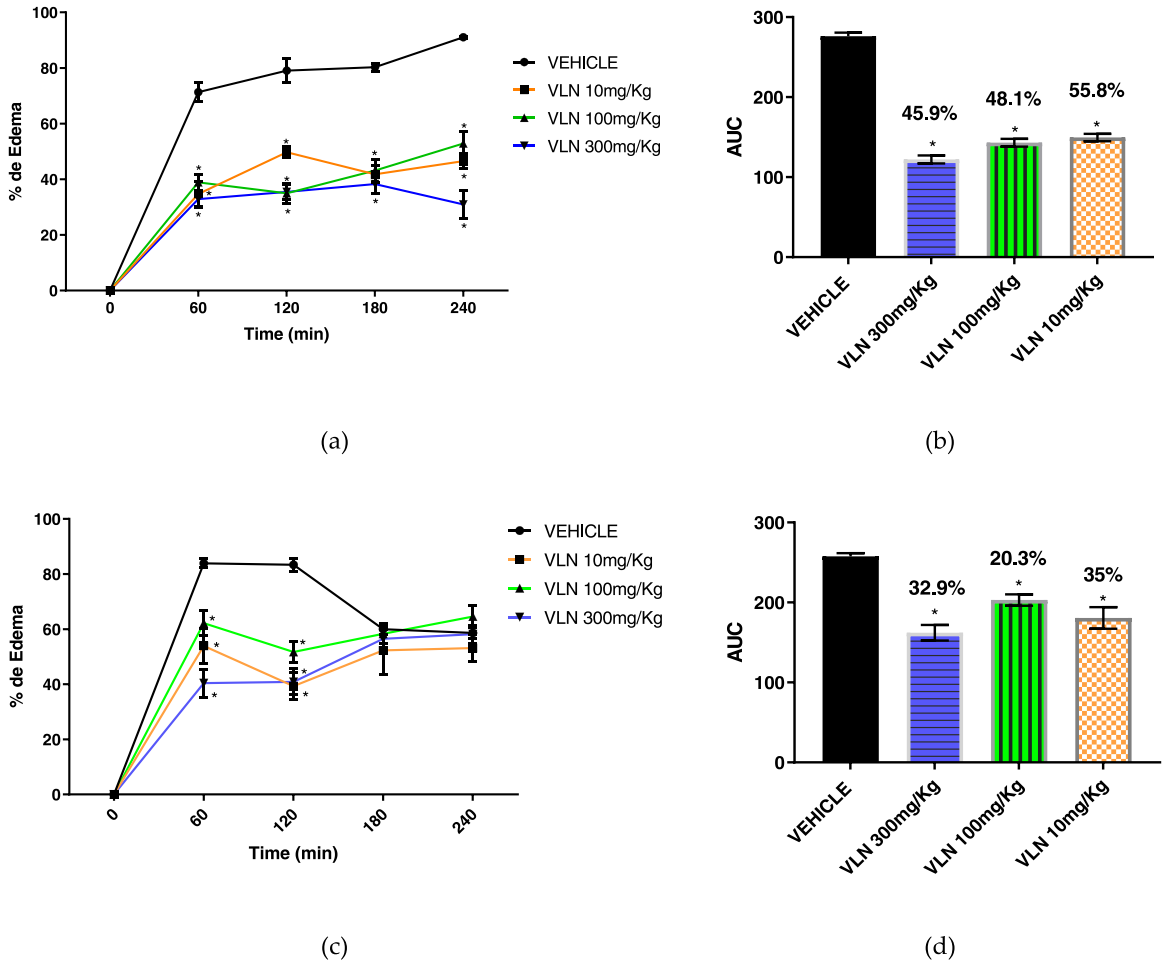

Fig. 1. Time-point analysis of treatment with different VLN doses on paw edema formation induced by carrageenan (**a**) or dextran (**c**) (n = 6 animals per group). These data are also represented as area under the curve (AUC) (**b** and **d**, respectively), showing the percent inhibition in relation to the untreated control. a4 = $p < 0.0001$ vs. Vehicle and a2 = $p < 0.01$ vs. vehicle. Statistical significance was determined with two-way ANOVA followed by Tukey's test.

3.4. Effects of VLN on cotton pellet-induced granuloma

Following the evaluation of the effects of VLN on acute inflammation, we investigated its effectiveness on granuloma formation, which is widely used as a chronic inflammation model in pharmacology. The administration of NTK (10 mg/Kg) significantly reduced both the granuloma weight ($p < 0.001$, Fig. 7A) and the concentration of total proteins ($p < 0.01$, Fig. 5B) in a magnitude of 21.1% and 32.15%, respectively, compared to the untreated mice (Fig. 7B).

