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NEUROPHARMACOLOGICAL PROFILE OF ETHANOLIC EXTRACT OF *ANACYCLUS PYRETHRUM* IN ALBINO WISTAR RATS

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ABSTRACT

The present study was carried out to evaluate CNS activity of ethanolic extract of roots of *Anacyclus pyrethrum* that includes general behavior studies, sedative, muscle relaxant, anxiolytic, nootropic activity and antidepressant studies in rats. The results reveal potential neuropharmacological activity of *Anacyclus pyrethrum* as nootropic and also having antidepressant property. Further neurochemical investigation can unravel the mechanism of action of plant drug with respect to nootropic and antidepressant activity. Preliminary investigation showed that ethanolic extract of *Anacyclus pyrethrum* has significant neuropharmacological activity.

INTRODUCTION: Herbal medicine emphasizes prevention of disease, rejuvenation of our body systems and it extends the life span and makes healthy life, balance and harmony [1]. Medicinal herbs are indispensible part of traditional medicine practiced all over the world due to easy acess, low cost and ancestral experience. *Anacyclus pyrethrum* (AP), family Asteraceae is used in traditional system of medicine and it is regarded as a tonic to the nervous system [2]. The roots contain anacyclin, pellitorine, hydrocarolin, inulin, traces of volatile oil and seasamin. *Anacyclus pyrethrum* is a perennial, procumbent herb, which is found throughout India. The plant roots are reported for anti-inflammatory [3], immunostimulating [4], anabolic, aphrodisiac activities [5]. However, no investigation reports exist pertaining to central nervous system activity, hence we decided to study in experimental animal models, the neuropharmacological effects of ethanolic extract of Anacyclus *pyrethrum* root that includes general behavior studies, sedative, muscle relaxant, anxiolytic, nootropic activity and antidepressant studies.

MATERIALS AND METHODS:

Plant Material: The roots of *Anacyclus pyrethrum* were procured from ayurvedic drug store in Trivandrum, Kerala and the sample was authenticated for their correct botanical identity by Professor Jayaraman, National Institute of Herbal Science, Chennai and specimen of the root has been be deposited in the department. Its voucher specimen no is 0997.

Preparation of Extracts: The roots of *Anacyclus pyrethrum* were powdered (500g) and ethanolic extract was prepared by simple maceration process using 2 L of ethanol. The ethanolic extract was evaporated under reduced pressure using rotavapor evaporator. The yield of the extract was 0.93%w/w. A suspension was prepared using 2%v/v tween 80 and administered orally.

Animals: Albino Wistar rats of either sex approximately of same age group having weight 150- 200g were used after they were acclimatized for a week under laboratory conditions. They were provided standard rodent pellet diet (Lipton India) and water *ad libitum*. The animals had free access to food and water and were maintained under 12: 12 hr light and dark cycle. All experiments were carried out during day time from 09.00 to 17.00 hr. The institutional animal ethical committee approved the protocol and care of animals was taken as per guidelines of committee for the purpose of control and supervision in experiments on animals (CPCSEA), representative of animal welfare, Govt. of India.

Preliminary Phytochemical Analysis: Preliminary phytochemical investigation was conducted as per procedure described by Kokate [6].

Acute Toxicity Study: Acute toxicity studies were performed according to the OECD 423 guidelines. Female Wistar rats weighing 100-150gm was selected and divided into groups containing three animals per group. The animals were fasted overnight and were provided with only water. All groups were administered 5mg/kg body weight ethanolic extract orally and observed for 14 days. If mortality was observed in one animal, then the same dose was repeated again to confirm toxic dose.

If mortality was not observed, the procedure was repeated for further higher doses such as 50, 300 & 2000mg/kg body weight. The treated animals were carefully observed individually for the toxicity signs and mortality. Parameters such as changes in skin and fur, eyes and mucous membranes, circulatory, respiratory, autonomic and central nervous system, behavioral pattern, tremors, convulsions salivation, diarrhoea, lethargy, sleep and coma were observed [7].

Neuropharmacological Activity:

General Behavior Studies: Evaluation of general behavioral profiles was performed by the method [8-9]. Albino Wistar rats were divided in to five groups (n=6). Ethanolic extract of *Anacyclus pyrethrum* was administered for first three groups at dose of 50, 100 and 200mg/kg p.o respectively. While the last two groups were administered diazepam (5mg/kg) as drug control and 2%v/v tween 80 as vehicle control. The animals were under observation for their behavioral changes if any, at 30 min intervals in the first one hour and at the hourly intervals for the next 4 hour for the following parameters.

