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Anxiolytic-like effect of the extract from *Bowdichia virgilioides* in mice

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Article

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Abstract: Current therapeutic for the treatment of anxiety is associated with a wild variety of side effects. The traditional use of plant extract to health care can indicate an important source of new pharmaceuticals. *Bowdichia virgilioides* Kunth, Fabaceae, is a plant commonly employed in the Brazilian folk medicine to treat inflammatory conditions. Nevertheless, despite its popular use there are no studies related to its possible neuropharmacological effect. Here, we investigated the possible anxiolytic effect of the extract of *B. virgilioides* after acute and sub-chronic treatment in mice. The aqueous extract from the stem barks of *B. virgilioides* (20, 200 or 400 mg/kg) was orally administered, and its anxiolytic effect was evaluated in the elevated plus maze, open-field and rota-rod tests. Diazepam was employed as standard drug. The aqueous extract treatment was effective in inducing anxiolytic effects with single acute treatment, a phenomenon that remained after chronic treatment. However, no changes in spontaneous locomotor activity or myorelaxant effect after aqueous extract treatment. The extract was either safe with no deaths in mice treated orally with 1000 mg/kg. These findings suggest that the aqueous extract from the stem barks of *Bowdichia virgilioides* has an acute and sub-chronic anxiolytic-like effect without compromising motor activity, demonstrating an advantage regarding to antidepressant drugs.

Introduction

Anxiety is considered a common emotional phenomenon in the human population, occurring in response to physiological and/or environmental factors. However, it can be a cause of disturbance in daily life and can express a pathological state (Clement & Chapouthier, 1998). Pharmacological treatment for anxiety disorder consists of the use of benzodiazepines and antidepressant drugs; however, although these treatments showing clinical efficacy, they have several problems. Benzodiazepines can lead to disturbing effects, such as amnesia, dependence liability, and sedation. The antidepressants can lead to sexual dysfunction, insomnia, and gastrointestinal disturbances (Mitte et al., 2005; Zarrindast et al., 2008). Thus, there is a need for the development of new anxiolytic drugs that lack these effects. In this context, medicinal plants continue to have anxiolytic potential (Carlini, 2003).

Several traditionally used plants exhibit pharmacological properties with great potential for therapeutic applications in the treatment of central nervous system disorders, such as anxiety disorders (Faustino et al., 2010). In folk medicine, some species belonging to the family Fabaceae, such as *Erythrina falcata* and *Melilotus officinalis*, are known to possess anxiolytic action (Almeida, 2010). Moreover, Ribeiro et al. (2006) in recent studies revealed anxiolytic-like effects of another member of Fabaceae family, *Erythrina velutina*, in animal models of anxiety. This study showed that acute and chronic administration of the hydroalcoholic extract of stem bark from *E. velutina* impaired inhibitory avoidance in the elevated T-maze test, which suggests an anxiolytic-like effect (Ribeiro et al., 2006).

Bowdichia virgilioides Kunth, Fabaceae, is popularly known as "sucupira-preta" in northeastern Brazil (Almeida et al., 1998). In folk medicine, its seeds are used in the treatment of rheumatism, arthritis, and skin diseases; its bark is employed as an antidiabetic agent and is utilized for healing in the topical treatment of inflammations (Albuquerque et al., 2007). There are few scientific studies dealing with the biological activity of this species, but some reports have demonstrated its antimalarial property (Deharo et al., 2001) as well as an important antinociceptive action in models of peripheral analgesia (Silva et al, 2010) and anti-inflammatory activity (Thomazzi et al., 2010). Previous investigations have described the presence of several components in this species, such as essential oil (Arriaga et al., 1998), alkaloids (Torrenegra et al., 1989),

flavonoids (Velozo et al., 1999), triterpenoids (Melo et al., 2001), and resins (Machado, 1936). Although these secondary metabolites has been reported as anxiolytic-like in previous studies, we found no reference on activity of aqueous extract from *B. virgilioides* on the central nervous system relating to anxiety as well as information on its acute toxicity. Since all these the secondary metabolites are possessed of an anxiolytic-like effect, it is reasonable to predict that extract crude that gathers them all may exert therapeutic effects on treat anxiety.

