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ORIGINAL ARTICLE

Evidence for the involvement of the GABAergic, but not serotonergic transmission in the anxiolytic-like effect of bisabolol in the mouse elevated plus maze

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Abstract Bisabolol (α-(−)-bisabolol) is a sesquiterpene which is a part of the essential oil of a variety of plants, but its common source is German chamomile. Several bioactivities including anti-inflammatory, anti-nociceptive, and anti-tumor effects were attributed to bisabolol. However, the neuropharmacological properties of bisabolol have not yet been reported. The present study evaluated behavioral effects of bisabolol using elevated plus maze (EPM), open field test (OFT), and rotarod test. Moreover, this study also examined whether the 5-HT_{1A} and $GABA_A$–benzodiazepine receptor systems are involved in the anxiolytic-like effects of bisabolol. After acute intraperitoneal treatment with bisabolol at the doses of 0.5, 1, 2, 5, and 10 mg/kg, OFT, EPM, and rotarod were utilized for investigating behavioral effects. Flumazenil, a benzodiazepine receptor antagonist, and WAY-100635, a 5-HT_{1A} receptor antagonist, were used to determine the action mechanism in the EPM. Bisabolol especially at the dose of 1 mg/kg was effective in increasing the total number of entries and time spent in the open arms of EPM while number of rearing and grooming in OFT was decreased in comparison to the control. In the rotarod, permanence time was decreased in the mice treated with the high doses of bisabolol. Pretreatment with flumazenil, but not WAY-100635, was able to reverse the effect of bisabolol 1 mg/kg in the EPM, indicating that the anxiolytic-like activity of bisabolol occurs via the GABAergic but not serotonergic transmission. The present study supports the idea that bisabolol may mediate its anxiolytic-like and sedative mechanisms involving $GABA_A$ receptors.

Keywords Bisabolol · Sesquiterpene · Locomotor activity · Anxiolytic-like · Sedative · GABAergic modulators

Introduction

Anxiety disorders are among the most common mental disorders that affect all age groups at some points in their lives (Grundmann et al. 2009). Pharmacotherapy is the most common treatment of this disorder but imposes significant economic costs and several undesirable side effects including delay in onset of action, sedation, dependence, and amnesia (Grundmann et al. 2009; Liu et al. 2015). For these reasons, there is a need for novel psychopharmacological agents that possess prompt onset of action as well as fewer side effects. Many researchers have focused on the arsenal of nature to find new leading compounds for the fight against mental disorders. Several plant-derived biological compounds particularly terpenoids have shown central nervous system activities (Melo et al. 2010; Costa et al. 2014; Kessler et al. 2014; Moreira et al. 2014).

Bisabolol (α-(−)-bisabolol) is a sesquiterpene which is a part of the essential oil of a variety of plants, but one of its common sources is German chamomile (*Chamomilla recutita* L.; Asteraceae) (Kamatou and Viljoen 2010). The use of chamomile as an herbal remedy with calming effects dates back to ancient times. Researchers have reported pharmacological activity of chamomile in animal models of anxiety (Yamada et al. 1996). In recent years, studies have also revealed clinically meaningful anti-depressant and anxiolytic activities of chamomile in human subjects (Amsterdam et al. 2009; Amsterdam et al. 2012). Can et al. (2012) have reported

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psychopharmacological profile of chamomile essential oil in mice. These authors demonstrated that chamomile essential oil represented an activity profile similar to that of the typical psychostimulants like caffeine (Can et al. 2012). Since a substantial amount of chamomile essential oil is composed of bisabolol, it can be considered as a functional part in the interaction between different constituents of the whole essential oil and might be responsible for some of chamomile essential oil biological activities. Previous studies have reported several bioactivities including anti-nociceptive, anti-inflammatory, and anti-oxidative effects for bisabolol (Aron de Miranda et al. 2010; Seki et al. 2011; Rocha et al. 2011). However, to our knowledge, the anxiolytic properties of bisabolol have not yet been reported. Thus, the present study investigated the behavioral effects of bisabolol using the elevated plus maze (EPM), the open field test (OFT), and the rotarod test. Moreover, this study also examined whether the 5-HT_{1A} and $GABA_A$–benzodiazepine receptor systems are involved in the anxiolytic-like effects of bisabolol in the EPM.