Awareness, Alertness and Spontaneous Activity: The awareness and alertness was recorded by visual measure of the animal's response when placed in a different position and its ability to orient itself without bumps or falls. Animals usually show a moderate degree of inquisitive behavior

Righting Reflex: Groups of rats were treated with the test compounds on the test day. After 15, 30 and 60 min, each rat was placed gently on its back on an undulated surface made of white iron and kept at 30^{o}C. If the animal remained on its back for 30 sec, it was considered as a loss of righting reflex.

Pinna Reflex: The reflex is examined by touching the centre of pinna with a hair or other fine instrument. The unaffected rat withdraws from the irritating hair.

Grip Strength: The grip strength test is used to assess muscular strength or neuromuscular function in rodents. It was measured by allowing the animal to grasp a pencil in the horizontal position and noting the time taken by the animal to drop the pencil on the table.

Touch Response: The touch response was recorded by touching the rat with a pencil or forceps at the various part of the body (i.e. on the side of the neck, abdomen and groin).

Pain Response: The pain response was graded when a small artery clamp was attached to the base of the tail, and response was noted.

Sound Response: Albino Wistar rats normally utter no sound, so vocalization may indicate a noxious stimulus.

Locomotor Activity: Locomotor activity (horizontal activity) was measured using actophotometer Rats were divided into five groups consisting of 6 per group. Three groups received the extract at a dose of 50, 100,200mg/kg body wt. The other two groups received control vehicle 2%v/v tween 80 and standard drug

(Diazepam 2 mg/kg, i.p). Locomotor activity is easily measured using actophotometer which operates on photoelectric cells connected with a counter.

When a beam of light falling on the photocell is cut off by the animal a count is recorded and displayed digitally. Each rat was placed individually in the activity cage floor for 10 min. The animals were placed in the actophotometer for recording the activity score after 60 min of drug and standard administration [10].

Effect on Motor Coordination: Rats were divided into five groups consisting of 6 rats per group. Three groups received the extract at a dose of 50, 100,200mg/kg body wt. The other two groups received control vehicle 2%v/v tween 80 and standard drug (Diazepam 2 mg/kg, i.p). All the groups of rats were trained to remain on the rota rod for three min. Only those rats which could balance themselves were selected for the study. The animals were discarded and replaced if they failed to do so. All the group animals were placed on the rota rod and the number of falls within 3 min was noted 60 mins after test drug administration and standard administration [11].

Assessment of Anxiolytic Activity in rats using the Holeboard Apparatus: Anxiety level were also evaluated in rats using a holeboard apparatus .The hole board apparatus consisted of wooden box (40×40×25 cm) with 16 holes (Diameter, 3cm) evenly distributed in the floor. The hole board was elevated to the height of 25 cm. The test was performed 60 min after administration of ethanolic extract of AP (50, 100,200mg/kg p.o), control vehicle2%v/v tween 80 and standard drug (Diazepam 2mg/kg, i.p). The number of head poking during 5 min period was recorded and the percentage decrease in head poking was also calculated [12]. An increase of the hole poking response reveals a positive anxiolytic like effect [13].

Nootropic Activity using Elevated Plus Maze: The nootropic activity was assessed using the elevated plus maze**.** Rats were divided into five groups consisting of 6 rats per group. Three groups received the extract at a dose of 50, 100,200mg/kg body wt. The other two groups received control vehicle2%v/v tween 80 and standard drug piracetam (200 mg/kg, p.o).The elevated plus maze considered is the exteroceptive behavioral model to evaluate learning and memory. The apparatus consisting of two open arms (50cms × 10cms) and two covered arms (50cms × 10cms × 40cms) extended from a central platform (10cms × 10cms) was elevated to a height of 50cms from the floor. On the first training day, each animal was placed at the end of an open arm facing away from the central platform.

Transfer latency (TL) was taken as the time taken by the rat to move into any one of the covered arms with all its four legs. TL was recorded on the training day. If the animal did not enter into the one of the arm within 90secs, it was gently pushed into one of the covered arms and the TL was assigned as 90secs. The animals was allowed to explore to the maze for 10secs and then returned to its home cage. Transfer latency was examined on 6^{th} day and after 24hrs on 7^{th} day of drug treatment. Significant reduction in transfer latency value indicates improvement in memory [14].

Assessment of Antidepressant Activity in rats using Forced Swim Test: Rats were divided into five groups consisting of 6 rats per group. Three groups received the extract at a dose of 50, 100,200mg/kg body wt. The other two groups received control vehicle2%v/v tween 80 and standard drug imipramine (15 mg/kg, p.o). Forced swim test is the most widely used pharmacological model for assessing antidepressant activity [15]. The development of immobility when rodents are placed in an un-escapable cylinder of water reflects the cessation of persistent escape directed behavior.

