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Pharmacological interaction of α-bisabolol and diclofenac on nociception, inflammation, and gastric integrity in rats

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Abstract

The combination of nonsteroidal anti-inflammatory drugs (NSAIDs) with herbal products having analgesic and anti-inflammatory effects may increase their beneficial effects and limit their side effects. In this study, the effects of an interaction between α-bisabolol and the NSAID, diclofenac on nociception (formalin test), inflammation (paw inflammation produced by carrageenan) and gastric injury in rat was assessed. Diclofenac, α-bisabolol, or diclofenac–α-bisabolol combinations produced antinociceptive and anti-inflammatory effects in rat ($p < .05$). The systemic administration of diclofenac, but not α-bisabolol, produced gastric damage while the diclofenac–α-bisabolol combinations produced limited gastric damage. Effective dose (ED_{40}) values were determined for each individual drug and analyzed isobolographically. The theoretical ED_{40} values for the antinociceptive (98.89 mg/kg) and the anti-inflammatory (41.2 mg/kg) effects differed from the experimental ED_{40} values (antinociception: 38.7 mg/kg and anti-inflammation: 13.4 mg/kg). We concluded that the interactions between diclofenac and α-bisabolol are synergistic. These data suggest that the diclofenac–α-bisabolol combinations can interact to produce minor gastric damage, thereby offering a safer therapeutic alternative for the clinical management of inflammation and/or inflammatory pain.

KEYWORDS

α-bisabolol, diclofenac, gastric damage, inflammation, nociception

1 | INTRODUCTION

α-bisabolol (IUPAC name: (2R)-6-methyl-2-[(1R)-4-methylcyclohex-3-en-1-yl] hept-5-en-2-ol) is a natural unsaturated monocyclic sesquiterpene alcohol. α-bisabolol is the primary constituent of the essential oils from several plants including German chamomile (*Matricaria recutita*), *Plinia cerrocampanensis* Barrie (Myrtaceae), *Stachys lavandulifolia* Vahl (Lamiaceae), and *Laserpitium zernyi* Hayek (Apiaceae) (Barreto et al., 2016; McKay & Blumberg, 2006; Popović et al., 2010; Vila et al., 2010). There are also reports regarding the antimicrobial, antioxidant, anti-inflammatory, antinociceptive, anti-spasmotic and anxiolytic effects of several of these plants (Barreto et al., 2016; McKay & Blumberg, 2006; Popović et al., 2010; Vila et al., 2010). α-bisabolol thus has diverse biological activities, such as antimicrobial (Vila et al., 2010), anti-tumor (da Silva et al., 2010), and gastroprotective activity (Rocha et al., 2010). Recently, α-bisabolol was found to have anti-inflammatory effects in the LPS-induced inflammatory response in RAW264.7 macrophages by downregulating expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) genes through inhibition of NF-κB and the transcription factors activator protein-1 (ERK and p38) signaling (Kim et al., 2011). Other studies found that α-bisabolol inhibited the inflammation induced by the

subcutaneous injection of croton oil, arachidonic acid and phenol in mice ear or carrageenan, 5-HT and dextran in mouse paw models (Leite et al., 2011; Rocha et al., 2011a; Tomić et al., 2014). Several reports have demonstrated an antinociceptive effect induced by α-bisabolol in pain-related behavioral responses to intraperitoneal cyclophosphamide, capsaicin, formalin, acetic acid, mustard oil, and intracolonic mustard oil (Leite et al., 2011; Leite, Fernandes, de Menezes, da Costa, & Campos, 2012). In other studies, α-bisabolol suppressed the nociceptive behaviors in a dose-independent manner in the footpad formalin test, paw mechanical inflammatory hypernociception, and orofacial pain tests (Barreto et al., 2016; Leite et al., 2012; Rocha et al., 2011a; Tomić et al., 2014).

Recently, our group demonstrated synergistic antinociceptive and anti-inflammatory effects produced by the *Matricaria chamomilla* ethanolic extract (MCE) in combination with the nonsteroidal anti-inflammatory drug (NSAID) diclofenac on the rat formalin paw test and the inflammation produced by carrageenan test, respectively (Ortiz et al., 2016, 2017). In addition, three terpenoids were the main constituents identified through ^{1}H-nuclear magnetic resonance: α-bisabolol, bisabolol oxide A, and guaiazulene (Ortiz et al., 2016). Furthermore, in those studies we evaluated the gastric injury produced by diclofenac at 3 and 6 hr, MCE and their combination in rat. Diclofenac, but not MCE, induced gastric damage (Ortiz et al., 2016, 2017). However, the highest dose of the diclofenac–MCE combination was associated with gastric injury, suggesting that systemic MCE may be interfering with the gastroprotective factors of chamomile (Al-Hashem, 2010; Rocha et al., 2011b). In this context, α-bisabolol can induce antinociceptive, anti-inflammatory, and gastroprotective effects in rodents (Barreto et al., 2016; Bezerra, Leal, Nogueira, & Campos, 2009; Leite et al., 2011, 2012; Kim et al., 2011; Rocha et al., 2011a,b). The aim of this work was to explore the effects from the interaction between α-bisabolol and diclofenac on rat paw inflammation by carrageenan injection, rat formalin-induced nociception and on rat gastric integrity.