3.5. In silico predictive interaction of VLN with COX-2 and H_1 receptor

The coupling protocols used in this study were validated by the mean-square deviation (RMSD) resulting from the comparison of the X-ray crystallography structures and the lower energy of the anchored structure. The differences between the structures of natural and anchored ligands were established at 0.80 Å and 0. 72 Å (for COX-2 and H1, respectively) demonstrating the accuracy of the coupling protocol. The binding score energies indicate a favorable interaction with a value range of − 8.6 kcal/mol for diclofenac and − 8.1 kcal/mol for VLN at the COX-2 binding site (Fig. 5A). Regarding the binding site in H1, the interaction energy with value of − 11 and − 8.0 kcal/mol for doxepin and VLN, respectively (Fig. 6A). Figs. 5B and 6D show the conformation of VLN and the control drugs (diclofenac and doxepin) with the best binding energy at the COX-2 and H_1 receptor binding sites, respectively.

The interaction maps between the ligands and the amino acids at the COX-2 binding site show two pockets of interaction. One of them has a hydrophobic characteristic formed by similar residues between the two interaction maps such as Tyr317, Gly495, Ser499, Leu321, Ala496, Val318 and Leu500 (Figs. 5B and 5D). On the other hand, only diclofenac formed hydrogen interactions with residues Ser-499 and Tyr-354 (Fig. 5C). There are 14 similar anchor residues responsible for stabilizing the COX-2/VLN complex through van der Waals, Alkyl and Alkyl-π interactions (Fig. 5E).

The present experimental findings demonstrate the involvement of eicosanoids and histamine in VLN-mediated anti-inflammatory effects. These findings suggest a potential interaction between VLN and the H1 receptor, due the existence of 10 similar anchor residues responsible for stabilizing the H1/VLN receptor complex. According to the interaction maps, such interaction occurs through Tyr108, Trp428, Phe432, Phe435 and Ala195 residues in both complexes (Figs. 6B and 6D) and is mediated by van der Waals, Alkyl, and Alkyl-π interactions (Fig. 6E). On the other hand, only doxepin showed Asp107-mediated hydrogen interactions (Fig. 6B). These findings suggest that alterations in the pharmacophoric structure of VLN could improve its interaction with others amino acids in the binding site.

The results demonstrated in the present research suggest that like NTK, investigated in our recently published work, VLN could interfere with inflammatory pathways stimulated by histamine and AA administration. In addition, since NTK is formed from the oxidative metabolism of VLN, it is hypothesized that the pharmacological properties of the precursor are conserved during the plant metabolism.