Methods

Animals

Male Swiss albino mice, weighing 25–30 g, were purchased from Pasteur Institute of Iran, North Research Center (Amol, Iran). The animals were housed in groups under controlled conditions of light (12-h light/dark cycle) and at room temperature. Food and water were available ad libitum. The experimental procedures were approved by the Animal Care and Use Committee of the Pasteur Institute of Iran. To minimize nonspecific stress, the mice were handled 3 min twice a day for 3 days before experiments. The animals were also familiarized with the laboratory environments before subjecting them to behavioral tests. All tests were performed with distinct groups of animals, and each animal was used only once.

Chemicals and treatments

Diazepam (Darupakhsh, Tehran, Iran) and buspirone HCl (Sigma-Aldrich, Munich, Germany) were used as standard anxiolytic drugs. Bisabolol ((−)-α-bisabolol or levomenol) and WAY-100635 (*N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]-ethyl]-*N*-(2-pyridinyl) cyclohexane carboxamide trihydrochloride) were obtained from Sigma (Sigma-Aldrich). Flumazenil (Sigma-Aldrich) was a gift from Dr. Bagherpour (Anesthesiologist, Sari, Iran). Tween 80® (1%, Sigma-Aldrich) in normal saline solution was used as a vehicle and solvent for all the drugs. Drug solutions were freshly prepared before testing and administered intraperitoneally (i.p.) in a volume of 0.1 ml/10 g mouse body weight.

Study design

To evaluate the anxiolytic-like effect of the bisabolol, the mice ($n = 7$ per group) were randomly assigned to eight experimental groups—vehicle (CON), diazepam 1 mg/kg (DZP), buspirone 10 mg/kg (BSP), bisabolol 0.5 mg/kg (BIS0.5), bisabolol 1 mg/kg (BIS1), bisabolol 2 mg/kg (BIS2), bisabolol 5 mg/kg (BIS5), and bisabolol 10 mg/kg (BIS10)—and received all the treatments 30 min prior to behavioral testing. Doses were selected according to the results of a pilot study performed in our laboratory which revealed that bisabolol at higher doses (100 and 200 mg/kg i.p.) altered locomotoractivity and produced marked sedation and muscle relaxation in mice.

In the second series of experiments, for determination of possible involved mechanism of anxiolytic-like activity, antagonism of GABA–benzodiazepine and 5-HT_{1A} transmissions were studied by pretreatment with flumazenil or WAY-100635. For this purpose, mice were assigned to 12 experimental groups ($n = 7$ per group) as follows: vehicle + vehicle (CON), vehicle + diazepam 1 mg/kg (DZP), vehicle + flumazenil 3 mg/kg (FLU), flumazenil 3 mg/kg + diazepam 1 mg/kg (FLU + DZP), vehicle + buspirone 10 mg/kg (BSP), vehicle + WAY-100635 1 mg/kg (WAY), WAY-100635 1 mg/kg + buspirone 10 mg/kg (WAY + BSP), flumazenil 3 mg/kg + buspirone 10 mg/kg (FLU + BSP), WAY-100635 1 mg/kg + diazepam 1 mg/kg (WAY + DZP), vehicle + bisabolol (BIS), WAY-100635 1 mg/kg + bisabolol (WAY + BIS), flumazenil 3 mg/kg + bisabolol (FLU + BIS). There was 15-min interval between two successive injections. Thirty minutes after the second injection, behavioral effects of different treatments were evaluated by using the EPM test. All of the experiments were done under blinded conditions.