2 | MATERIALS AND METHODS

2.1 | Animals

Male Wistar rats (7–9 weeks: 180–220 g) from our own breeding facilities were used in this study; animals were housed in regular plastic cages at 22–24 °C temperature, under a 12 hr light-dark cycle, with free access to food (Standard Purina chow diet) and purified water ad libitum. Efforts were made to minimize animal suffering and to reduce the number of animals used. Each rat was used in only one experiment and at the end of the experiments, they were euthanized in a CO_2 chamber. All experiments followed the Guidelines on Ethical Standards for Investigation in Animals (Zimmermann, 1983), and the protocol was approved by the Institutional Animal Care and Use Committee (CINVESTAV, IPN, Ciudad de México, Mexico).

2.2 | Drugs and compounds

Diclofenac, α-bisabolol, carrageenan, and formaldehyde were purchased from Sigma (St. Louis, MO). Diclofenac and carrageenan were dissolved in saline for the biological tests. α-bisabolol was diluted in 1% Tween 80.

2.3 | Measurement of antinociceptive activity

The rat paw 1% formalin test was used to assess nociceptive and antinociceptive effects (Ortiz et al., 2016; Ortiz, 2017). Fifty microliters of 1% formalin were injected subcutaneously (s.c.) in the right hind paw, and flinching behavior quantified. The nociceptive behavior showed a biphasic pattern (Ortiz et al., 2016; Ortiz, 2017). The number of flinches yielded a biphasic curve, and the area under the curve (AUC) was calculated for both phases.

At different times before the formalin insult, animals were orally administered with vehicles or increasing doses of diclofenac (10, 18, 30, and 56 mg/kg at 30 min before), α-bisabolol (30, 56, 100, and 180 mg/kg at 60 min before), or the diclofenac–α-bisabolol combinations (12.4, 24.7, 49.5, and 98.9 mg/kg). The α-bisabolol and diclofenac doses were selected from pilot experiments and similar studies (Ortiz et al., 2016; Rocha et al., 2011a). Investigators were blinded to the treatments that the rats had received.

2.4 | Measurement of carrageenan-induced paw inflammation

Inflammation and anti-inflammation were evaluated using the carrageenan-induced paw edema test (Bignotto et al., 2009; Ortiz, 2017; Ortiz et al., 2017). The volume of the paw was measured with a plethysmometer (Ugo Basile Model 7140 Comerio, Italy). Subsequent, vehicles or different doses of diclofenac (3, 10, 18, and 30 mg/kg), α-bisabolol (10, 30, 56, and 100 mg/kg), or α-bisabolol–diclofenac (5.1, 10.3, 20.6, and 41.2 mg/kg) combinations were administered orally. At different times after compound administration, 100 μL of a 1% carrageenan solution were administered subcutaneously (s.c.) into the paw. The paw volumes were measured at 1, 2, 3, 4, 5, and 6 hr after carrageenan administration (Bignotto et al., 2009; Ortiz, 2017; Ortiz et al., 2017). All treatments were administered in a volume of 4 mL/kg.

2.5 | Measurement of gastric damage

Independent groups of animals were given an oral (p.o.) dose of vehicle or treatment. The different treatments were diclofenac (10–56 mg/kg), α-bisabolol (30–180 mg/kg), or diclofenac–α-bisabolol combinations (12.4–98.9 mg/kg). The doses employed in these experiments were the same as those used in the systemic antinociception experiments. The rats were euthanized 3 hr after drug administration. Other groups of animals orally received vehicle or a different dosage of drug, including diclofenac (3–30 mg/kg), α-bisabolol (10–100 mg/kg), or a diclofenac–α-bisabolol combination (5.1–41.2 mg/kg). The doses employed in these experiments were the same doses used in the anti-inflammation experiments. Rats were euthanized 6 hr after drug administration. To evaluate gastric damage, the number of hemorrhagic lesions in the stomach of the tested rats was scored as previously reported (Ortiz, 2017; Ortiz et al., 2016, 2017).

TABLE 1 Diclofenac–α-bisabolol combinations doses

Systemic antinociception		Systemic anti-inflammation	
Diclofenac mg/kg	α-bisabolol mg/kg	Diclofenac mg/kg	α-bisabolol mg/kg
ED_{40}/2: 23.10	ED_{40}/2: 75.8	ED_{40}/2: 13.90	ED_{40}/2: 27.25
ED_{40}/4: 11.55	ED_{40}/4: 37.9	ED_{40}/4: 6.95	ED_{40}/4: 13.63
ED_{40}/8: 5.78	ED_{40}/8: 18.95	ED_{40}/8: 3.48	ED_{40}/8: 6.81
ED_{40}/16: 2.89	ED_{40}/16: 9.48	ED_{40}/16: 1.74	ED_{40}/16: 3.41

ED, effective dose.