Considering that current therapeutics for the treatment of anxiety, such as benzodiazepinics, have well-known benefits for treating anxiety, and its use is associated with different side effects, including tolerance that limits their use, the replacement with herbal remedies could provide real cure over long time treatment. Therefore, the objective of the present work was to evaluate the anxiolytic effect of the aqueous extract from *Bowdichia virgilioides* in mice.

Materials and Methods

Plant material

The stem barks of *Bowdichia virgilioides* Kunth, Fabaceae, were collected in March 2006 at the Arboretum of the Federal University of Alagoas, Brazil. The plant was taxonomically identified by the expert in Botanics Rosangela P. Lyra Lemos of the Alagoas Environment Institute (Instituto de Meio Ambiente de Alagoas), and a voucher specimen was deposited in the IMA herbarium situated in Maceió, Alagoas, Brazil, under number MAC29914.

Preparation of the plant extract

The stem barks of *B. virgilioides* were dried at ambient temperature and triturated. The aqueous extract of *B. virgilioides* (AEBv) was prepared by infusing 50 µg of powdered plant material for 20 min using 300 µmL of boiling water. After filtration, the extract was concentrated until total solvent evaporation was achieved by means of a rotary evaporator, and lyophilization was accomplished. The yield of the infusion was 17.2%. The extract was reconstituted in 0.9% NaCl solution (vehicle) before the treatments at the required doses.

Animals

Male Swiss mice (20-25g) were obtained from the Central Animal Housing facilities of the Federal University of Alagoas. The animals were maintained in cages under standard conditions of controlled light (light/dark cycle of 12 h) at 22±2 °C and access to food and water *ad libitum*. All animal experimentation was carried out in accordance with institutional ethics guidelines of the Federal University of Alagoas (license number: 010095/2009-67).

Drugs and treatments

Animals were randomly assigned to one of two administration regimes: Acute or sub-chronic. Within each treatment, animals received by gavage (*p.o.*) a volume of 200 µL, and were further divided into five treatment groups (n=9/treatment): *B. virgilioides* extract (AEBv, 20, 200 or 400 mg/kg), diazepam (5 mg/kg, *i.p.*; Roche, Brazil), and vehicle (NaCl, 0.9 %).

For the acute treatment groups, animals received a single dose with AEBv (20, 200 or 400 mg/kg), diazepam or vehicle. One hour after the treatment, each animal was conducted to behavioral tests. Animals in the sub-chronic groups, received daily treatments for seven days and were tested 24 h following the final dose. The *B. virgilioides* extract dose and the protocol treatment were based on preliminary studies (Thomazzi et al., 2010; Silva et al., 2010; Anuradha et al., 2008; Harsha & Anilakumar, 2013) with different extract doses administered to mice with some modifications.

Behavioral tests

To assess the effect of AEBv on the behavior of the studied animals, two types of behavioral models were investigated: elevated plus maze test and open-field test. The animals were used only once for the behavioral test, and the apparatus was cleaned with 10% alcohol between each experiment.

Elevated plus maze test

Behavior in the elevated plus maze (EPM) is used to assess exploration, anxiety, and motor behavior. The possible anxiolytic effects of the aqueous extract of AEBv were assessed, basically using the same method described by Komada et al. (2008). The EPM consists of four arms, 40 cm long and 20 cm wide, arranged in such a way that the two arms of each type were opposite to each other. The maze was elevated 50 cm above the floor. Two arms were enclosed by walls 30 cm high and the other two arms were exposed.

