INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com
ISSN 2230 – 8407

Research Article

EVALUATION OF ANTI-ANXIETY ACTIVITY OF *ANACYCLUS PYRETHRUM*

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Article Received on: 10/11/17 Approved for publication: 20/12/17

DOI: 10.7897/2230-8407.0812249

ABSTRACT

Aim of this study is to investigate anti-anxiety effect of ethanolic extract of *Anacyclus pyrethrum* using various experimental animal models. The anti-anxiety potential of the plant was demonstrated using a variety of models, such as Elevated plus maze test, Open field apparatus test and Light/dark exploration test. Ethanolic extract of *Anacyclus pyrethrum* at 250mg and 500mg/kg have shown significant anti-anxiety activity in all the models. Present findings will be definitely helpful in the management of anxiety with cost-effective and free from toxic effects.

Keywords: *Anacyclus pyrethrum,* anti-anxiety, elevated plus maze test.

INTRODUCTION

There is a need of new drugs for the treatment of anxiety because current anti-anxiety treatments like benzodiazepines, having several concerns associated with its use, mainly the increased incidence of dependence, tolerance, abuse liability, psychomotor impairment and potentiating the other CNS depressant drugs[1]. The various plants are being investigated in complementary alternative medicines for anxiety[2, 3]. Many Researchers are in the search of an alternate, more specific and cost-effective therapy with least adverse reactions for the treatment of anxiety[4]. Literature survey reveals the traditional use of natural products as anxiolytics for the management of neurological disorders and which is gaining a lot of interest.

In due regard, we have undertaken present herbal drug research aimed to investigate the Anacyclus *pyrethrum* for the anxiolytic activity which is known to possess anti-anxiety property in Ayurvedic system of medicine, but lacks preclinical evidence in experimental animals.

MATERIALS AND METHODS

Plant material

The *Anacyclus pyrethrum* roots were collected from local market after identification and authentication by Dr. M. B. Mulimani, Professor of Botany, S.B Arts and K.C.P. Science College, Bijapur, Karnataka. A voucher specimen (AP04) has been deposited at the herbarium of Dept. of Pharmacology, HSK College of Pharmacy, Bagalkot.

Preparation of extract

Fresh roots were air dried, pulverized to a coarse powder by using grinder and passed through a 40-mesh sieve. Then the powdered material was packed into Soxhlet column and extracted with ethanol. Then the extract was concentrated using rotary flash evaporator and percentage yield of the same was recorded.

Preliminary phytochemical screening

Test extract was subjected to preliminary phytochemical screening for the detection of various phytoconstituents. Tests for the presence of phytoconstituents were performed by following the standard procedures described in the literature[5].

Total Phenolic Content

The total phenolic content of *Anacyclus pyrethrum* extract was determined UV spectrophotometrically using folin-ciocalteu method. Aliquots (0.1 ml) of the extract was mixed with 0.5 ml of folin-ciocalteu reagent and made up to 3 ml with distilled water. After 3 min, 2 ml of sodium carbonate (20%) was added and mixed thoroughly. The sample was then incubated for 5 min at 50°C and cooled. The absorbance was measured at 650 nm against the blank. The total phenolic content was expressed as mg gallic acid equivalent per gm of extract. The coefficient of determination was $r^2 = 0.9968$.

Total Flavonoid Content

The total flavonoid content of *Anacyclus pyrethrum* was estimated using aluminium chlroride method. A volume of 0.5 ml of $AlCl_3$ ethanol solution (2 %) was added to 0.5 ml of sample solution. After 1 h incubation at room temperature, the absorbance was measured at 420 nm. Extract samples were evaluated at a final concentration of 0.1 mg/ml. The total flavonoid content was calculated as mg quercetin equivalent per gm of extract. The coefficient of determination was $r^2 = 0.9965$[6].