2.6 | Data analysis

2.6.1 | Antinociception

The AUC of the systemic antinociceptive effects produced by each individual and combined drug regimen was calculated as previously described (Ortiz, 2017; Ortiz et al., 2016, 2017). The percentage of antinociception for the second phase of the assay was determined according to the following equation (Ortiz, 2017; Ortiz et al., 2016, 2017):

$$\%\,\text{antinociception} = \left[\left(AUC_{vehicle} - AUC_{post-drug}\right)/AUC_{vehicle}\right] \times 100$$

2.6.2 | Anti-inflammation

Differential paw volume (DPV) data were determined using the following formula: DPV = paw volume during 6 hr – basal paw volume. The percentage of anti-inflammation for each drug treatment was calculated according to the following equation (Ortiz, 2017):

$$\%\,\text{anti-inflammation} = \left[\left(DPV_{vehicle} - DPV_{post-drug}\right)/DPV_{vehicle}\right] \times 100.$$

2.6.3 | Compound combinations

The effective doses 40 (ED_{40}) for antinociception and anti-inflammation were calculated as reported by Tallarida (2000). The interactions between diclofenac and α-bisabolol were characterized by isobolographic analysis (Ortiz, 2017; Ortiz et al., 2016, 2017). The concomitant administration of two compounds (diclofenac + α-bisabolol) was determined based on the ED_{40} value of each individual compound. Experimental dose–response curves were obtained with the compound combinations as shown in Table 1, and the experimental ED_{40} values were calculated from these curves for each combination. The theoretical ED_{40} values of the drug mixtures were calculated from the sum of the effects of each individual drug (e.g., diclofenac + α-bisabolol combination theoretical ED_{40} = diclofenac ED_{40}/2 + α-bisabolol ED_{40}/2). These theoretical ED_{40} values were statistically compared with the experimentally obtained ED_{40} values, respectively (Ortiz, 2017; Ortiz et al., 2016, 2017; Tallarida, 2002). To enhance the analysis of the results, an interaction index (γ) value was obtained using the following formula: γ = experimental ED_{40} of combination/theoretical ED_{40} of combination. The γ value indicated the type of pharmacological interaction as follows: $\gamma = 1$, additive interaction; $\gamma > 1$, antagonism; $\gamma < 1$, synergistic interaction (Ortiz, 2017; Ortiz et al., 2016, 2017; Tallarida, 2002).

2.7 | Statistical analyses

Dose–response curves were evaluated for significance using the one-way ANOVA and Dunnett's test. The comparison between theoretical and experimental values was assessed using the Student's *t* test (Ortiz, 2017; Ortiz et al., 2016, 2017; Tallarida, 2000, 2002). Experimental ED_{40} values significantly inferior to the theoretical ED_{40} values, respectively, indicated a synergistic interaction. The results were considered statistically significant when $p < .05$.

3 | RESULTS

3.1 | Antinociceptive activity produced by diclofenac and α-bisabolol

Peripheral administration of formalin produced a flinching behavior indicative of nociception. The systemic administration of diclofenac or α-bisabolol produced a dose-dependent antinociceptive effect during the second phase ($p < .05$; Figure 1). The ED_{40} values for systemic

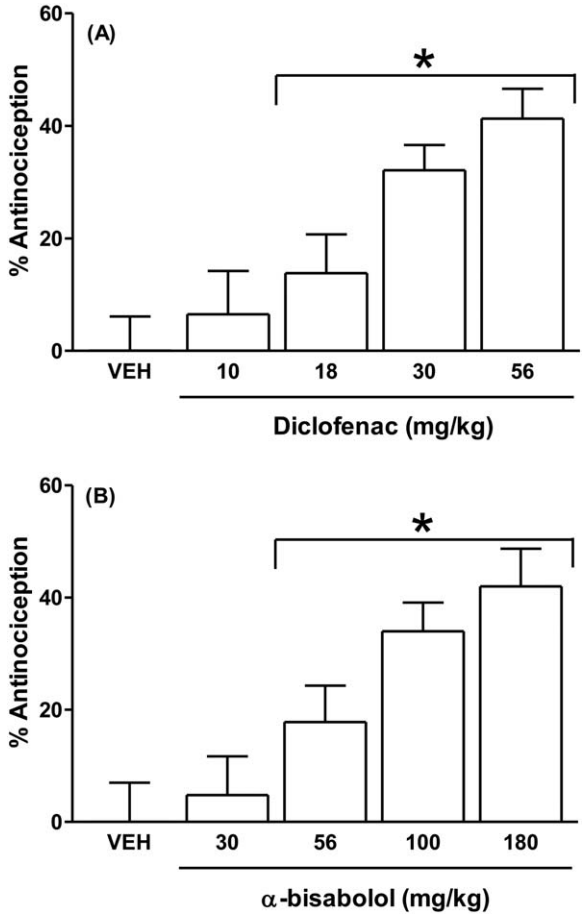

FIGURE 1 Systemic antinociceptive effect of diclofenac and α-bisabolol in the 1% formalin test. Prior to the injection of formalin, rats were pretreated with a systemic administration of: (A) vehicle (VEH) or diclofenac, and (B) VEH or α-bisabolol. Data are expressed as the percent of antinociception on the second phase. Each point corresponds to the mean ± *SEM* of 6–10 animals. *Significant difference from vehicle group ($p < .05$) as determined by the analysis of variance followed by Dunnett's test

