Evaluations of antidepressant activity of *Anacyclus pyrethrum* root extract

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The present study was designed to screen antidepressant activity of *Anacyclus pyrethrum* (AP) root extract. An experiment was designed by different method such as Locomotor activity, Haloperidol-induced catalepsy, Forced swim test (FST), Tail suspension test (TST), Clonidine-induced hypothermia and Reserpine-induced hypothermia on Swiss male albino mice. Standard root extract of *Anacyclus pyrethrum* (AP root extract) showed an increase in ambulatory behaviour indicating a stimulant effect of the photoactometer. AP root extract produces a significant antidepressant effect in both FST and TST as they reduced the immobility. AP root extract was found to be effective in reversing hypothermia produced by clonidine and reserpine. In our study, we found that AP root extract inhibited haloperidol-induced catalepsy. These study suggest that AP root extract might produce antidepressant effect by interaction with adrenergic and dopamine receptor thereby increasing the level of noradrenaline and dopamine in brains of mice.

Key words: *Anacyclus pyrethrum,* antidepressant activity, forced swim test and tail suspension test

INTRODUCTION

Depression is a heterogeneous disorder that affects a person's mood, physical health and behaviour. Patient with major depression have symptoms that reflect changes in brain neurotransmitter, specifically norepinephrine (NE), serotonin and dopamine. The prevalence of major depression in the general population is estimated to suffer from depression. An estimated 5.8% of men and 9.5% of women experience the depressive episodes in their lifetime. Suicidal tendency remains one of the common outcomes of depression, with depressive illness being responsible for 60% of the death toll.[1-4]

Despite the advent of new molecules in the pharma cotherapy of depression, it is unfortunate that this disorder goes undiagnosed and untreated. Although the currently prescribed molecules provide some improvement in the clinical conditions of the patient.[5,6]

On other hand, medicinal plants are of great value in the field of treatment and cure of the disease with least risk and low side-effect profiles. Practical experiences and several modern research studies have clearly shown that therapy using it is better than using synthetic chemicals, being safer besides having synergetic effect of their active ingredient and the presence of certain mineral and salt. Ayurveda, the ancient traditional system of medicine, mentions a number of single and compound drug formulations of plant origin that are used for the treatment of psychiatric disorders.[7]

Anacyclus pyrethrum (AP), family Compositae, is a perennial, procumbent herb, which is found throughout India. AP root contains essential oils and an alkaloid pellitorine that is intensely pungent constituent with a mixture of isobutyl amide. Traditionally, AP plant is used in traditional system of medicine and it is regarded as a tonic to the nervous system.[8] The antibacterial and anti-inflammatory activities are reported of the AP root.[9,10]

Conditions of chronic inflammation exacerbate the sickness and depression like behaviours that develops in response to acute peripheral inflammation. Patients with major depression have been found to exhibit evidence of an activated innate immune response as reflected by increased biomarkers of inflammation, including innate immune cytokines, acute-phase proteins, chemokines and adhesion molecules.[11]

As AP possesses anti-inflammatory activity, it might be useful in depression.

MATERIALS AND METHODS

The AP root hydroalcoholic extract procured from Green Chem Pvt. Ltd. Banglore, and the roots of AP plant was extracted using alcohol and water. The extraction

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was repeated untill the active principles completely extracted into the medium. The extract was combined and concentrated to an optimum volume and purified. The aqueous layer was dried and powder was collected. Haloperidol and Imipramine was procured from Torrent Pharmaceuticals Ltd. Ahmedabad, Amitriptyline procured from Sun Pharmaceuticals Ltd, Baroda, Clonidine procured from Intas Pharmaceuticals Ltd. and Amphetamine procured from Department of Pharmaceutical Sciences, M.S. University, Baroda, India. Zendopa was procured from Ranbaxy India. Swiss male albino mice weighing 22-25 g were obtained from National Institute of Toxicology (Pune, India) and housed in groups of five under standard laboratory conditions of temperature and light/dark cycle. They had free access to standard pellet chow and water. Experiments were conducted between 09.00 and 16.00 hours. The animals were acclimatized to the laboratory conditions for not less than 10 days before behavioural experiment. All experiment work done in Laboratory of Pharmacology of Dr. D. Y. Patil Institute of Pharmaceutical Science and Research, Pimpri, Pune-4110018. Animals used in this study were treated and cared for in accordance with the guidelines recommended by the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) and the experimental protocol was approved by Institutional Animal Ethical Committee (IAEC/05-06/P-15).

Experimental Design

Locomotor activity

Test group mice were pretreated 30 minutes before an experiment with AP root extract (50, 100, 200 mg/kg p.o.) dissolved in distilled water, the standard group mice were pretreated with amphetamine (1 mg/kg i.p.) and the control group mice were pretreated with vehicle (distilled water) and placed in the digital photoactometer (INCO, Ambala, India) one by one, which consist of a cage which is 30 cm long and 30 cm deep with wire mesh at the bottom. A continuous beam of light from about six lights was made to fall on corresponding photoelectric cell; the photoelectric cell got activated when an animal crossed the beam of light and thereby cut-off the rays of lights falling on it. These cutoffs were counted for a period of 10 minutes and the figure was taken as a measure of the locomotor activity of the animal.[12]