Experimental animals

In the present study, albino mice (20 – 30 g) of either sex were used. These animals were procured from BLDEA's Sri B.M.Patil Medical College, Hospital and Research Center, Bijapur. Before initiation of experiment, the rats were acclimatized for a period of 10 days under standard environmental conditions such as temperature (26 $\pm$ 2^{0}C), relative humidity (45-55%) and 12 hr dark/light cycles. All the animals were fed with rodent pellet diet (VRK Nutritional industries, Pune, India) and water was allowed *ad-libitum* under strict hygienic conditions. Ethical clearance for performing the experiments on animals was obtained from Institutional Animal Ethics Committee (IAEC clearance No. BPC/IAEC/20).

Determination of Acute toxicity (LD_{50})

The acute toxicity of *Anacyclus pyrethrum* extract was determined in female albino mice; this is because literature surveys of conventional LD50 tests show that, females are generally slightly more sensitive. In this study, the animals were fasted for 4 hr before the experiment. After dosing, food but not water was withheld for further 1 hr. Mortality and general behaviour of the animals observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hr, with special attention given during the first 4 hr, and daily thereafter, for a total of 14 days. Fixed dose method of OECD Guideline No. 423; (Annexure-2d: Starting dose is: 2000 mg/kg b.w) was followed for toxicity study. Based on the results of the study, 1/5th, 1/10th and 1/20th of LD_{50} cut off value were selected as screening doses for investigation of anti-stress activity[7].

Evaluation models for anxiolytic activity of *Anacyclus pyrethrum*

Elevated Plus-Maze (EPM)

The plus-maze apparatus consisting of two open arms (35 x 6 cm) and two closed arms (35 x 6 x 15 cm) extending from a central platform and were elevated to a height of 45 cm above the floor.

Albino mice of either sex weighing between 20-30 g were divided into 05 groups of 06 mice in each were fasted overnight prior to the test but water was supplied *ad libitum*.

Group I - Normal control was receive vehicle.
Group II - Diazepam (1 mg/kg, p.o.)
Group III - EEAPR (Ethanolic extract of *Anacyclus pyrethrum* root) 125 mg/kg, p.o.
Group IV - EEAPR 250 mg/kg, p.o.
Group V - EEAPR 500 mg/kg, p.o.

All the groups were received vehicle, standard and different test doses respectively once daily for 10 days. On 10th day one hour after the treatment, each mouse was individually placed on the center of the elevated plus maze with its head facing the open arm. During the entire experiment, mice were allowed to socialize. Every Precaution was taken out to ensure that no external stimuli, other than the height of the plus-maze could invoke maze anxiety. During the 5 min experiment, following behaviour of the mice was recorded

- ✓ Number of entries into the open arm and time spent in the open arm.

Open- Field apparatus test (OFT)

Albino mice (20-30 g) of either sex were divided into 05 groups of 06 mice in each were fasted overnight prior to the test but water was supplied *ad libitum*.

Group I - Normal control was received vehicle.
Group II - Diazepam (1 mg/kg, p.o.)
Group III - EEAPR 125 mg/kg, p.o.
Group IV - EEAPR 250 mg/kg, p.o.
Group V - EEAPR 500 mg/kg, p.o.

Group I was maintained as normal control received vehicle only once daily for 7 days, group II was received diazepam (1 mg/kg, p.o.), and Groups III, IV and V were treated with different doses of test extracts p.o. respectively once daily for 7 days. On 7th day 60 min after administration of the vehicle, standard and test extract, and each mouse was placed in the center of open field arena and the following parameters were recorded during a test session of 5 min.

- ✓ **Ambulation:** Measured in terms of the number of squares crossed by the animal and **Rearing:** Number of times, the animal stood on its hind limbs[8].

Light–dark test (LDT)

The light dark test is the sensitive model commonly used to detect activity in anxiety related disorders. This apparatus consists an acrylic box (40 cm × 60 cm × 20 cm) divided into light and dark chambers. The light chamber (40 cm × 40 cm) was painted white and connected via an opening (7 cm) at floor level to the dark chamber (40 cm × 20 cm), which was painted black. A lamp with a 60-W white light was placed 40 cm above the light chamber.

Albino mice (20-30 g) of either sex were be divided into 05 groups of 06 mice in each fasted overnight prior to the test but water was given ad *libitum*.