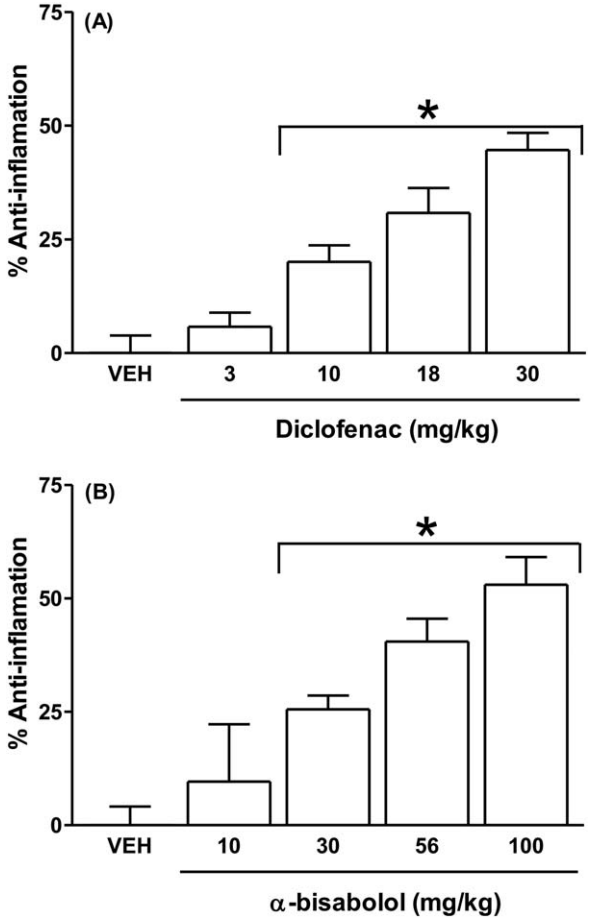

FIGURE 2 Systemic anti-inflammatory effect of diclofenac and α-bisabolol in the carrageenan test. Prior to the injection of carrageenan, rats were pretreated with a systemic administration of: (A) vehicle (VEH) or diclofenac, and (B) VEH or α-bisabolol. Results are shown as the % of the effects. Data are presented as the mean ± *SEM* for 6–10 animals. *Significantly different ($p < .05$), using the ANOVA and the Dunnett's test

diclofenac and α-bisabolol were 46.2 ± 5.4 mg/kg and 151.6 ± 12.8 mg/kg, respectively.

3.2 | Anti-inflammatory activity produced by diclofenac and α-bisabolol

Peripheral administration of carrageenan produced a significant increase in paw volume (inflammation). The systemic administration of diclofenac or α-bisabolol produced a dose-dependent anti-inflammatory effect ($p < .05$; Figure 2). The ED_{40} values for the individual anti-inflammatory activity of systemically administered diclofenac or α-bisabolol were 27.8 ± 4.8 mg/kg or 54.5 ± 4.4 mg/kg, respectively.

3.3 | Interactions between diclofenac and α-bisabolol

Fixed-dose ratio combinations of diclofenac and α-bisabolol were assayed and used to construct two dose–response curves (Figures 3 and 4). The final experimental ED_{40} values were 38.7 ± 5.9 mg/kg and 13.4 ± 2.6 mg/kg, for systemic antinociception and anti-inflammation, respectively. These values were lower ($p < .05$) than the theoretical ED_{40} values: 98.89 ± 9.0 mg/kg and 41.2 ± 4.3 mg/kg, for the systemic antinociception and anti-inflammation, respectively. The occurrence of synergistic interactions is shown in Figures 3 and 4 by the experimental ED_{40} values being below the additive effect threshold. Furthermore, the interaction index (γ) values for the diclofenac–α-bisabolol combinations [0.39 ± 0.1 (systemic antinociception) and 0.33 ± 0.1 (anti-inflammation)] were different from unity ($p < .05$). These results suggest that at systemic level, the interaction between diclofenac and α-bisabolol is synergistic in terms of antinociceptive and anti-inflammatory actions.

3.4 | Gastric injury

For both intervals (Figures 5 and 6), the systemic administration of diclofenac, but not α-bisabolol, caused hemorrhagic erosion of the