Open field test (OFT)

This test is used to evaluate general locomotor activity and anxiety-like behavior in rodents. The open field device consisted of an acrylic glass (30 × 30 × 15 cm), which was divided into nine squares. For testing, each mouse was placed in the central square and the number of squares crossed with the four paws; rearing and grooming behaviors were measured for 5 min (Neto et al. 2013). After each trial, the device was wiped with a 70% ethanol solution to remove any traces left from the previous animal.

Elevated plus maze (EPM)

This test has been widely used to measure anxiety-like behavior in rodents. The apparatus for mice consisted of two open arms (30 × 5 cm^2) and two closed arms (30 × 5 × 25 cm^3) connecting by a 5 × 5-cm^2 central platform (Costa et al. 2014). The maze was placed to height of 45 cm in a quiet room. Half an hour after

treatment the mouse was placed at the center of the plus maze with its face directing towards one of the close arms, then some parameters such as number of entries in open and closed arms and time spending in each of them were recorded for 5 min.

Rotarod

This test has been approved for evaluation of muscle relaxation and motor coordination of treatments. For this test, each animal was placed on a 2.5-cm-diameter bar turning at 12 rpm, which was set 25 cm above the floor. For each mouse, the time of permanence on the bar for 1 min was recorded (do Vale et al. 2002).

Statistical analysis

All results are expressed as mean ± SEM (standard error of mean) values. The data were analyzed by means of analysis of variance (ANOVA) followed by Student–Newman–Keuls post hoc test. All analyses were performed using SPSS software V. 18 (SPSS Inc., Chicago, IL, USA). Values of $p < 0.05$ were considered statistically significant.

Results

Effects of bisabolol on the OFT

OFT was carried out to determine locomotor activity and possible effect of bisabolol on exploratory behavior of mice. As shown in Fig. 1a administration of diazepam and buspirone did not change the overall locomotor activity, but the number of rearing and grooming behavior was reduced in mice (Fig. 1b, c). In the bisabolol-treated mice, at the doses of 0.5, 1, and 2 mg/kg, any significant change in locomotion has not been observed while at the higher doses, 5 and 10 mg/kg, significant decrease in the number of crossing (43 and 75% in comparison with the control group) was seen (Fig. 1a). In the case of rearing and grooming behaviors, treatment with bisabolol at all doses resulted in significant decrease in comparison to the control group (Fig. 1b, c).

Effect of bisabolol on the EPM test

The results of the possible anxiolytic-like activity of bisabolol assessed by EPM in mice are shown in Fig. 2. One-way ANOVA indicated a significant difference in the number of open arm entries and the percent of time spent in the open arms among the groups. Results showed that in BIS0.5, BIS1, and BIS2 groups, the number of open arm entries (NEOA) was increased (65, 92, and 60%, respectively), as compared to the control (Fig. 2a). Results demonstrated that the time spent in open arms (TSOA) was 1.5- and 1.9-fold increased in BIS0.5- and BIS1-treated mice, respectively, in comparison with the controls (Fig. 2b).The same behavioral alterations were observed in DZP- and BSP-treated mice in comparison with the CON in EPM (Fig. 2a, b).