Group I - Normal control received vehicle only
Group II - Diazepam (1 mg/kg, p.o.)
Group III - EEAPR 125 mg/kg, p.o.
Group IV - EEAPR 250 mg/kg, p.o.
Group V - EEAPR 500 mg/kg, p.o.

The treatment was given once daily for 7 days. On 7th day 60 min after administration of the vehicle, standard drug and test extract to different groups, each mouse was placed in the light chamber facing the opening into the dark chamber, and the following observations were recorded manually during a 5-min trial:

- ✓ Time spent in the light compartment, number of squares crossed and duration of Immobility[9].

Statistical analysis

The data obtained from the above findings were subjected to statistical analysis following one-way ANOVA followed by Tukey's Kramer Multiple Comparison Test to assess the statistical significance of the results using GraphPad Prism-5 software. p-values less than 0.05 were considered as statistically significant.

Table 1: Effect of A*nacyclus pyrethrum* root extract on the elevated plus-maze behaviour in mice

Sl.No	Treatment	Dose	% Entry into open arm	% time spent in open arm
1.	Control	---	42.47±2.51	37.93±2.07
2.	Std (Diazepam)	1 mg/kg p.o.	78.29±5.32***	74.77±4.29***
3.	EEAPR	125 mg/kg p.o.	44.02±3.88^{ns}	49.52±3.31^{ns}
4.	EEAPR	250 mg/kg p.o.	64.37±4.73*	60.74±4.89**
5.	EEAPR	500 mg/kg p.o.	70.06±4.62**	68.58±3.47***

Values are Mean SEM, (n=6), ns Non-significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ as compared to control.

Table 2: Effect of A*nacyclus pyrethrum* root extract on the behaviour of animal in open field test

Sl.No	Treatment	Dose	No. of squares crossed	Rearing
1.	Control	---	122.47±2.51	10.38±1.49
2.	Std (Diazepam)	1 mg/kg p.o.	178.29±5.32***	28.82±2.64***
3.	EEAPR	125 mg/kg p.o.	134.02±3.88^{ns}	14.77±2.90^{ns}
4.	EEAPR	250 mg/kg p.o.	145.37±3.73**	22.58±3.03*
5.	EEAPR	500 mg/kg p.o.	164.06±4.62***	26.39±3.55**

Values are Mean SEM, (n=6), ns Non-significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ as compared to control.

Table 3: Effects of A*nacyclus pyrethrum* root extract on the light dark test behaviour in mice

Sl.No	Treatment	Dose	Time spent in Lighted box	No. of squares crossed	Duration of Immobility
1.	Control	---	97.49±11.72	10.90±1.64	38.48±2.21
2.	Std (Diazepam)	1 mg/kg p.o.	188.20±14.41***	31.03±2.25***	18.98±2.08***
3.	EEAPR	125 mg/kg p.o.	143.06±13.53^{ns}	16.48±2.62	28.33±2.65*
4.	EEAPR	250 mg/kg p.o.	166.78±12.38**	24.37±2.28**	24.52±2.46**
5.	EEAPR	500 mg/kg p.o.	182.94±13.44***	28.66±3.03***	20.97±2.67***

Values are Mean SEM, (n=6), ns Non-significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ as compared to control.

RESULTS

Effect of A*nacyclus pyrethrum* root extract on the elevated plus-maze behaviour in mice

The vehicle-treated mice (Normal control) had spent more time in closed arm and showed less entries in open arm compared to closed arm of the maze during 5 min. Animal treated with diazepam (Standard) showed significant ($p<0.001$) increase in the percentage of open arms entries as well as time spent in open arm. Oral administration of ethanol extract of *Anacyclus pyrethrum* (250 and 500 mg/kg) exhibited significant increase in the percentage of number of open arm entries and time spent in open arm [Table 1]. Whereas, at 125 mg/kg has also demonstrated increase in the percentage of open arms entries as well as time spent in open arm but results were found statistically non-significant.