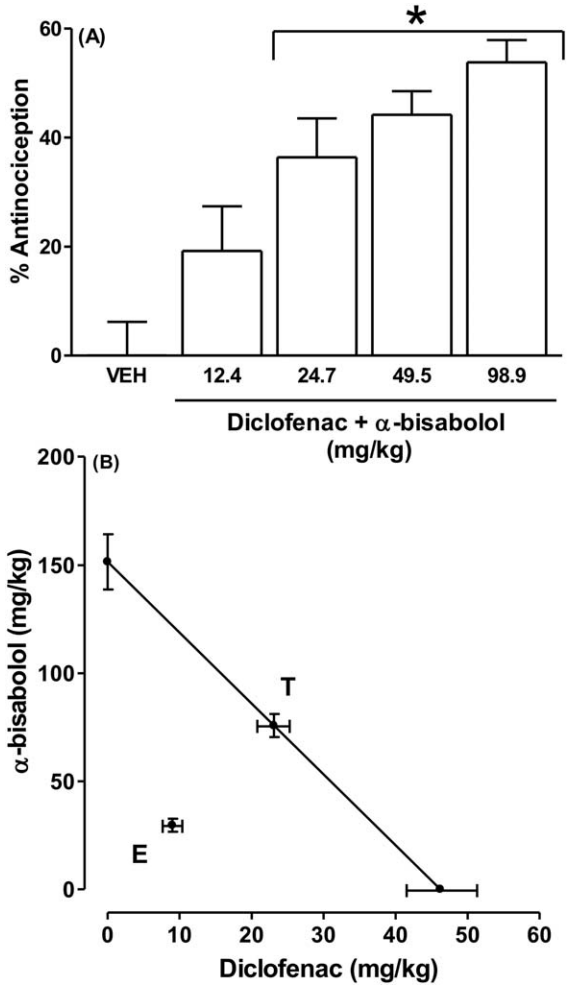

FIGURE 3 Drug interaction study: (A) Systemic antinociceptive effect of the diclofenac–α-bisabolol combination; rats were pretreated with a systemic administration of vehicle (VEH) or the diclofenac–α-bisabolol combination, before formalin injection. (B) Isobologram showing the systemic interaction between diclofenac and α-bisabolol in the formalin test. The oblique line between the *x* and *y* axes is the theoretical additive line. The point in the middle of this line, indicated by "T", is the theoretical additive point calculated from the individual drug ED values. The experimental point indicated by "E," is the actual observed ED value for this combination. Horizontal and vertical bars indicate *SEM*

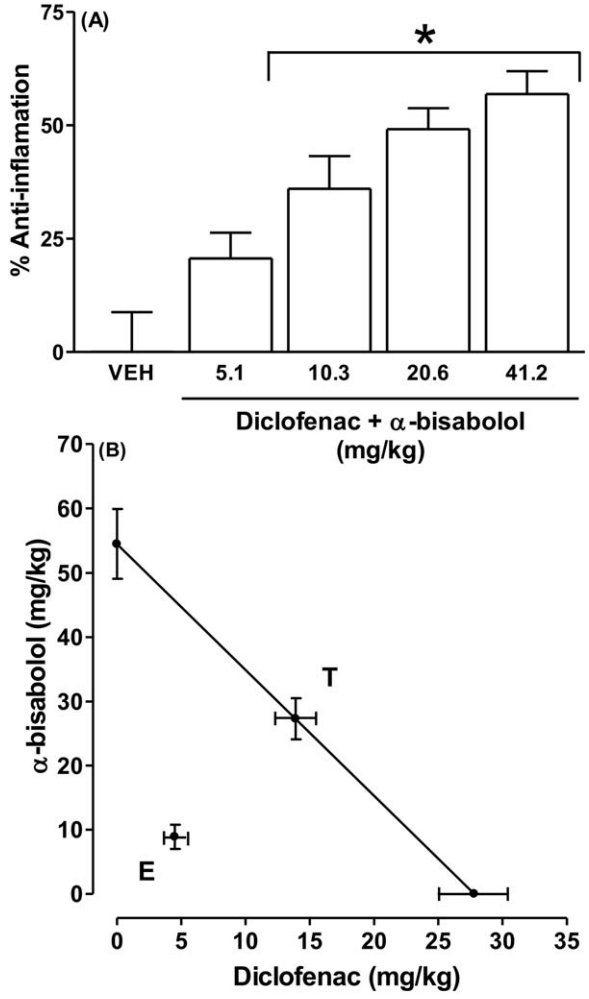

FIGURE 4 Drug interaction study: (A) Systemic anti-inflammatory effect of the diclofenac–α-bisabolol combination; rats were pretreated with a systemic administration of vehicle (VEH) or the diclofenac–α-bisabolol combination, before carrageenan injection. (B) Isobologram showing the systemic interaction between diclofenac and α-bisabolol in the carrageenan test. The oblique line between the *x* and *y* axes is the theoretical additive line. The point in the middle of this line, indicated by "T," is the theoretical additive point calculated from the individual drug ED values. The experimental point indicated by "E," is the actual observed ED value for this combination. Horizontal and vertical bars indicate the *SEM*

stomach ($p < .05$). At 3 hr, the diclofenac–α-bisabolol combination elicited no gastric damage ($p > .05$) (Figure 5). The level of gastric damage caused by the diclofenac (13.9 mg/kg), α-bisabolol (27.25 mg/kg) combination (at hour 6) was different from that measured in vehicle-treated animals ($p < .05$) (Figure 6).

