Academic Sciences Asian Journal of Pharmaceutical and Clinical Research
Vol 5, Suppl 4, 2012 ISSN - 0974-2441

Research Article

EVALUATION OF ANTI-ANXIETY ACTIVITY OF *PLECTRANTHUS AMBOINICUS* (LOUR.) ON RATS

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Received: 1 January 2012, Revised and Accepted: 7 February 2012

ABSTRACT

The purpose of this study was to characterize the putative anxiolytic-like effects of the aqueous and alcoholic extract of leaves of *Plectranthus amboinicus* (AQPA & ALPA) using the elevated plus maze (EPM) and the light-dark test (LDT) in rats. Control rats were treated with an equal volume of 2% acacia suspension, and positive control rats with diazepam (2mg/kg). Single treatments of the aqueous/alcoholic extract of *Plectranthus amboinicus* (250 & 350 mg/kg, i.p.) significantly increased the time-spent and entries into open arms of the EPM, and reduced the time-spent and entries into the closed arms versus saline controls ($P < 0.05$). In the light-dark test, AQPA & ALPA (250 and 350 mg/kg, i.p.) and diazepam (2 mg/kg, i.p.) prolonged the time spent in the light area and entries in to light area. Neither diazepam nor the AQPA & ALPA extract produced any overt behavioral change or motor dysfunction in the EPM and LDT. These results indicate that AQPA & ALPA extract is an effective anxiolytic agent.

Keywords: Anxiolytics; Diazepam; Elevated plus maze; Light-dark test; *Plectranthus amboinicus*

INTRODUCTION

Anxiety affects one-eighth of the total population world-wide and has become an important area of research interest in psychopharmacology during this decade[1]. Benzodiazepines are the major class of compounds used in anxiety and they have remained the most commonly prescribed treatment for anxiety. However, the realization that benzodiazepines present a narrow safety margin between the anxiolytic effect and those causing unwanted side effects has prompted many researchers to evaluate new compounds in the hope that other anxiolytic drugs will have less undesirable effects[2]. The recognition of anxiolytic effects of non-benzodiazepine azapirones agents, which act as 5-HT_{1A} partial agonists, such as buspirone, gepirone, and ipsapirone and their therapeutic role in clinical anxiety and mood disorders has further focused attention on the 5-HT_{1A} receptor[3]. Although the azapirones interact with other neurotransmitter systems, such as the dopaminergic and noradrenergic and they display nanomolar affinity for 5-HT_{1A} receptor sites[4]. However, the anxiolytic effects of azapirones follow a time course observed with antidepressants where therapeutic effects are delayed for 3-4 weeks, which is unlike the rapid effects observed with benzodiazepine anxiolytics[4]. On the basis of these considerations, it was the purpose of this study to characterize the anxiolytic-like activity of an AQPA & ALPA extract prepared from the leaves of *Plectranthus amboinicus* (PA, Lamiaceae). The genus *Plectranthus* contains about 350 species, being found in Tropical Africa, Asia and Australia[5]. The *Plectranthus amboinicus* (Lamiaceae) is a native species from Asia and distributed in America. It is use frequent in folk medicine against inflammations and respiratory infections[6]. Previous pharmacological studies showed that *P. amboinicus* possesses antiepileptic, antioxidant[7], leishmanial[8] and antimicrobial properties[9, 10, 11]. The leaves of this specie contain essential oils, flavonoids, terpens and cinnamic derivates[12]. These phytocompounds possess anti-inflammatory and chemotherapy effects[13]. In Brazil, especially in the Northeast Region, the use of this medicinal species is a practice widely employed, mainly in the form of crude extracts and infusions, to the treatment of several diseases including inflammations, and cancer[14]. Thus, in the present study, the anxiolytic activity of an AQPA & ALPA extract prepared from the leaves of PA was examined using the elevated plus maze (EPM) and Light-dark test (LDT) in rat. In addition, it was of interest to investigate which receptor systems are involved in the anxiolytic-like effects of AV through the co-administration of the benzodiazepine-antagonist flumazenil and the 5-HT_{1A} receptor antagonist WAY-100635[15]. We report here the results of *in vivo* experimental studies with the AQPA & ALPA extract from the leaves of *P. amboinicus* in Elevated plus maze and Light-dark test.