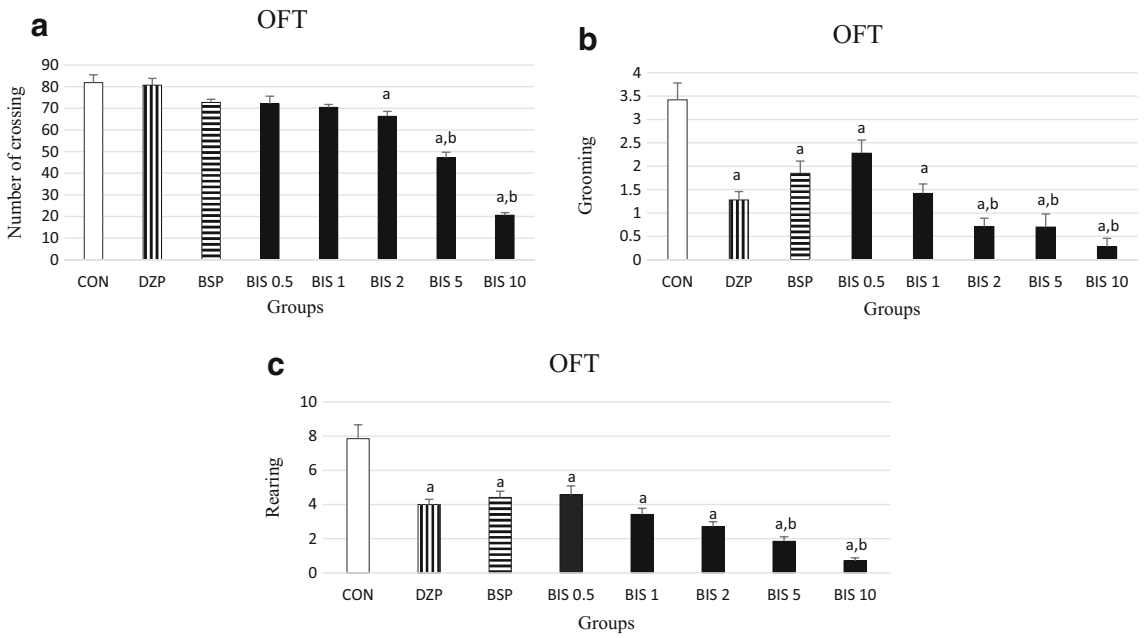

Fig. 1 Effects of bisabolol (BIS) and of two reference drugs on the number of squares crossed (**a**), grooming (**b**), and rearing (**c**) in the open field test (OFT). Five doses of BIS (0.5–10 mg/kg) were compared to diazepam (DZP, 1 mg/kg) and buspirone (BSP, 10 mg/kg). Values are presented as mean ± SEM for seven mice per group. ANOVA yielded $F_{7,42} = 64.7$ ($p < 0.0001$) for **a**, $F_{7,42} = 16.18$ ($p < 0.0001$) for **b**, and $F_{7,42} = 24.12$ ($p < 0.0001$) for **c**. [a]$p < 0.001$ vs. control; [b]$p < 0.05$ vs. BSP and BIS0.5 (ANOVA followed by Student–Neuman–Keuls test)

Effect of bisabolol on the rotarod

Administration of bisabolol at the higher doses resulted in significant alteration in rotarod motor coordination (Fig. 3). Permanence on rotarod decreased in BIS5 (48%)- and BIS10 (60%)-treated groups in comparison to the CON but did not change in the diazepam and buspirone groups (Fig. 3).

The role of GABA or 5-HT1$_A$ transmission on anxiolytic-like activity of bisabolol in the EPM test

In order to determine whether anxiolytic-like activity of bisabolol occurs through the GABAergic or serotonergic transmissions, mice before receiving bisabolol (1 mg/kg, this dose was selected as the most efficacious anxiolytic based on the results of the first experiments) were pretreated with flumazenil or WAY-100635. As shown in Fig. 4a, b, DZP, BSP, and BIS produced significant increase in the NEOA and TSOA, while FLU and WAY did not show any significant difference in comparison with the CON. As expected, FLU and WAY antagonized the effects of DZP and BSP in the EPM, respectively. Pretreatment with WAY did not have any effect on anxiolytic-like action of BIS, but pretreatment with FLU reversed the effects of BIS in the EPM.