Mice received AEBv (20, 200 or 400 mg/kg, *p.o.*), or vehicle once per day for seven days to sub-chronic treatment, while that to acute treatment 1h after oral administration (AEBv or vehicle) was conducted the behavior test. The positive control diazepam (5 mg/kg, *i.p.*) was given once 30 min before the test. After treatment, each animal was placed at the center of the maze facing one of the enclosed arms. During a 5 min test period, the number of open and enclosed arms entries, as well as the

time spent in open arms was recorded. Entry into an arm was defined as the point when the animal places all four paws into the arm.

Open-field test

The locomotor and exploratory behavior was assessed in an OFT. The open-field was made of glass cube and surrounded by walls 30 cm in height. The floor of the open-field, 10 cm in length and 10 cm in width, was divided into nine squares. Animals were evaluated 1 h after a single oral dose of vehicle, AEBv (20, 200 or 400 mg/kg) or diazepam. Each animal was placed individually at the center of the apparatus and observed for 5 min to record the locomotor (expressed by squares entered and crossing with the four paws) and exploratory activities (expressed by the number of rearing on the hind limbs).

Rota rod test

To discard possible nonspecific effects of the AEBv on motor coordination or on muscle relaxation the mice were tested on the rota-rod. The animals were trained on the apparatus for two days before the experiment. On the day of the experiment, mice were treated with AEBv (200 or 400 mg/kg, *p.o.*) or diazepam (5 mg/kg, *i.p.*) and tested in the rota-rod from 60 min after their administration. After treatment, the motor performance time (s) was recorded with a stopwatch for up to 240 s (de Oliveira et al., 2012).

Acute toxicity test

The acute toxicity test for AEBv was carried out to evaluate any possible toxicity. Mice (n=6) of either sex were tested by administering at only dose