4 | DISCUSSION

In the last few decades, interest in herbal medicine has grown given issues with side effects associated with conventional therapeutics. The empirical use of medicinal plants and natural products has passed from generation to generation for thousands of years with a wide range of biological activities produced by the constituents of herbal products have been elucidated (Atanasov et al., 2015; Cragg & Newman, 2013). α-bisabolol, the primary constituent of the essential oil from several plants has diverse biological activities e.g., antimicrobial (Vila et al., 2010), antitumor (da Silva et al., 2010), antinociceptive, anti-inflammatory, and gastroprotective activities (Barreto et al., 2016; Leite

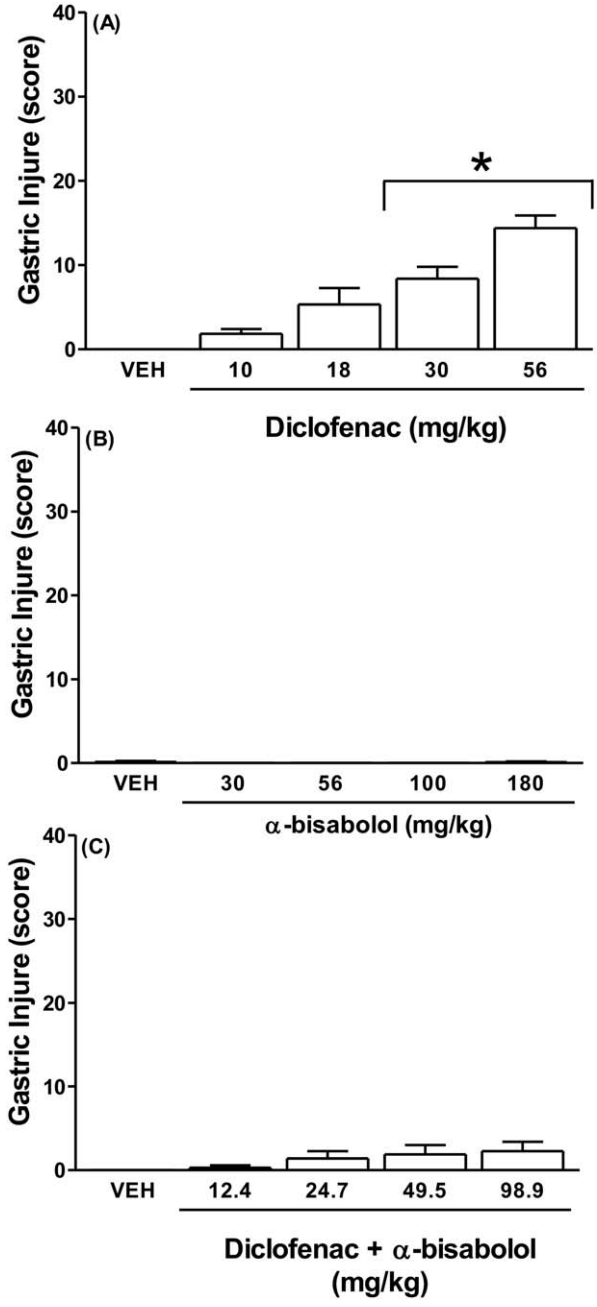

FIGURE 5 Gastric injury produced by diclofenac, α-bisabolol and the diclofenac–α-bisabolol combination. Rats were pretreated with an oral administration of: (A) vehicle (VEH) or diclofenac, (B) VEH or α-bisabolol, and (C) VEH or a diclofenac–α-bisabolol combination; they were euthanized 3 hr later. The stomach was removed and the extent of hemorrhagic damage was scored. Data are expressed as the score of gastric injury. Each point corresponds to the mean ± *SEM* of 6–10 animals. *Significantly different ($p < .05$), from the vehicle group as determined by the analysis of variance followed by Dunnett's test

et al., 2011, 2012; Rocha et al., 2010, 2011a, 2011b). In this study, we report systemic antinociceptive and anti-inflammatory effects induced by α-bisabolol, diclofenac, and the diclofenac–α-bisabolol combination.

4.1 | Antinociceptive effect of diclofenac, α-bisabolol, and diclofenac–α-bisabolol combination

The late phase of the formalin test can be blocked by NSAIDs such as diclofenac or ibuprofen (Ortiz, 2017; Ortiz et al., 2012). Diclofenac, like other NSAIDs, decreases prostaglandin synthesis by nonselective inhibition of COX in the injured tissues and the central nervous system (Gan, 2010). However, other prostaglandin-independent mechanisms may be involved in the actions of diclofenac, e.g., activation of biguanide-dependent mechanisms, potassium channels (K_{Ca}, K_v, and K_{2P}), the nitric oxide-cGMP pathway, inhibition of proton-gated channels, and antagonism of NMDA receptors (Gan, 2010; Ortiz, 2011, 2013; Ortiz et al., 2012; Veale et al., 2014; Voilley, 2004).

Antinociceptive effects are associated with α-bisabolol administration (Barreto et al., 2016; Leite et al., 2012; Rocha et al., 2011a). It inhibited 5-lipoxygenase (5-LO), decreasing leukotriene levels (Baylac & Racine, 2003) and also downregulated expression of iNOS and COX-2 (Kim et al., 2011). Docking simulations predicted that COX could be inhibited by α-bisabolol (Ortiz et al., 2016). In support of this finding α-bisabolol treatment of RAW264.7 macrophages inhibited LPS-induced prostaglandin-E_2 release via inhibition of COX-2 (Kim et al., 2011; Srivastava, Pandey, & Gupta, 2009). Therefore, inhibition of COX and 5-LO by α-bisabolol can decrease prostaglandin and leukotriene production thus modulating formalin-induced nociception.