MATERIALS AND METHODS

Plant material

The fresh leaves of *P. amboinicus* were collected from local gardens of Salem, India during the month of December 2009. Dr. S. Ravi Kumar, Department of Botany, Gov. Art and Science Collage, Salem, Tamil Nadu, India, performed the botanical identification.

Plant extracts

Crude aqueous and alcoholic extracts (AQE & ALE) were prepared by maceration of 500 gm. remnants of the leaves, with water and ethanol 70% (v/v), for 72 h. The obtained extract was filtered and concentrated in a rotary evaporator at 45 °C under reduced pressure, yielding 24.79% & 4.53% w/w respectively.

Animals

Wistar albino rats, weighing 260–270 g, were obtained from the animal house of the Department of Pharmacology of the Truba Institute of Pharmacy, Bhopal, India. Animals were housed at four per cage, allowed free access to water and food, and maintained under constant temperature (23±1 ^{0}C) and humidity (60±10%) under a 12-h light/dark cycle (light on 07.30–19.30 h). Animal treatment and maintenance were conducted in accordance with the Principles of Laboratory Animal Care (NIH publication no. #85-23 revised 1985).

Experimental design

A total number of 36 rats were divided into six groups of six rats each:
Group I: control (2% acacia solution, 2ml/kg)
Group II: standard (Diazepam, 2mg/kg)
Group III: Test (Aq. Extract of *P. amboinicus* (250mg/kg B.Wt)
Group IV: Test (Aq. Extract of *P. amboinicus* (350mg/kg B.Wt)
Group V: Test (Alcoholic Extract of *P. amboinicus* (250mg/kg B.Wt)
Group VI: Test (Alcoholic Extract of *P. amboinicus* (350mg/kg B.Wt)

Procedures

Elevated plus-maze test

The elevated plus-maze comprised two open (50 cm×10 cm×25 cm) and two enclosed (50 cm×10 cm×40 cm) arms that radiated from a central platform (10 cm×10 cm) to form a plus sign. The maze was constructed of black painted wood. A slight raised edge on the open arms (0.25 cm) provided additional grip for the animals. The plus-maze was elevated to a height of 50 cm above floor level by a single central support. Four 25W red fluorescent lights arranged as a cross

at 100 cm above the maze were used as the source of illumination[16]. The experiment was conducted during the dark phase of the light cycle (9:00–14:00 h). The trial was started by placing an animal on the central platform of the maze facing an open arm. The number of entries into, and the time spent in, each of the two types of arm, were counted during a 10 min test period. The percentage open arm entries and percentage open arm time were used as indices of anxiety. A rat was considered to have entered an arm when all four paws were on the arm. The apparatus was cleaned thoroughly between trials with damp and dry towels. All behavioral recordings were carried out with the observer unaware of the treatment the rat had received[17].

Light dark test

The apparatus consisted of two 20 cm×10 cm×14 cm plastic boxes: one was dark and the other was transparent. The mice were allowed to move from one box to the other through an open door between the two boxes. A 100W bulb placed 30 cm above the floor of the transparent box was the only light source in the room. A mouse was put into the light box facing the hole. The transitions between the light and the dark box and time spent in the light box were recorded for 5 min immediately after the mouse stepped into the dark box[18, 19]. The apparatus was cleaned thoroughly between trials. All behavioral recordings were carried out with the observer unaware of the treatment the mice had received.

Statistical analysis

All analyses were performed using SPSS V11.5 software for Windows. All the data were given as means±S.E.M. Data were analyzed by one-way ANOVA. Whenever ANOVA was significant, further comparisons between vehicle- and drug-treatment groups were performed using the Dennett's *t*-test. The level of statistical significance adopted was $P < 0.05$.