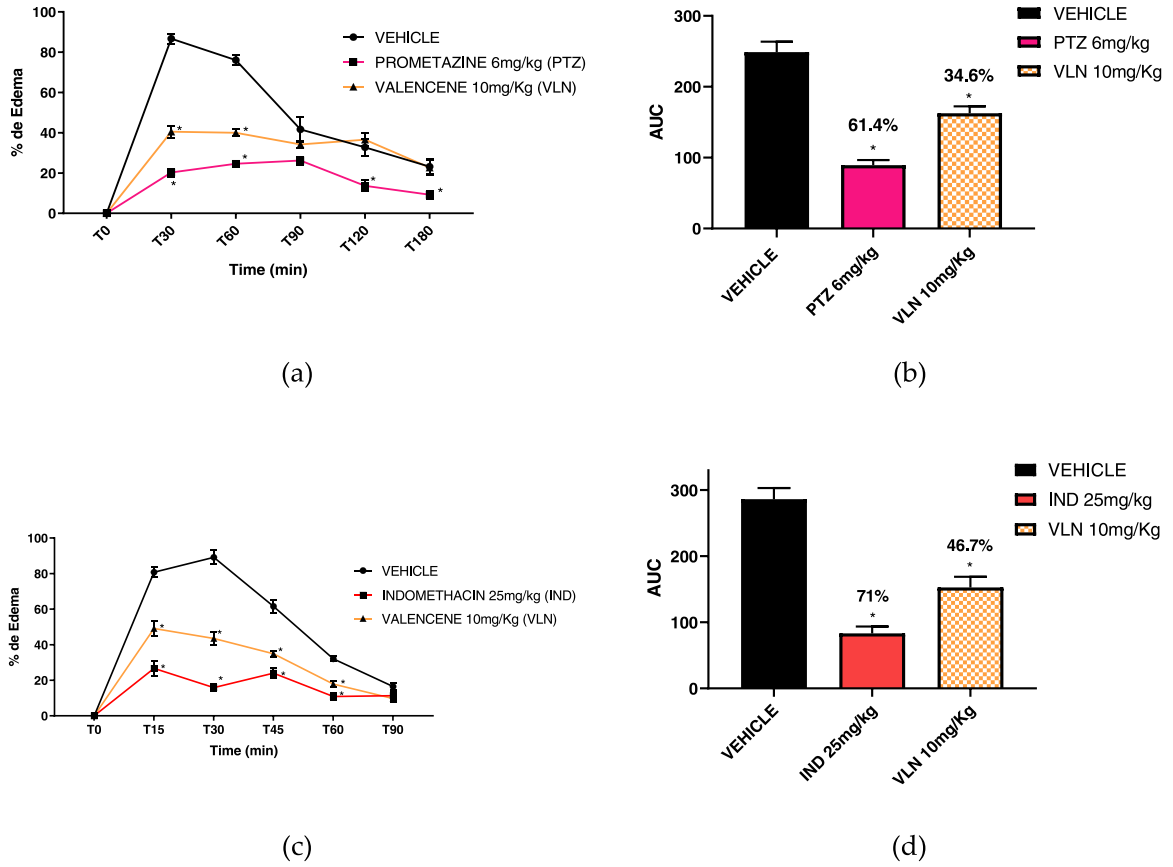

Fig. 2. The antiedematogenic action of VLN-mediated paw edema inhibition (n = 6 animals per group) induced by histamine (**a**) or arachidonic acid (**b**). The result is expressed as a percentage of edema induced in relation to the untreated control. Statistical significance was determined with two-way ANOVA followed by Tukey's test. a4 = $p < 0.0001$ vs. vehicle and a2 = $p < 0.01$ vs. vehicle.

4. Discussion

The present study evaluated the pharmacological properties of VLN in murine models of acute and chronic inflammation, demonstrating that this sesquiterpene has promising anti-inflammatory effects through the inhibition of edema formation, leukocyte recruitment and activation, and the production of inflammatory mediators. The results show that VLN is an orally active substance at a dose of 10 mg/Kg, which presented effects comparable to those observed with the administration of a dose up to 30-fold higher.

The antiedematogenic activity observed in a model of paw edema induced by carrageenan and dextran suggest a possible action of VLN in inflammatory pathways mediated by vasoactive amines and AA metabolites, since these mediators play crucial roles in the development of acute inflammation events [25,26]. To confirm our hypothesis, we induced paw edema through the administration of histamine and AA. Histamine is a major mediator in immediate reactions, stimulating the development of inflammatory edema via its H_1receptor [27]. On the other hand, AA-derived mediators such as PGE_2 and LTB_4 contribute significantly to a series of events that result in edema formation, hyperalgesia, and leukocyte recruitment [28,29]. The inhibitory effects demonstrated by VLN in these two models support the evidence of a possible interference of this sesquiterpene in the above-described inflammatory pathways. These results are corroborated by the in-silico data demonstrating a favorable interaction of VLN with both the histamine H_1 receptor and the COX-2 enzyme, indicating that the sesquiterpene could inhibit both the activation of H_1 receptors (acting as an antagonist) and the synthesis of prostaglandins (via COX-2 inhibition).

Previous research has identified similar mechanism of action for other sesquiterpenes [30,31]. An in vitro assay indicated that the anti-inflammatory effects of carvacrol result from COX-2 inhibition [32]; Tintino et al., 2018, demonstrated that β-caryophyllene presents antiedematogenic effects that are associated with the modulation of signaling pathways activated by histamine and AA [33]; NTK, which is metabolically related to VLN, was found to modulate neuroinflammation via inhibition of COX-2 activity [18]. The active site of COX-2 has a lipophilic interaction pocket that is important for the action of inhibitors, which favors the interaction of compounds with hydrophobic or aromatic groups, such as terpenes [34,35]. Similar interactions are also observed in VLN and doxepin interaction maps presented in this study, allowing us to raise the hypothesis that the VLN interferes simultaneously with the two pharmacological targets (H_1 and COX-2).

Earlier reports demonstrated that essential oils of species such as *Hyptis martiusii* [36] and *Croton rhamnifolioides* [37] have anti-inflammatory activities that, at least partially, dependent on H_1 receptor inhibition. Studies with terpenoid compounds isolated as safranal have shown that this compound possibly acts as a competitive antagonist of histamine H1 receptors [38]. The terpenoid pulegone showed an anti-histaminic effect that were comparable to those of mepyramine and dexchlorpheniramine [39]. Studies with isopulegone [40] and,8-cineole [41] indicated that these compounds can interfere with the biosynthesis of eicosanoid and the action of histamine.