The apparatus consisted of transparent cylinder (50cmhigh x20cmwide) filled to 30 cm depth with water at room temperature. In pre test, rats are placed in cylinder for 15 min 24 hr prior to 5min swim test. Extracts and standard dose was administered 1hr prior to swim test Duration of immobility was recorded during 5min swimming test, a rat was judged to be immobile when it floated in a upright position, making small movements to keep its head above water. Increase in active response such as climbing or swimming and reduction in immobility are considered as behavioral profile consistent with antidepressant like action [16].

Statistical Analysis: The data are expressed as mean ± SEM. Statistical analysis was done using one way

analysis of variance (ANOVA) followed by Dunnet's test.

RESULTS: The preliminary phytochemical screening carried out on ethanolic extract of *Anacyclus pyrethrum* revealed the presence of phytoconstituents such as alkaloids, tannins, flavonoids, saponins, fixed oils. The extract did not produce any toxic symptoms of mortality up to dose level of 2000mg/kg body weight in rats and hence the drugs were considered safe for further pharmacological screening. Rats treated with extract of *Anacyclus pyrethrum* and submitted to general behavioral profile studies did not show any difference in their behavior. They were alert, with normal grooming, touch response, sound response, pain response and motor activity, grip strength were normal. The animals showed no signs of depression during the observation period. However, the standard drug diazepam caused a significant depression of all these responses compared with the ethanolic extract of *Anacyclus pyrethrum*.

The ethanolic extract of AP in a dose of (50mg/kg, 100mg/kg and 200mg/kg, p.o) did not produce statistically any significant reduction in locomotor activity as compared to the control animals receiving only the vehicle. Diazepam treated groups revealed a statistically significant decrease in locomotor activity as compared to the control. Results were shown in **table 1**.

TABLE 1: EFFECT OF ETHANOLIC EXTRACT OF ANACYCLUS PYRETHRUM AND DIAZEPAM ON LOCOMOTOR ACTIVITY IN RATS, USING ACTOPHOTOMETER APPARATUS

Treatment	Locomotor activity (scores) in 10 min	
	Before treatment	After treatment
Control (vehicle, p.o)	466.16±11.79	450.83±8.72
AP (50mg/kg p.o)	477.66±5.49	483.16±6.49
AP (100mg/kg p.o)	491.5 ± 28.60	416.16 ± 28.42
AP (200mg/kg p.o)	486.83 ± 12.93	403.33 ± 20.11
Standard (Diazepam 2mg/kg i.p)	499.6 ± 23.98	4.16 ± 0.79*

Statistical significance test was done by ANOVA followed by Dunnet's 't' test (n=6); Values are mean ± SEM of 6 animals per group; *P<0.01vs control;AP-*Anacyclus pyrethrum*

There was no statistically significant increase in number of falls within 3 min after the treatment with ethanolic extract at AP which suggests that the extract does not have muscle relaxant property (Results shown in **table 2**). However, diazepam treated groups showed an increase in the number of falls as compared to the control.

TABLE 2: EFFECT OF ETHANOLIC EXTRACT OF *ANACYCLUS PYRETHRUM* AND DIAZEPAM ON MUSCLE RELAXANT ACTIVITY IN RATS, STUDIED USING ROTA ROD APPARATUS

Treatment	Number of falls in 3 min	
	Basal reading	After treatment
Control (vehicle, p.o)	7±0.57	8.33±0.33
AP (50mg/kg p.o)	9.3±0.49	7.66±0.61
AP (100mg/kg p.o)	8.5±0.34	9.6±0.55
AP (200mg/kg p.o)	8.5±0.80	7.5±0.56
Standard (Diazepam 2mg/kg i.p)	9.16±0.70	16.5±0.76*

Statistical significance test was done by ANOVA followed by Dunnet's 't' test (n=6); Values are mean ± SEM of 6 animals per group; *P<0.01vs control; AP-*Anacyclus pyrethrum*

The statistical analysis of data obtained indicated that the groups treated with ethanolic extract at dose level of 50mg/kg, 100mg/kg and 200mg/kg, p.o, show no significant increase in number of head poking compared to the control group. However diazepam treated group showed significant increase in exploratory activity thus indicating anxiolytic activity.. Results are shown in table 3.

TABLE 3: EFFECT OF ETHANOLIC EXTRACT OF *ANACYCLUS PYRETHRUM* AND DIAZEPAM ON ANXIETY INDUCED IN RATS USING HOLEBOARD APPARATUS

Treatment	Number of head pokings
Control (vehicle, p.o)	9.33 ± 1.35
AP (50mg/kg p.o)	9.83 ± 1.01
AP (100mg/kg p.o)	8.00 ± 1.18
AP (200mg/kg p.o)	7.16 ± 1.13
Standard (Diazepam 2mg/kg i.p)	1.83 ± 0.30*

Statistical significance test was done by ANOVA followed by Dunnet's 't' test (n=6); Values are mean ± SEM of 6 animals per group; *P<0.01; AP-*Anacyclus pyrethrum*

Transfer latency reflected retention of learned task or memory. AP treated animals at a dose of 50mg/kg, 100mg/kg and 200mg/kg, p.o showed dose dependant decrease in transfer latency on 6^{th} and 7^{th} day when compared to the control group. Higher dose of AP 200mg/kg p.o more significantly enhanced learning and memory when subjected to elevated plus maze test.