RESULTS AND DISCUSSIONS

The plant (*P. amboinicus*) containing alkaloids, saponin, kardenolids, bufadienolids also polyphenol and two flavonoids were identified as 4', 7-dimethoxy-5, 6-dihydroxyflavone (M10) and chrysosplenetin (M11)[20]. Flavonoids may be responsible for the neuropharmacological activity of the plant[21]. In the present study, we used the EPM & light dark model of anxiety to evaluate the anxiolytic effects of the aqueous and alcoholic extract of *P. amboinicus* this is a model which uses the natural fear of rodents to avoid open and elevated places. The ratio of open and closed area entries reflects a specific effect on anxiety, provided there is no concomitant change in the total number of entries (open + closed), however, this is not totally true for diazepam which increases preference for the open areas i.e. total entries . As expected, diazepam produced significant increases in time spend and in number of entries into the open arms & light chamber. Diazepam also increased the total number of entries. These data are in agreement with the results of other studies, where diazepam and other benzodiazepines have been shown to produce anxiolytic effects in a variety of anxiolytic screening procedures, including EPM and light dark model. The behavioral alterations induced by the *P. amboinicus* plant extract in the EPM provided anxiolytic effect because the *P. amboinicus* leaves extract at a dose of 350 mg/kg (AQPA & ALPA) significantly increased the arm entries in open arms and decreased the time spent and arm entries in the closed arms in a similar fashion; diazepam increased the time spent and arm entries in the open arms (Table no: 1 and Fig no: a & b) and the Light-dark model also provided anxiolytic effect because *Plectranthus amboinicus* leaves extract at a dose of 350 mg/kg (AQPA & ALPA) significantly increased the entries in light chamber and decreased the time spent and entries in the dark chamber in a similar fashion, diazepam increased the time spent and entries in the light chamber(Table no: 2 and Fig no: c & d). However, unlike many other plant extracts where an anxiolytic effect was accompanied by sedative action, increase in the dose of *P. amboinicus* exerted stimulation rather than sedation. In this study, the number of entries into open arm and time spent into open arm were taken & the number of entries in light chamber and time spent in light chamber were taken as a measure of anxiety by elevated plus maize and light dark method.

Table: 1 Anti anxiety activity of *Plectranthus amboinicus* on rat by using Elevated plus-maze model.

Group	Treatment	Dose	No. of entries in open arm	Time spend in open arm
Ist	Control (2% acacia suspension)	0.5 ml	4.35± 0.35	3.58± 0.35
IInd	Diazepam	2mg/kg	8.27±0.57**	7.49±0.45**
IIIrd	Test (2% acacia suspension of aq. Extract of P. amboinicus)	250mg/kg	4.97±0.41*	5.49±0.45*
IVth	Test (2% acacia suspension of aq. Extract of P. amboinicus	350mg/kg	5.45±0.41*	6.29±0.47**
Vth	Test (2% acacia suspension of alcoholic extract of P. amboinicus	250mg/kg	4.45±0.39*	4.69±0.32*
VIth	Test (2% acacia suspension of alcoholic extract of P. amboinicus	350mg/kg	6.37±0.49**	5.67±0.49*

All value are given in mean±SEM, *$P < 0.05$, **$P < 0.01$ as compare with the control group (one way ANOVA followed by Dunnett's test).

Table: 2 Anti anxiety activity of *Plectranthus amboinicus* on rat by using Light-dark test.

Group	Treatment	Dose	No. of entries in light chamber	Time spend in light chamber
Ist	Control (2% acacia suspension)	0.5 ml	2.49± 0.35	4.35± 0.49
II nd	Diazepam	2mg/kg	5.35±0.47**	7.65±0.45**
IIIrd	Test (2% acacia suspension of aq. Extract of P. amboinicus)	250mg/kg	3.25±0.45*	4.34±0.39*
IVth	Test (2% acacia suspension of aq. Extract of P. amboinicus	350mg/kg	3.97±0.47*	5.67±0.37**
Vth	Test (2% acacia suspension of alcoholic extract of P. amboinicus	250mg/kg	2.95±0.39	4.67±0.48*
VIth	Test (2% acacia suspension of alcoholic extract of P. amboinicus	350mg/kg	4.27±0.37**	5.95±0.49**

All value are given in mean±SEM, *$P < 0.05$, **$P < 0.01$ as compare with the control group (one way ANOVA followed by Dunnett's test).