There are multiple reports on synergistic interactions between diclofenac and analgesics, neuromodulators or antihistamines in animal models (Jiménez-Andrade et al., 2003; Picazo, Castañeda-Hernández, & Ortiz, 2006; Ortiz, 2017) and with plant extracts (Acosta-Madrid et al., 2009; De Paz-Campos et al., 2014; Ortiz et al., 2016), e.g., a synergistic antinociceptive interaction with a diclofenac–MCE combination in the rat formalin test (Ortiz et al., 2016).

In this study, the isobolographic analysis demonstrated a synergistic interaction between diclofenac and α-bisabolol in systemic antinociception with diclofenac-mediated effects (Gan, 2010; Ortiz, 2011, 2013; Ortiz et al., 2012; Voilley, 2004) synergistically interacting with those of α-bisabolol (Baylac & Racine, 2003; Kim et al., 2011; Ortiz et al., 2016; Srivastava et al., 2009).

4.2 | Anti-inflammatory effects induced by diclofenac, α-bisabolol, and the diclofenac–α-bisabolol combination

Diclofenac relieves inflammation, swelling, and stiffness in patients with arthritis and other inflammatory affections (Gan, 2010). Diclofenac decrease prostaglandin synthesis via nonselective inhibition of COX (Gan, 2010) and possesses other mechanism of action mechanisms (Gan, 2010; Ortiz, 2011, 2013; Ortiz et al., 2012; Veale et al., 2014; Voilley, 2004) that reduce the leucocyte and neutrophil count and myeloperoxidase (MPO) levels in carrageenan-induced inflammatory exudate (Mathew, Jacob, Durgashivaprasad, Reddy, & Unnikrishnan, 2013). Diclofenac can also reduce the capacity of rolling, sticking, and migrating of leukocytes after a carrageenan stimulus (Martinez, Aparecida De Oliveira, & Fortes, 1999). The anti-inflammatory effects of diclofenac are abrogated in adrenalectomized rats, suggesting a role of

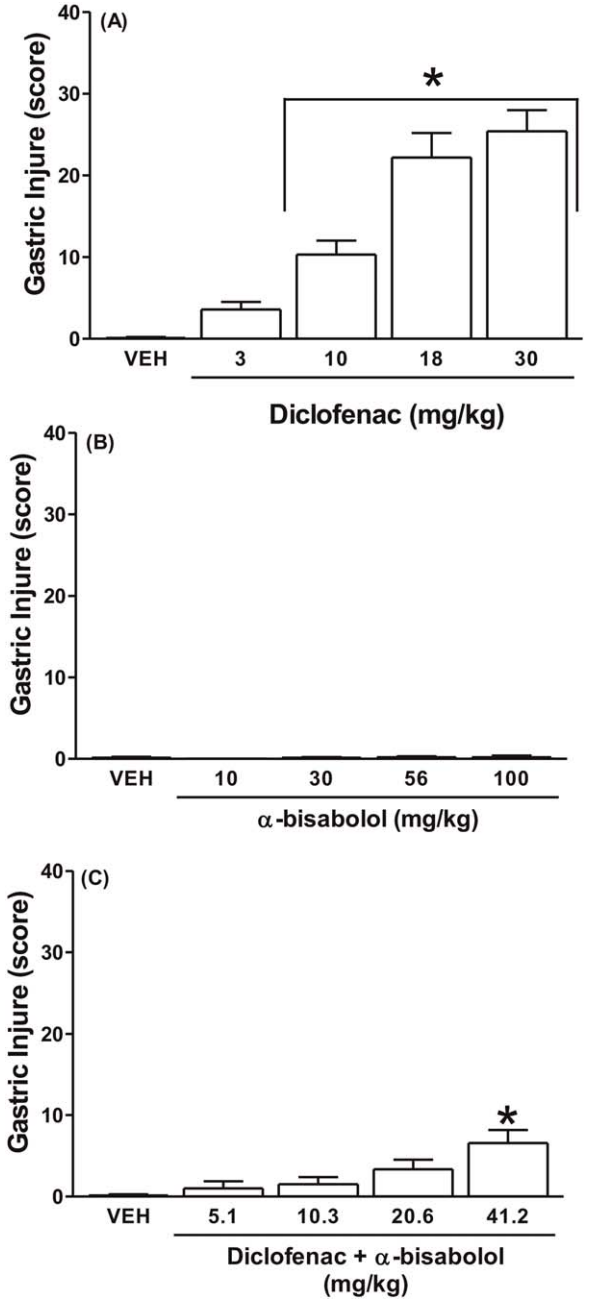

FIGURE 6 Gastric injury produced by diclofenac, α-bisabolol and the diclofenac–α-bisabolol combination. Rats were pretreated with an oral administration of: (A) vehicle (VEH) or diclofenac, (B) VEH or α-bisabolol, and (C) VEH or a diclofenac–α-bisabolol combination; they were euthanized 6 hr later. The stomach was removed and the extent of hemorrhagic damage was scored. Data are expressed as the score of gastric injury. Each point corresponds to the mean ± *SEM* of 6–10 animals. *Significantly different ($p < .05$), from the vehicle group as determined by the analysis of variance followed by Dunnett's test

adrenaline and prednisolone in its anti-inflammatory effects (Suleyman, Halici, Cadirci, Hacimuftuoglu, & Bilen, 2008).