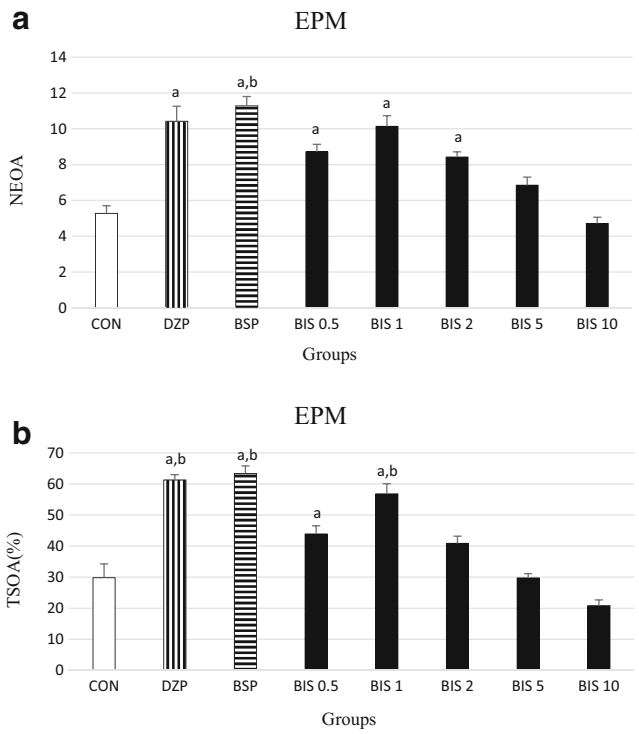

Fig. 2 Effects of bisabolol (BIS) and of two reference drugs on the number of entries in open arms (NEOA) (**a**) and percent of time spent in open arms (TSOA %) (**b**) in the elevated plus maze (EPM). Five doses of BIS (0.5–10 mg/kg) were compared to diazepam (DZP, 1 mg/kg) and buspirone (BSP, 10 mg/kg). Values are presented as mean ± SEM for seven mice per group. ANOVA yielded $F_{7,42} = 22.15$ ($p < 0.0001$) for **a** and $F_{7,42} = 35.88$ ($p < 0.0001$) for **b**. $^{a}p < 0.01$ vs. CON; $^{b}p < 0.05$ vs. BIS0.5 (ANOVA followed by Student–Neuman–Keuls test)

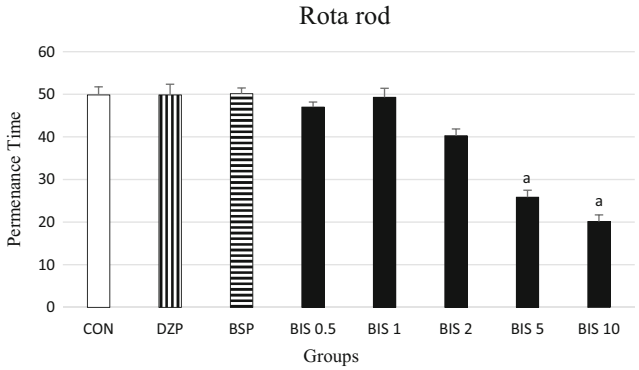

Fig. 3 Effects of bisabolol (BIS) and of two reference drugs on the permanence time in the rotarod test. Five doses of BIS (0.5–10 mg/kg) were compared to diazepam (DZP, 1 mg/kg) and buspirone (BSP, 10 mg/kg). Values are presented as mean ± SEM for seven mice per group. ANOVA yielded $F_{7,42} = 45.66$ ($p < 0.0001$). $^{a}p < 0.001$ vs. CON (ANOVA followed by Student–Neuman–Keuls test)

Discussion

Sesquiterpenes have been identified as the active constituents present in several medicinal plants, with a wide range of biological activities including anti-infective, anti-oxidant, anti-inflammatory, and anti-cancer effects (Kamatou and Viljoen 2010; Manayi et al. 2016). Although the activity of compounds like monoterpenes is well established, this activity for sesquiterpenes has only been reported in a limited number of studies (Manayi et al. 2016). However, to the best of our knowledge, there are no reports of the behavioral effects of bisabolol. In the present research, the sedative and anxiolytic effects of bisabolol were examined in murine animal model using OFT, EPM, and rotarod test, which are validated models for testing central nervous system effects (Costa et al. 2014).