Piracetam 200mg/kg p.o treated for 6 days decrease transfer latency on 6^{th} day and after 24 hrs on 7^{th} day as compared to the control group, indicating

improvement in learning and memory. Results are shown in **table 4**.

TABLE 4: EFFECT OF ETHANOLIC EXTRACT OF *ANACYCLUS PYRETHRUM* AND PIRACETAM ON LEARNING AND MEMORY USING ELEVATED PLUS MAZE APPARATUS

Treatment	Transfer latency (secs)	
	Day 6	Day 7
Control (vehicle, p.o)	24.83±1.35	24.66±1.11
AP (50mg/kg p.o)	18.33±1.47*	15.08±0.42*
AP (100mg/kg p.o)	16.33±0.80*	15.16±0.70*
AP (200mg/kg p.o)	9.16±0.30*	10±0.57*
Piracetam (200mg/kg)	11.16±0.30*	10.33±0.80*

Statistical significance test was done by ANOVA followed by Dunnet's 't' test (n=6); Values are mean ± SEM of 6 animals per group; *P<0.001vs control; AP-*Anacyclus pyrethrum*

The effect of extract at dose level of 50mg/kg, 100mg/kg and 200mg/kg, p.o and impiramine on active behaviors in forced swim test of rats is shown in **table 5**. Ethanolic extract significantly shortened the immobility time in comparison to the control values. However impiramine treated group showed significant reduction in immobility time as compared to the control.

TABLE 5: EFFECT OF ETHANOLIC EXTRACT OF *ANACYCLUS PYRETHRUM* ON IMMOBILITY PERIOD IN FORCED SWIM TEST

Treatment	Immobility time (mean± SEM)
Control (vehicle, p.o)	35±1.6
AP (50mg/kg p.o)	30±1.50
AP (100mg/kg p.o)	22.33±1.18*
AP (200mg/kg p.o)	12.66±0.84*
Imipramine (15mg/kg i.p)	8.5±0.42*

Statistical significance test was done by ANOVA followed by Dunnet's 't' test (n=6); Values are mean ± SEM of 6 animals per group; *P<0.01 vs. control; AP-*Anacyclus pyrethrum*

DISCUSSION: General behavior studies suggest that ethanolic extract does not possess any neurotoxicity. The extracts of *Anacyclus pyrethrum* were found to have no effect on the locomotor activity. Locomotor activity is considered as an index of alertness and a decrease in the activity would indicate sedative activity. Experimental findings suggest the extracts did not demonstrate any effect on the muscle coordination, as indicated by the findings with respect to the rota rod.

In our investigation, the extracts did not produce any significant change or increase in the exploratory activity of the rats in the hole board method, hence, we can conclude that the extract does not possess anxiolytic activity.

Generally most of the anxiolytic agents have an adverse effect on memory as seen with the benzodiazepines, commonly used as anxiolytics [17].

Our findings indicated that ethanolic extract treated rats show remarkable dose dependent reduction in transfer latency, indicating significant improvement in memory, thus demonstrating nootropic activity. This facilitatory effect on learning and memory was observed only after treatment for a period of 7 days. This probably may be attributed to the involvement of neurotransmitters since the building of memory is augmented only when the levels of neurotransmitters are attenuated on repeated administration of the extracts. There is ample evidence demonstrating that the central cholinergic system, serotonergic transmission and noradrenaline function play a vital role in the cognitive function of the brain [18].

Moreover, the lack of effect on locomotor activity works to the advantage of the plant demonstrating nootropic activity. The forced swimming test demonstrated that ethanolic extract of *Anacyclus pyrethrum* clearly acted as antidepressant in rats .The reduction of immobility was comparable to observed effects after administration of reference antidepressant drug imipramine, a putative catecholaminergic involvement in the antidepressant like effects of *Anacyclus pyrethrum* extracts could be suggested.

The present findings indicate improvement of learning acquisition and observed antidepressant property of *Anacyclus pyrethrum* root extract, there by validating its claim as a nervine tonic in the Indian system of medicine. Considering the lack of need of drugs with proven effect in improving learning, specific memory improving and antidepressant effect of *Anacyclus pyrethrum* can be of enormous interest for further neurochemical investigation which can unravel the mechanism of action of plant drug with respect to activity.

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