Lin and colleagues [16] The sesquiterpenes VLN, NTK, and caryophyllene oxide-oxide inhibited the production of leukotrienes by RBL-2H3 cells, indicating inhibition of AA metabolism, possibly through a direct action on 5-lipoxygenase. The inhibitory effect of VLN on edema formation, leukocyte recruitment, and MPO production in the peritonitis model reinforces its action on eicosanoid production and histamine signaling since these mediators have a key role in increasing vascular

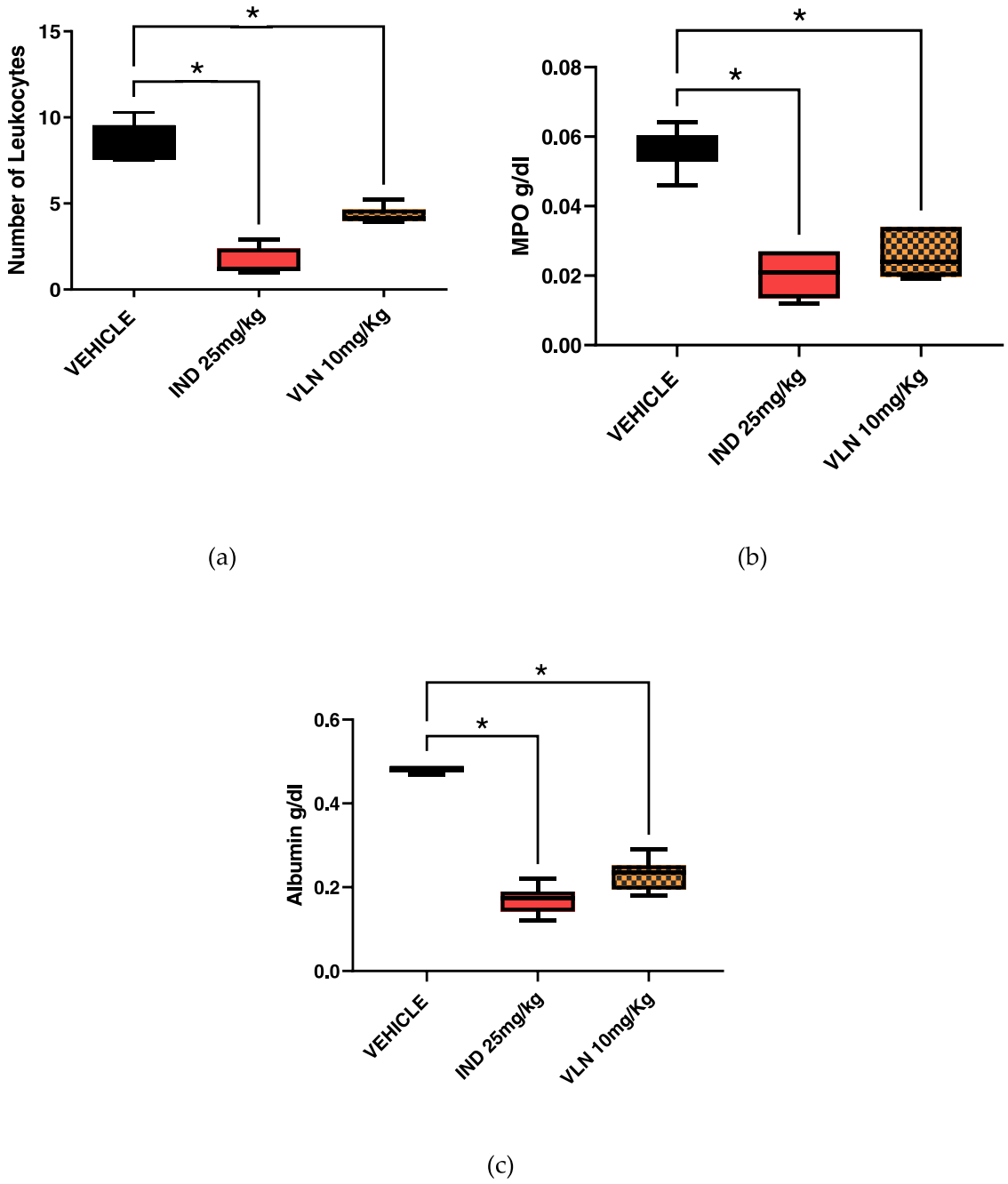

Fig. 3. The effects of VLN on carrageenan-induced peritonitis (n = 6 animals per group). **a**) The number of total leukocytes; **b**) concentrations of myeloperoxidase (MPO), and **c**) concentrations of albumin in the peritoneal fluid of mice. a4 = $p < 0.0001$ vs. vehicle; a3 = $p < 0.001$ vs. vehicle and a2 = $p < 0,01$ vs. vehicle. Statistical significance was determined with T test.

permeability and migration of leukocytes from the activation of adhesion molecules in both leukocytes and the endothelium [42]. This hypothesis is strengthened by evidence demonstrating that pretreatment with VLN inhibited the expression/production of I-CAM induced by TNF-α and interferon-gamma (IFN-γ) [43].