Effect of A*nacyclus pyrethrum root extract* on open field test

In the open field test (OFT), diazepam and extract (*Anacyclus pyrethrum* 250 and 500 mg/kg p.o.) treated mice showed significant increase in the number of rearings and number of squares crossed during 5-min interval as compared to vehicle-treated control group. Whereas, the test extract at 125 mg/kg p.o. showed statistically non-significant results [Table 2].

Effects of A*nacyclus pyrethrum root extract* on Light dark test

Treatment with diazepam significantly increased the time spent ($P < 0.001$) in light box as well as the number of crossings between the light and dark boxes, but duration of immobility was significantly reduced. *EEAPR* treated mice also exhibited dose dependent significant increase in the time spent in light box and the number of crossings between light and dark boxes. The duration of immobility was also significantly reduced as compared to the vehicle treated group.

DISCUSSION

The results of the present study showed that EEAPR administered by oral route to mice produced a significant anxiolytic effect in three well-consolidated anxiety animal models: EPM, OFT and LDT. In these animal models, the anxiety-related behaviours in mice were significantly decreased indicates that anxiety in mice was relieved after treatment with extract.

In the present study, administration of EEAPR has produced an increase in time spent by mice in the illuminated side on the LDT indicating an anxiolytic effect of plant, which was confirmed by the increase in time spent in the open arms on the EPM.

The Elevated plus maze test is one of the most widely used validated model to study anti-anxiety agents. This test is based upon on the natural conflict between the tendency to avoid potential dangerous area and to explore a new environment. It used to evaluate psychomotor performance and emotional aspects of mice and rats. Results of our study on the elevated plus maze after treatment with ethanol extract of *A. pyrethrum* revealed the anxiolytic activity, since significant attenuation of anxiety like behaviour (increases the time spent in open arm) in EPM, most representative indices of anxiolytic activity[10]. Time spent on the central platform appears to be related to decision making and/or risk assessment, and the total arm entries is a measure reflecting changes in anxiety or in general activity[11].

The OFT is used to evaluate the animal emotional state. The open field model examines anxiety-related behaviour characterized by the normal aversion of the animal to an open, bright area. Thus, animals removed from their acclimatized cage and placed in environment express anxiety and fear, by showing alteration in all or some parameters. Anxiolytic treatment

reduces such fearful behaviour of animals in open field[12]. Statistical analysis of the data obtained from these experiments supported anxiolytic activity of plant extract as its effect shows significant increase in the number of rearings, number of assisted rearings and number of squares crossed, as compared to the vehicle-treated group, which indicates its anxiolytic effect.

The anxiolytic activity was also observed in the light/dark test. LDT is an ethological-based approach-avoidance conflict test. It is sensitive to drugs that affect anxiety. In this test, the number of transitions between the light and dark compartments as well as the time spent in the light compartment is recognized as anxiety indices, despite the transition parameter being highly dependent on locomotor activity[9]. Mice treated with ethanol extract of *A. pyrethrum* showed increase in the time spent in the light compartment and also changes in the numbers of shuttle crossings, confirming the activity upon the main anxiolytic parameter. The observed anxiolytic effect of the title plant may be due to the agonistic effect on GABA/benzodiazepine receptor complex, or 5-HT1A receptor or antagonize the 5-HT1B receptor[13, 14].

Earlier reports on the chemical constituents of plants and their pharmacology suggest that plants containing flavonoids, alkaloids, phenolic compounds, saponins and tannins possess activity against many CNS disorders[15]. Investigations on the phytochemical screening of EEAPR revealed the presence of alkaloids, glycosides, steroids, saponins, tannins, phenolic compounds and flavonoids. It is possible that the mechanism of anxiolytic action of title plant could be mediated by these phytochemicals.

ACKNOWLEDGEMENT

We would like to thank the Management, BVVS's HSK College of Pharmacy, Bagalkot for providing necessary facilities to perform this research.

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Cite this article as:

Patil Virupanagouda P *et al*. Evaluation of anti-anxiety activity of *Anacyclus pyrethrum*. Int. Res. J. Pharm. 2017;8(12):46-49 http://dx.doi.org/10.7897/2230-8407.0812249

Source of support: Nil, Conflict of interest: None Declared

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