Fig. a : Effects of AQPA & ALPA in the elevated plus-maze test in rat. Results are expressed as means±S.E.M. (*n* = 6). The following parameters are shown: no of entries in open arm. **P* < 0.05, ***P* < 0.01, compared with vehicle-treated animals.

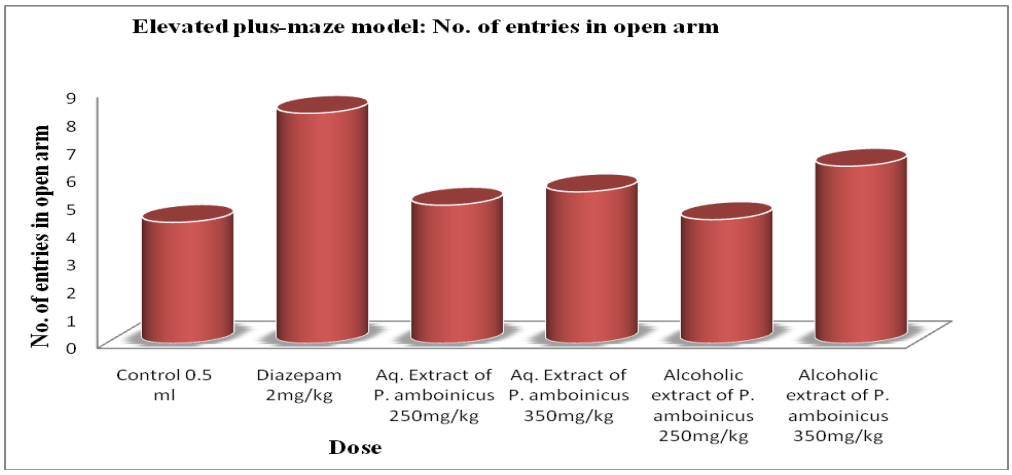


Fig. b : Effects of AQPA & ALPA in the elevated plus-maze test in rat. Results are expressed as means±S.E.M. (*n* = 6). The following parameters are shown; time spent in open arms. **P* < 0.05, ***P* < 0.01, compared with vehicle-treated animals.

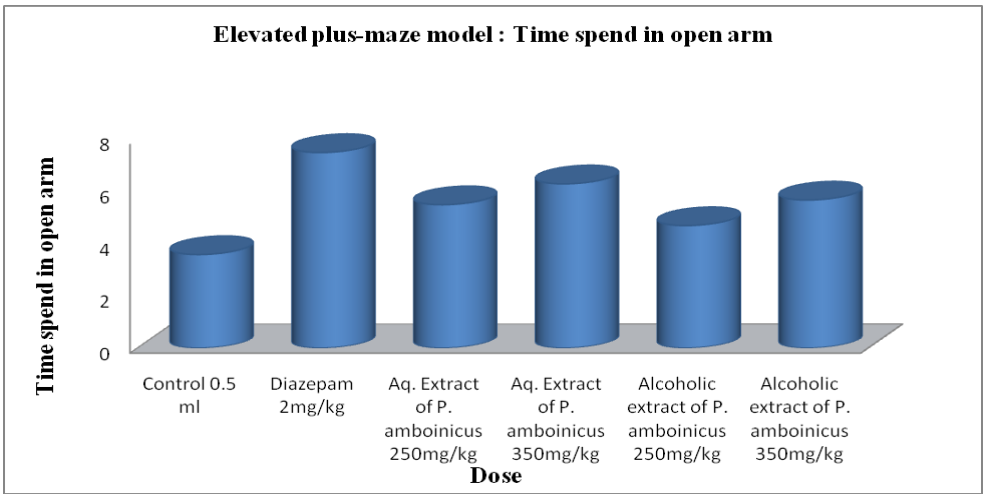


Fig. c. Effects of AQPA& ALPA in the light-dark test in rate. Results are expressed as means±S.E.M. (*n* = 6). The following parameters are shown; no of entries in light chamber. **P* < 0.05, ***P* < 0.01, compared with vehicle-treated animals.