The inhibition of IL-1β and TNF-α production in a pleurisy model indicates an important mechanism by which VLN inhibits acute inflammatory responses. These cytokines are produced especially by activated macrophages and resident cells and activate signaling pathways in a wide variety of cells, with repercussions for increased vascular permeability, leukocyte migration, and many other inflammatory events [44]. In addition, evidence demonstrates that these cytokines can induce the expression of COX-2 [45], providing a link between the results obtained in the pleurisy assay and the other events observed in the acute inflammatory response.

Yang and collaborators (2016) used a dermatitis model to investigate the anti-allergic effects of VNL. The topical administration of this compound significantly was found to decrease the secretion of IgE in the serum, as well as inhibited the production of a variety of proinflammatory cytokines, including IL-1β, IL-6, and IL-13 in local skin lesions and spleen tissue. The authors also confirmed that VNL significantly decreased synthesis and expression of chemokines associated with dermatitis in keratinocyte lineage cells (HaCaT) stimulated with TNF-α and IFN-γ [43].

Following the characterization of VLN in acute inflammation, we attempted to evaluate its effects using a chronic model. Our results

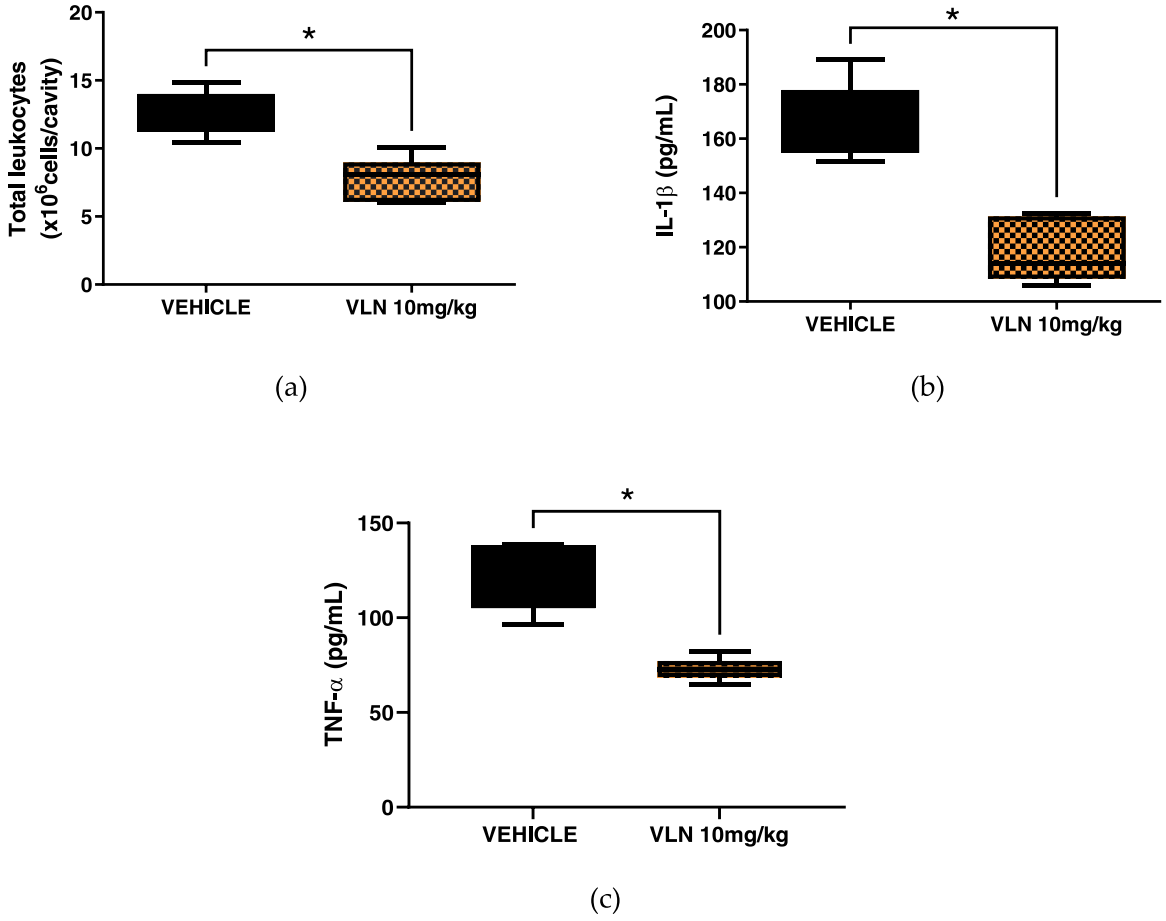

Fig. 4. The effects of VLN on carrageenan-induced pleurisy (n = 6 animals per group). **a**) The number of total leukocytes; **b**) concentrations of Interleukin (IL)− 1β, and **c**) concentrations of Tumor Necrosis Factor (TNF)-α in the pleural lavages of mice. A3 = $p < 0.001$ vs. vehicle; a2 = $p < 0.01$ vs. vehicle and a1 = $p < 0.05$ vs. vehicle. Statistical significance was determined with T test.