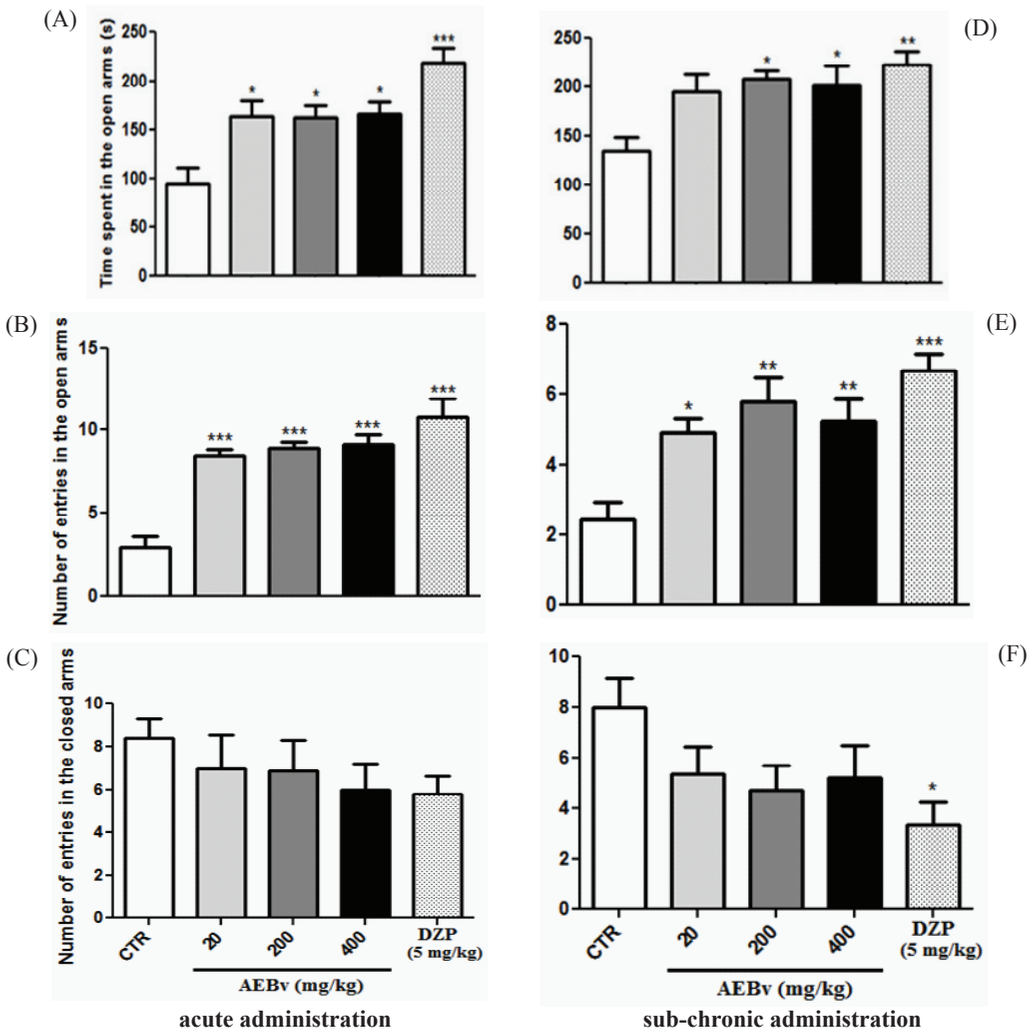

Figure 1. Effects of the aqueous extract from stem barks of *Bowdichia virgilioides* (AEBv) in the absolute time spent in the open arms (A and D), number of entries in the open arms (B and E), and number entries into closed arms (C and F) during 5 min of exposure to the elevated plus maze. Animals were acutely (A, B and C) or sub-chronically treated (D, E and F) with saline 0.9% NaCl (control group), AEBv (20, 200, and 400 mg/kg), or diazepam (DZP: 5 mg/kg) prior to the test. Data are presented as mean±SEM (n=9 per group). $^{*}p<0.05$, $^{**}p<0.005$, $^{***}p<0.0001$ compared to the control group.

1000 mg/kg (*p.o.*) of AEBv, while the control group received distilled water (10 mL/kg). All the groups were observed for any gross effect for the first 4 h and then mortality was observed after 24 h (Bruce, 1985).

Statistical analysis

Analysis and graphs construction were performed using the software GraphPad Prism version 5.00 (GraphPad Prism Software, Inc.). The results were analyzed by one-way ANOVA followed by Tukey's test, and data are presented as mean±SEM. Values of $p \leq 0.05$ were considered significant.

Results

As shown in Figure 1, acute treatment with AEBv at doses of 20, 200 or 400 mg/kg, *p.o.*, induced a sedative effect in mice when assessed by the elevated pluz maze test (EPM). The results (Figure 1A and B) showed that the time the animals remained in the open arms ($p<0.05$) and the number of entrances into the open arms ($p<0.001$) were significantly increased than compared with the controls. Diazepam (5 mg/kg, *i.p.*), used as a positive control, produced a statistically significant increase in both parameters. The other behavioral parameter evaluated in the EPM, such as entries on enclosed arms, was not significantly altered by any dose of AEBv than compared to control group (Figure 1C). These effects were similar to those obtained for diazepam (Figure 1C).

The sub-chronic treatment with AEBv showed a significant increase in the time that the animals remained in the open arms and the number of entrances into the open arms (Figure 1D and E). In addition, as well as in acute treatment, the sub-chronical protocol did not affect the number of entries on enclosed arms (Figure 1C).

In the open-field test, both treatments with AEBv (20, 200 or 400 mg/kg, *p.o.*) acute (Figure 2A and B) or sub-chronic (Figure 2C and D) did not was able to alter the number of crossings and rearings, as compared to control group. Moreover, mice treated with one only dose of diazepam showed alterations in the locomotor activity when compared with the control group (Figure 2A). In addition, no difference was observed in locomotor activity between groups after acute and sub-chronic treatment.