Our results suggest that bisabolol at low doses has anxiolytic-like effects on mice without altering locomotor activity, but at the higher doses, motor coordination may be impaired and sedation occurs. Firstly, we studied the effect of bisabolol on the exploratory activity of mice in the OFT. Diazepam and buspirone have been used as standard anxiolytics, and different doses of bisabolol were used for screening potential anxiolytic-like action. DZP and BSP did not decrease the locomotor activity of mice, but BIS at the higher doses altered locomotor function of mice and decreased the number of crossing and also the numbers for rearing and grooming. Our results are in line with do Vale et al. (2002) who reported central effects of some terpenoid compounds like citral, myrcene, and limonene in mice open field test which led to decreasing the number of crossings and also numbers for rearing and grooming (do Vale et al. 2002). It is accepted that rearing is a function of the excitability level of

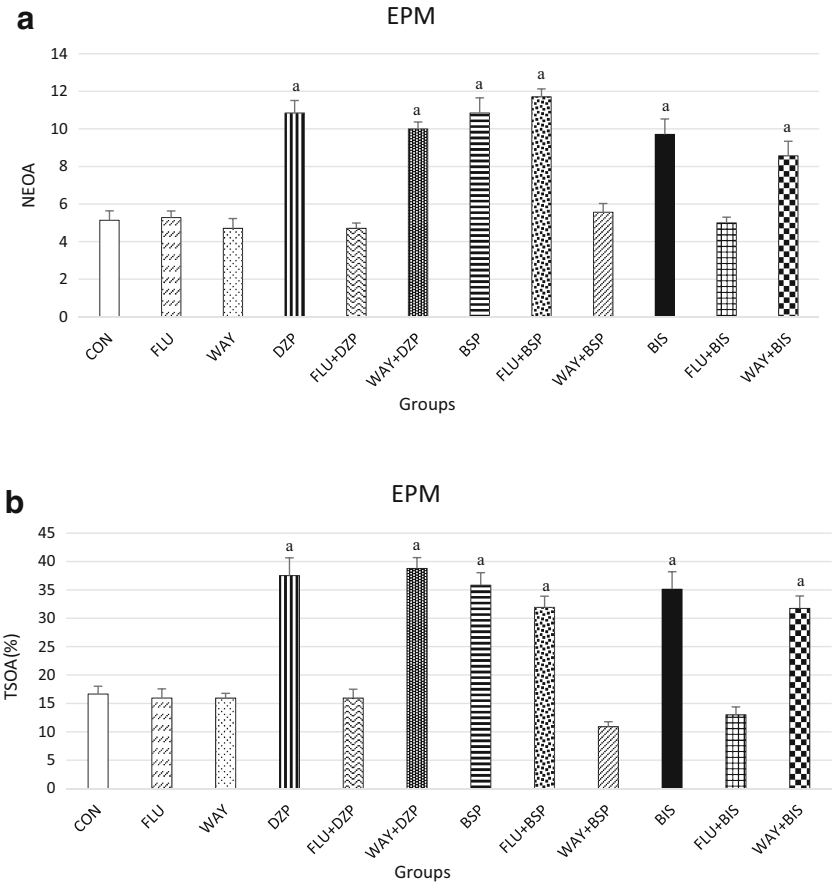

Fig. 4 Effects of pretreatment with receptor antagonists on the number of entries in open arms (NEOA) (**a**) and time spent in open arms (TSOA) (**b**) in the elevated plus maze (EPM) in mice. Flumazenil (FLU, 3 mg/kg), WAY100635 (WAY, 1 mg/kg) or their vehicle was administered either alone or was followed by administration of diazepam (DZP, 1 mg/kg), buspirone (BSP, 10 mg/kg), bisabolol (BIS, 1 mg/kg), or their vehicle. Values are presented as mean ± SEM for seven mice per group. ANOVA yielded $F_{11,66} = 44.32$ ($p < 0.0001$) for **a**, and $F_{11,66} = 30.75$ ($p < 0.0001$) for **b**. $^{a}p < 0.001$ vs. CON (ANOVA followed by Student–Neuman–Keuls test)

the central nervous system (de Almeida et al. 2012), and reduction of rearing and grooming in the bisabolol-treated mice confirms the central effects of bisabolol.