demonstrated that VLN treatment was effective in inhibiting granuloma formation. Marques and collaborators (2019) demonstrated that this compound inhibited the production of IL-1β, TNF-α and nitric oxide (NO) by RAW 264.7 macrophages, without affecting cell viability [6]. The same study showed that the compound inhibited the activation and migration of macrophages associated with the proliferative response in a granuloma model, corroborating the data of the present study. Accordingly, Yang and collaborators demonstrated that VLN inhibited the expression of the mRNA of IL-1β and IL-6 RAW 264.7 cells [43]. Additionally, Tsoyi et al. (2011) reported that the species *Cyperus rotundus* and its constituents VLN and NTK increased the expression of heme oxygenase-1 (HO-1) in addition to inhibiting iNOS expression and NO production, increasing the survival of septic mice [15], highlighting the therapeutic potential of VNL in chronic diseases in which the activation of macrophages plays an important pathophysiological role.

5. Conclusions

The results of the present study demonstrated that VLN has anti-inflammatory activity in a murine model of acute inflammation that is characterized by the inhibition of both edema formation and leukocyte migration and activation. These effects result, at least partially, from the inhibition of the production of MPO, IL-1β, and TNF-α. In addition, in silico analysis indicates the inhibition of COX-2 activity and histamine H1 receptor antagonism as potential mechanisms by which VLN modulates acute inflammation. The anti-inflammatory properties of VLN were preserved in a granuloma model, indicating a possible anti-proliferative effect. Nevertheless, the effects of this compound on chronic inflammation remain to be better understood and new assays using binding titration experiments and determination of the Kd value are necessary to establish the involvement of H_1 receptor and COX-2 as part of the anti-inflammatory mechanism by valencene. In conclusion, VLN inhibited acute and chronic inflammatory responses in mice, indicating that this sesquiterpene has therapeutic potential for the treatment of inflammatory diseases.

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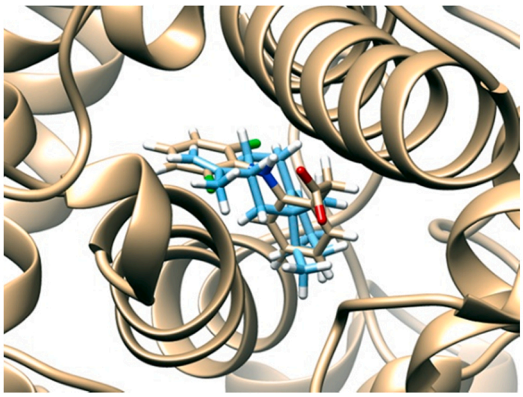
CRediT authorship contribution statement

L.B.R.D. and I.R.A.d.M.: Conceptualization. **I.S.A., T.M.D.**: Methodology. **J.R.F.**: Software. **A.O.B.P.B.M. and F.R.S.P.**: Validation. **M.R. C.d.O.**: Formal analysis. **C.P.S.Jr.**: Investigation. **B.K.**: Resources. **N.C. M. and J.R.G.S.d.A.**: Writing – original draft. **I.R.A.d.M. and L.J.Q.Jr.**: Supervision. **I.R.A.d.M., H.D.M.C. and B.K.**: Project administration. **B. K.**: Funding acquisition. All authors have read and agreed to the published version of the manuscript.



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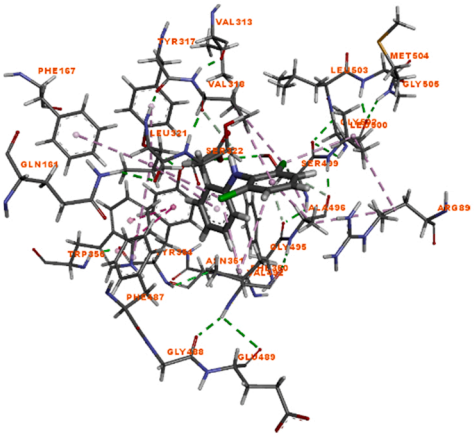