The rota-rod test was carried out to measure locomotor impairment in mice treated acutely or repeatedly with AEBv. In this study, AEBv-treated mice did not show any significant motor performance alterations with the dose of 200 or 400 mg/kg *p.o.* (223.63±11.58 s and 209.88±11.90, respectively) as compared to saline-treated animals (187.25±34.54 s) in the rota-rod test. As expected, diazepam (positive control, 5 mg/kg, *i.p.*) reduced the motor performance time of mice after 30 min of treatment

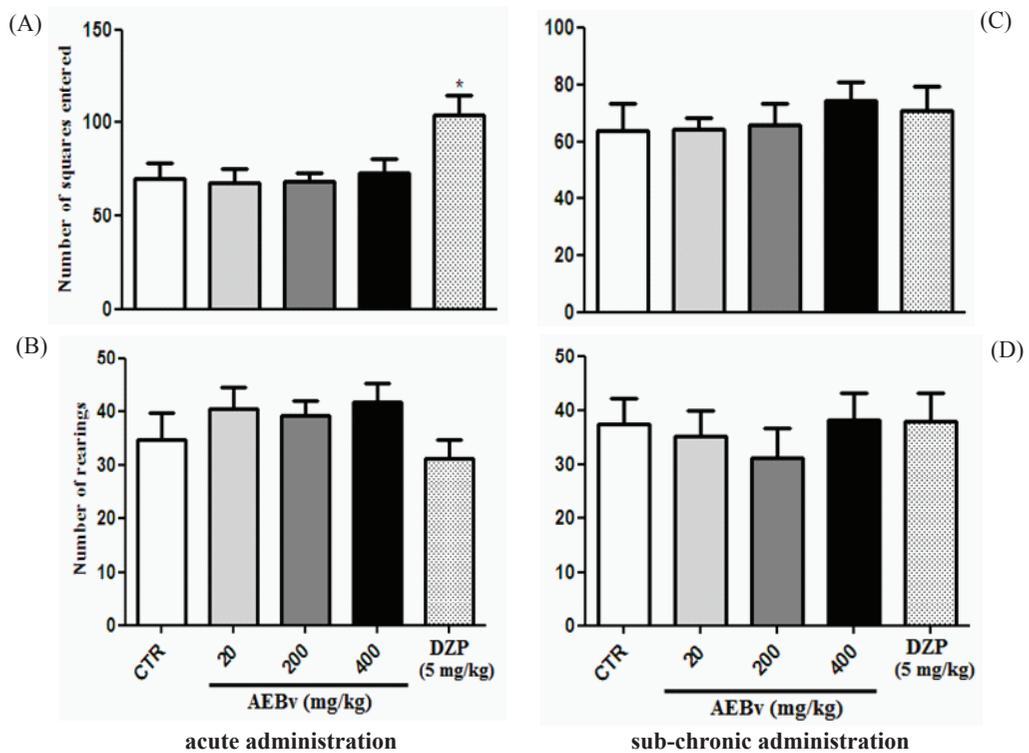

Figure 2. Effects of the aqueous extract from stem barks of *Bowdichia virgilioides* (AEBv) on the total number of squares entered (A and C) and number of rearings (B and D) during 5 min of exposure to the open-field test. Animals were acutely (A, B) or sub-chronically treated (C, D) with saline 0.9% NaCl (control group), AEBv (20, 200, and 400 mg/kg), or diazepam (DZP: 5 mg/kg) prior to the test. Data are shown as mean±SEM (n=9 per group). $^{*}p<0.01$ compared to the control group.

(29.69±18.21 s). Furthermore, no gross behavior change or mortality was observed at the tested doses and, therefore, the extract is considered to be safe up to the dose of 1000 mg/kg.

Discussion

Despite its use in traditional Brazilian medicine to treat pain, inflammatory diseases, or even act in wound healing, *Bowdichia virgilioides* Kunth, Fabaceae, has not yet been evaluated for its activity on the CNS. Furthermore, to our knowledge there are no reports about sedative or anxiolytic effects of *B. virgilioides*. Thus, the aim of the present study was evaluate, for the first time, the anxiolytic properties produced by aqueous stem bark extract from *B. virgilioides* (AEBv) in behavioral models.