Hendriks et al. (1985) have demonstrated the central nervous depressant activity of valerenic acid in the mouse. Valerenic acid is a sesquiterpene and the major constituent of valerian root extract. These authors have reported a decrease in locomotility of mice and increase in the pentobarbital induced sleeping time after administration of valerenic acid. From these data, they have concluded that valerenic acid has central nervous system depressant properties (Hendriks et al. 1985). Benke et al. (2009) described the anxiolytic action of valerenic acid in the mouse EPM and light/dark box. They have demonstrated that neurons expressing β3 containing $GABA_A$ receptors are a cellular substrate for the anxiolytic action of valerian extracts (Benke et al. 2009).

In order to further substantiate the anxiolytic-like effect of bisabolol, a second behavioral paradigm was studied. Rodents when placed in a new environment demonstrate fear and anxiety. EPM is designed based on the natural fear of openness and elevation in rodents. Increases in open arm parameters are the most representative indices of anxiolytic-like effects (File 2001). Findings of the EPM showed that bisabolol, especially at the dose of 1 mg/kg was able to increase the number of entries as well as the percent of time spent in the open arms. Similar findings have been reported from several plant-derived compounds like 1,4-cineole, phytol, and gallic acid (Gomes et al. 2010; Costa et al. 2014; Mansouri et al. 2014).

In the third behavioral paradigm, acute injection of bisabolol dose-dependently decreased the permanence time on the rotarod test. Muscle relaxation, the main parameter assessed by the rotarod test, was seen at the higher doses of bisabolol. Taking the results all together, it can be suggested that bisabolol especially at the highest dose could have sedative and central nervous depressive effects. In line with our findings, citral, myrcene, and limonene have been reported to be capable of decreasing permanence time on the rotarod bar (do Vale et al. 2002). By contrast, for the monoterpene 1,4-cineole, also known as eucalyptol at all doses, no changes was observed in the rotarod test as compared to the control (Gomes et al. 2010). On the other hand, it has been reported that phytol, a diterpene present in green leaves of various medicinal plants, especially at the high doses, significantly impaired the rotarod performance of mice (Costa et al. 2014). As it could be expected, diazepam and buspirone, two reference anxiolytic compounds, increased the number of entries as well as the percent of time spent in the open arms of the EPM. Data obtained from the rotarod test showed that these compounds did not cause any change in the permanence time of mice on the rotarod bar.

Since bisabolol represented central nervous activity, we decided to determine the possible underlying mechanism. The anxiolytic-like effect of bisabolol in the EPM was significantly reduced by pretreatment with flumazenil but not WAY-100635. Flumazenil, a benzodiazepine receptor antagonist, reversed the effects of bisabolol on the number of entries as well as time spent in the open arms, suggesting that this compound might act through the GABAergic system. Consistent with our results, it has been shown that several terpenoids exert their anxiolytic-like effects via GABAergic system (Melo et al. 2010; Costa et al. 2014; Kessler et al. 2014; Moreira et al. 2014).

In summary, the present study showed that acute administration of bisabolol leads to anxiolytic-like effects in mice. Parameters observed in the open field and rotarod tests support the idea that bisabolol, especially at higher doses, possibly possesses depressor activity on the central nervous system. Furthermore, our results suggest that the anxiolytic-like effects of bisabolol are related to an interaction with the $GABA_A$ receptor, probably at the receptor subtypes that mediate benzodiazepines effects. Considering low toxicity of bisabolol as it has been granted by the Food and Drug Administration (FDA) with Generally Regarded as Safe (GRAS) status (Kamatou and Viljoen 2010), additional studies with chronic administrations are needed to prove this activity and fully clarify the involved mechanisms.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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