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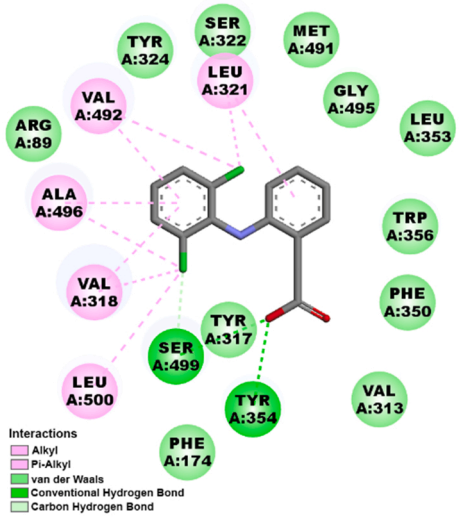

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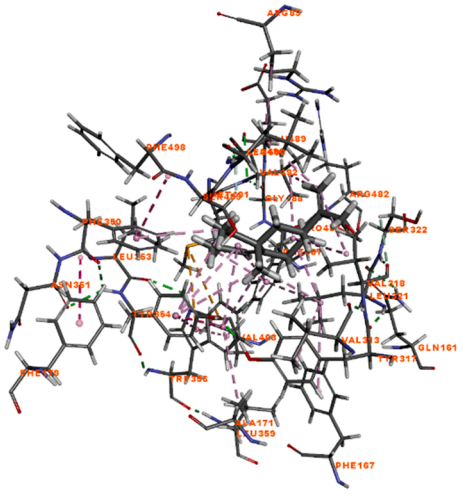

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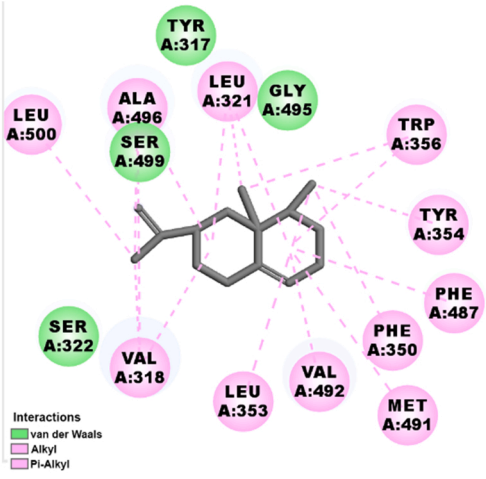
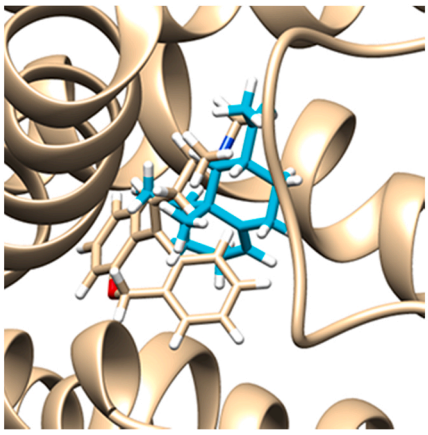


(e)

Fig. 5. Superimposition of poses of best energy of VLN (blue color) and diclofenac into the COX-2 enzyme binding site (a), Interactions of docked ligand diclofenac into binding site (b), Interaction map of amino acids in the binding pocket of COX-2 enzyme with diclofenac (c), Interactions of valencene into binding site (d) and Interaction map of the binding pocket of COX-2 enzyme with valencene (e).



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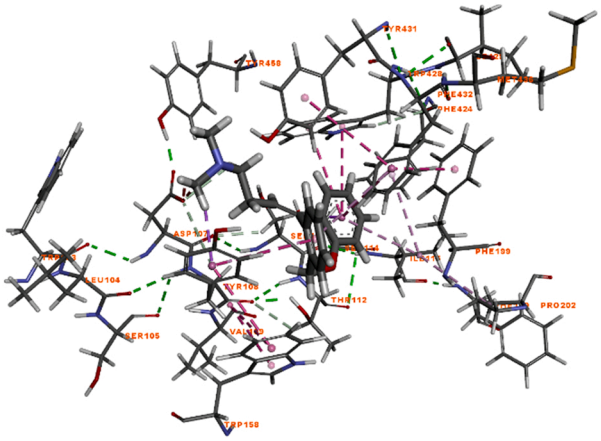

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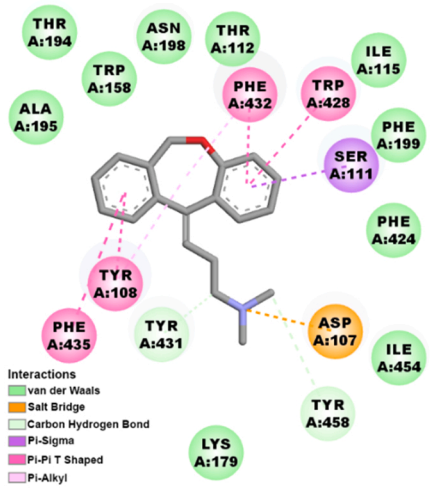