The present study showed that acute or sub-chronic administration of AEBv, in the elevated plus-maze test, induced an increase in the exploration and the time spent into the open arms (*i.e.*, anxiolytic-like action) in a non-dose-related way. The number of entries into the enclosed arms did not changed by the oral treatment with the AEBv in comparison to the control values. The elevated plus-maze is a well-accepted, experimental animal model typically used to test the effectiveness of anxiolytics drugs (Pellow et al., 1985; Hogg, 1996). Drugs that increase the open arms exploration are considered anxiolytics and the reverse holds true for anxiogenic compounds (Handley & McBlane, 1993). As expected, diazepam increased the activity in the open arms of the elevated plus-maze apparatus, confirming its anxiolytic actions.

It is widely accepted that benzodiazepinic after a continuous administration promotes anxiolytic effect. However, this drug also produces effects as anticonvulsants, and also produces sedation and myorelaxant effects after chronicle usage (Melo et al., 2006). In this study, we observed that the anxiolytic effects of EABv were achieved after acute treatment, not being necessary the use of the extract for a prolonged period. Thus, the extract can exert an acute effect which can be very interesting since first choice drugs as antidepressant induce its therapeutic effect only after the third week of continuous use.

Data in the literature demonstrated that drugs that alter general motor activity may give false-positive/negative results in the plus maze test (Treit & Fundytus, 1988). Therefore, in order to verify the relation between the anxiolytic effect of AEBv and a locomotor activity alteration, it was decided to use, in our protocol, the open field test, a classic animal model used to assess the autonomic effects of drugs and the general activity of animals (Prut & Belzung, 2003). In this test, the groups that received treatment acute or sub-chronical with AEBv at doses which produced an anxiolytic-like effect did not alter locomotor activity phenomenon that was different of animals diazepam-treated, suggesting that this plant may not produce undesirable sedative side effects.

A deficit in motor coordination would very likely affect performance in the plus maze and open field tests. In this way, we aimed to investigate the effects of AEBv in the rota rod test, a classic animal model used to evaluate peripheral neuromuscular blockage. The findings showed that AEBv, in contrast to diazepam, had no significant effect on the motor coordination of animals on the rota rod test, suggesting that the anxiolytic-like effect might not be exerted through peripheral neuromuscular blockage, but rather, elicited centrally.

Presently, there are no reported active constituents from the stem barks aqueous extract of this plant; however, a large number of compounds, including alkaloids (Barbosa-Filho et al. 2004), lupeol, sitosterol and stigmasterol (Melo et al. 2001), have been reported in the literature. The anxiolytic effect of alkaloids (Martínez-Vázquez et al., 2012), flavonoids (Li et al., 2011) and saponins (Wei et al., 2007) has been previously reported and, therefore, we suggest that these anxiolytic effects may be due to the above constitutes. Moreover, cannot be discarded a possible mechanism of action anxiolytic of *B. virgilioides* mediated by a synergistic action of these phytochemicals.

In summary, even that the anxiolytic-like effect observed after sub-chronic treatment has exerted a more significant effect, we cannot discard the anxiolytic-like effect observed after acute treatment with the extract. This result shows an advantage over the commonly used drugs in the treatment of anxiety disorders. Thus, this is the first time that an anxiolytic-like effect that does not affect the locomotor activity is suggested for AEBv, at the doses tested herein. Further studies are underway, in order to elucidate the possible mechanisms involved in the anxiolytic activity of this extract.

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Authors' contributions

LFAV and MDSR performed experiments, and participated in the experimental design, data analysis, and writing the manuscript. ARAB participated in preparation of the plant extract. IMMNV participated in open-field test. JPS participated of both tests rota-rod and acute toxicity. EB participated in the data analysis and in writing the manuscript. SS participated in designing the experiments, data analysis and writing the manuscript. All the authors have read the final manuscript and approved the submission.

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