The present results showed that the α-bisabolol reduced the inflammation associated with the administration of carrageenan in the paw of the rats and are in agreement with the anti-inflammatory effects induced by α-bisabolol following subcutaneous injection of croton oil, arachidonic acid, and phenol in mice ear or carrageenan, 5-HT and dextran in mouse paw (Leite et al., 2011; Rocha et al., 2011a). Inhibition of 5-LO and COX by α-bisabolol decreased leukotriene and prostaglandin production, respectively, the consequences of which may be associated with decreasing carrageenan-induced paw inflammation (Baylac & Racine, 2003).

Isobolographic analysis demonstrated that a diclofenac–α-bisabolol combination was synergistic in terms of the anti-inflammatory effect in rats. The effects of diclofenac potentially involve decreasing neuronal and cellular excitability via the blockade of proton-gated channels, NMDA receptors and activation of K^+ channels (Gan, 2010; Ortiz, 2011, 2013; Ortiz et al., 2012; Veale et al., 2014; Voilley, 2004) that lead to decreased functionality of inflammatory cells such as leucocytes and neutrophils (Martinez et al., 1999; Mathew et al., 2013; Suleyman et al., 2008). Conversely, α-bisabolol has been demonstrated to reduce the LPS-induced COX-2 mRNA and protein expression (Srivastava et al., 2009; Srivastava, Shankar, & Gupta, 2010). In addition, α-bisabolol inhibited prostaglandin and leukotriene release by the blockade of COX and 5-LO, respectively (Baylac & Racine, 2003; Kim et al., 2011; Ortiz et al., 2016; Srivastava et al., 2009). The potential participation of these various mechanisms induced a synergism in the anti-inflammatory effect of the diclofenac–α-bisabolol combination.

4.3 | Gastric damage produced by the diclofenac, α-bisabolol, and the diclofenac–α-bisabolol combination

The prostaglandins produced by COX-1 are a major factor in controlling mucus and bicarbonate secretion in stomach. Gastrointestinal damage induced by NSAIDs and by ethanol involve inhibition of prostaglandin and reactive oxygen species (ROS) production (Rocha et al., 2011a,b; Yeomans, Hawken, Brailsford, & Naesdal, 2009). Strategies to reduce or prevent these gastropathic effects involve the use of selective COX-2 inhibitors (celecoxib), co-administration of acid suppressive agents (proton pump inhibitors, H_2-receptor antagonists) or the reintroduction of mucosal prostaglandins by using prostaglandin analogs, e.g., misoprostol or enprostil (Lazzaroni & Porro, 2009; Yeomans et al., 2009). Another strategy possible is the combination of herbal agents or their constituents with NSAIDs. Previously, we found that the oral administration of citral (a monoterpene that occurs naturally in herbs, plants, and citrus fruits) that did not elicit gastric damage in rats (Ortiz, Ramírez-Montiel, González-García, Ponce-Monter, & Castañeda-Hernández, 2010) decreased the gastric damage produced by the NSAID, naproxen. Diclofenac–α-bisabolol induced gastroprotection in ethanol-induced gastric injury in mice (Barreto et al., 2016; Leite et al., 2011, 2012; Rocha et al., 2010, 2011a, 2011b). This effect an effect associated with reduction in lipid peroxidation, superoxide dismutase activity and neutrophil migration (Rocha et al., 2011a,b). In contrast, in two prior studies the gastric injury at 3 and 6 hr produced by diclofenac, MCE and their combination in rat was studied (Ortiz et al., 2016, 2017). Diclofenac, but not MCE, induced gastric damage. However, the highest dose diclofenac–MCE combination resulted in gastric injury (Ortiz et al., 2016, 2017) suggesting that systemic MCE may be interfering with unknown gastroprotective factors in components of chamomile (Al-Hashem, 2010; Rocha et al., 2011b). In this study, the gastric damage produced by diclofenac and diclofenac–α-bisabolol was evaluated at 3 and 6 hr after administration. In this case, diclofenac, but not diclofenac–α-bisabolol, produced gastric damage (Figures 5 and 6). No gastric damage ($p > .05$) was observed 3 hr after administration of the diclofenac–α-bisabolol combination (Figure 5). However, after 6 hr and at the highest dose of diclofenac (13.9 mg/kg) and α-bisabolol (27.25 mg/kg) combination a modest but significant increase gastric damage was observed in the rat (Figure 6). These results agree with the incomplete and significant gastroprotection of diclofenac–α-bisabolol in the gastric damage produced by absolute ethanol and the ethanol–indomethacin combination in rodents (Bezerra et al., 2009; Rocha et al., 2011a,b). The gastrointestinal safety of this combination in human patients in clinical situations awaits validation.

5 | CONCLUSIONS

The interactions observed in this study suggests that the diclofenac–α-bisabolol combinations may have an improved anti-inflammatory and analgesic profile than diclofenac alone. Additionally, diclofenac–α-bisabolol was able to decrease gastric damage associated with diclofenac alone suggesting that the co-administration of diclofenac and α-bisabolol will offer benefits at the clinical level. Further research into the efficacy and gastric security resulting from the administration of this combination in different animal species and clinical situations is required.

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