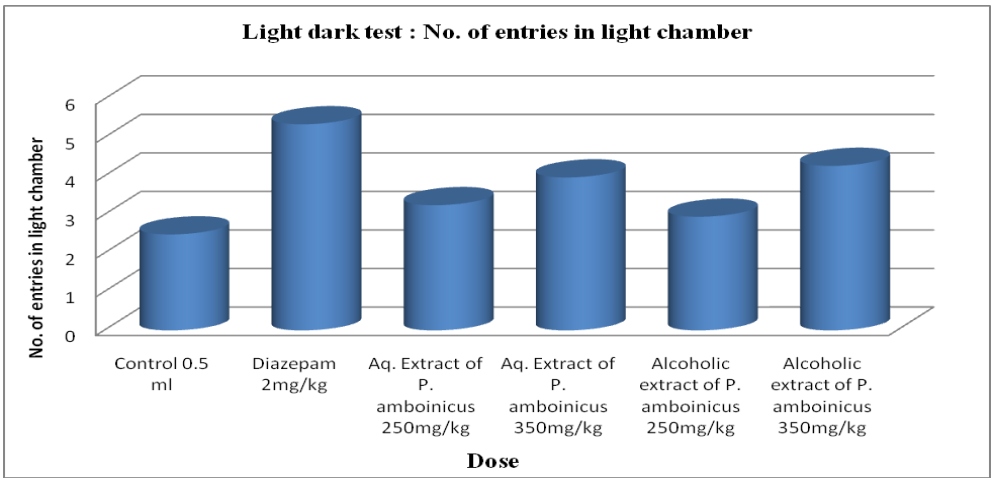
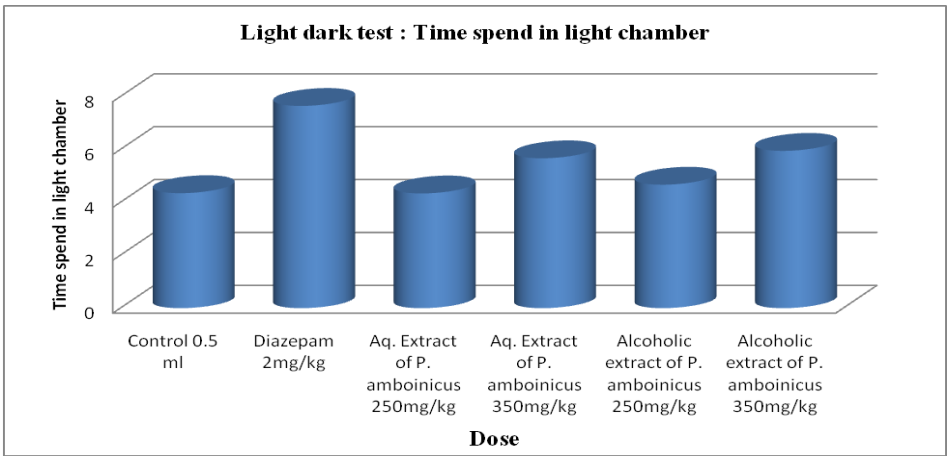


Fig. d. Effects of AQPA & ALPA in the light-dark test in rate. Results are expressed as means±S.E.M. (*n* = 6). The following parameters are shown; times spend in light chamber. **P* < 0.05, ***P* < 0.01, compared with vehicle-treated animals.



CONCLUSION

The findings in this study suggest that the *P. amboinicus* possess Anti- anxiety activity. The results have been obtained carefully from the controlled experiments model with laboratory animals. The statistical validity of the findings has been proven and they provide a scientific foundation for the use of the biologically active ingredients of *P. amboinicus* in anxiety for explain the clinical importance of the *P. amboinicus*.

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