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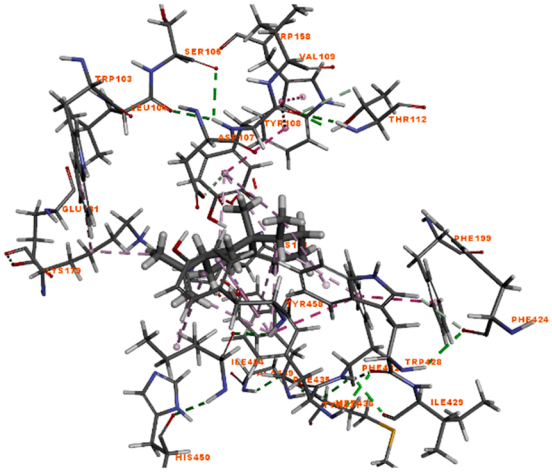

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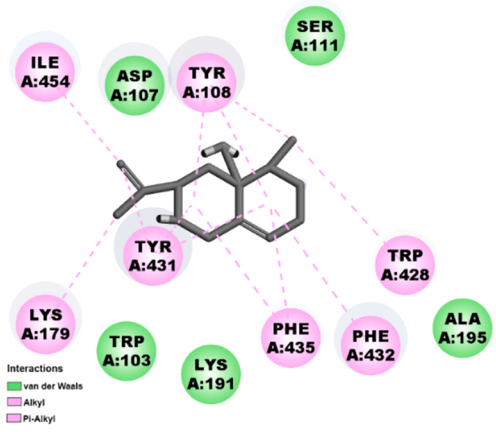

(e)

Fig. 6. Superimposition of poses of best energy of VLN (blue color) and doxepin into the binding pocket of the H1 receptor (a) 3D structure of the doxepin binding site,), Interaction map of amino acids in the binding pocket of the H_1 receptor and doxepin (c), (d) 3D structure of valencene binding site, Interaction map of binding pocket of the H_1 receptor and valencene (e).

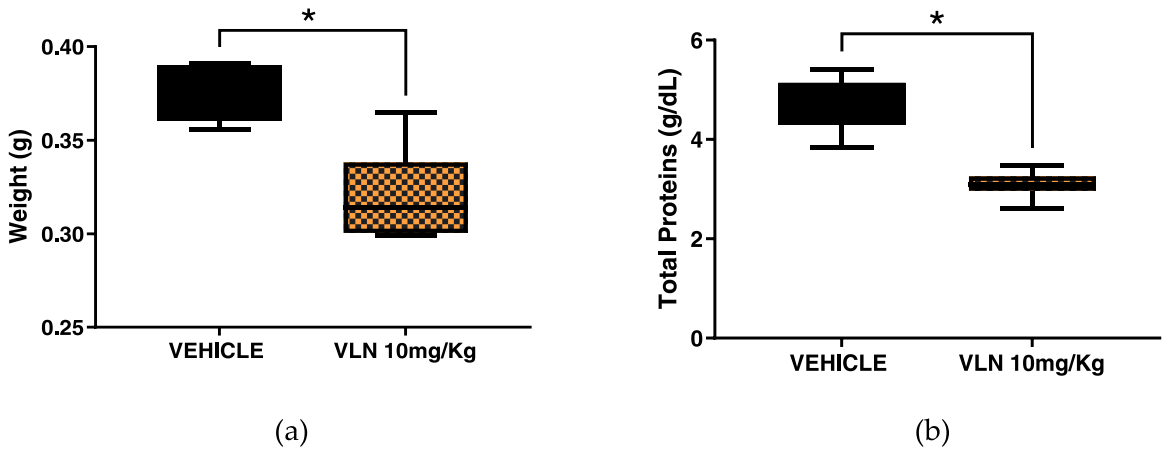

Fig. 7. The effects of VLN treatment on cotton pellet-induced granuloma in mice (n = 6 animals per group). **a**) Final weight of the granuloma. **b**) Protein concentration in the homogenates. a3 = $p < 0.001$ vs. vehicle and a2 = $p < 0.01$ vs. vehicle. Statistical significance was determined with T test.

Conflict of interest statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Institutional Review Board Statement

The animal study protocol was conducted according to the guidelines for animal testing (Nih Guide for the Care and Use of Laboratory Animals, Nih - National Institute of Health -USA, 1996; Federal Law No. 11,794/2008 and the National Council for Control of Animal Experimentation (CONCEA)). The protocols used in this study were approved by the review board for animal experimentation (CEUA) of the Regional University of Cariri, (protocol number 100/2019.2).

Informed Consent Statement

Not applicable.

Data availability

Not applicable.

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