Haloperidol-induced catalepsy

The AP root extract treatment was given to groups of mice 30 minutes before haloperidol (1 mg/kg). Animals were observed for severity for catalepsy using 'Bar test'. The phenomenon is measured as the time; the mouse maintained an imposed position with both front limbs extended and resting on 2.5 cm high glass bar (0.9 cm diameter). The animals were observed for the duration of catalepsy at 5, 15, 30, 60, 90 and 120 minutes after the treatment. The cutoff time was 10 minutes.[13]

Laboratory Model for Assessment of Antidepressant Activity

Forced swim test

Behaviour despair was proposed as a model to test antidepressant activity by Porsolt *et al.* Mice were forced to swim individually in a glass jar ($25\times12\times25$ cm^3) containing fresh water of 15 cm height and maintained at 25°C after an initial 2 minute period of vigorous activity, each animal assumed a typical immobile posture. A mouse was considered to be immobile when it remained floating in the water without struggling, making only minimum movements of its limb necessary to keep its head above water. The total duration of immobility was recorded during the next 4 minutes of a total 6 minute test.[14]

The changes in the immobility duration were studied after 30 minutes of administration of the AP root extract (50, 100, 200 mg/kg p. o.) in test group, imipramine (16 mg kg i. p.) in the standard group and vehicle in the control group.

Tail suspension test

The total duration of immobility induced by tail suspension was measured according to the method described by Steru *et al.* as a facile means of evaluating potential antidepressant. Mice were suspended on the edge of a table 50 cm above the floor by the adhesive tape placed approximately 1 cm from tip of the tail. Immobility time was recorded during a 6 minute period. Animal was considered to be immobile when it did not show any movement of the body and hanged passively.[15]

The changes in the immobility duration were studied after 30 minutes of administration of the AP root extract (50, 100, 200 mg/kg p o.) in the test group, imipramine (16 mg/kg i. p.) in the standard group and vehicle in the control group.

Clonidine-induced hypothermia

Animal received Clonidine (0.1 mg/kg i.p.) 30 minutes after the administration of vehicle or AP root extract. The rectal temperature was noted in degree centigrade with rectal probe connected to a thermometer after every 30 minutes for 120 minutes.[16]

Reserpine-induced hypothermia

Mice were randomized according to their basal rectal temperature and thereafter they were treated with Reserpine (2 mg/kg s.c.). After 18 hours of Reserpine treatment, AP root extract (50,100,200 mg/kg p.o.) and Amitriptyline (16 mg/ kg i.p.) were administered to respective test and standard groups. The rectal temperature was noted in degree centigrade with rectal probe connected to a thermometer after every 30 minutes for 120 minutes.[17]

RESULTS

Effect of AP Root Extract on Locomotor Activity

AP (50,100,200 mg/kg) increased locomotor activity significantly ($P<0.001$) as shown in Table 1, suggesting a psychostimulant effect.

Effect of AP Root Extract on Haloperidol- induced Catalepsy

In vehicle-treated mice, Haloperidol produces maximum catalepsy at 60 minutes. In AP root extract-treated test animals, significant reduction in duration of catalepsy was observed ($P<0.001$) shown in Table 2.

Effect of AP Root Extract on Forced Swim Test and Tail Suspension Test

The behavioural score of immobility in control, standard drugs and AP root extract-treated groups are shown in Figure 1. Single dose administration of AP root extract with different dose range in mice showed a decrease in immobility time as compared to control ($P<0.001$) and the effect was qualitatively comparable to the standard antidepressant drug.

Effect of AP Root Extract on Clonidine-induced Hypothermia

In vehicle-treated animals, clonidine produced fall in rectal temperature and the peak effect was observed 60 minutes after its administration. As shown in Figure 2, AP root extract and amitriptyline reversed the hypothermic effect produced by clonidine significantly ($P<0.01$ and $P<0.05$, respectively).

Effect of *AP* Root Extract on Reserpine-induced Hypothermia

Reserpine produced hypothermia in all experimental groups. After 60 minutes of treatment with AP root extract and Amitriptyline, Reserpine-induced hypothermia got reversed significantly ($P<0.01$) as shown in Figure 3.

DISCUSSION

In the present study, AP root extract was evaluated for

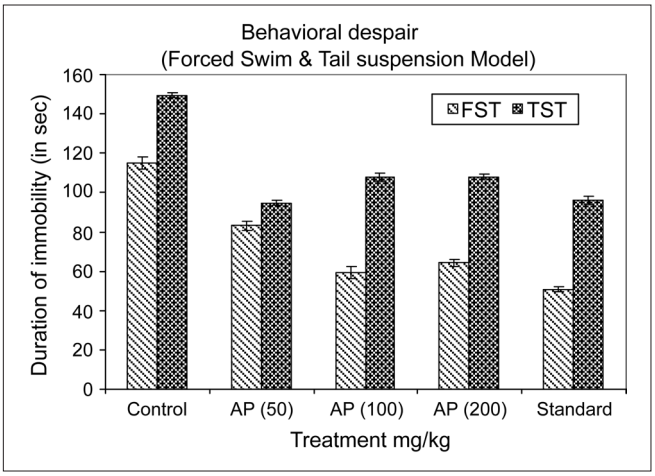

Figure 1: Effect of *Anacyclus pyrethrum* root extract on immobility period in forced swim test and tail suspension test. Control vehicle; AP 50 - AP root extract (50 mg/kg p.o); AP 100 - AP root extract (100 mg/kg p.o) AP 200 - AP root extract (200 mg/kg p.o); Standard Imipramine (15 mg/kg i.p.)

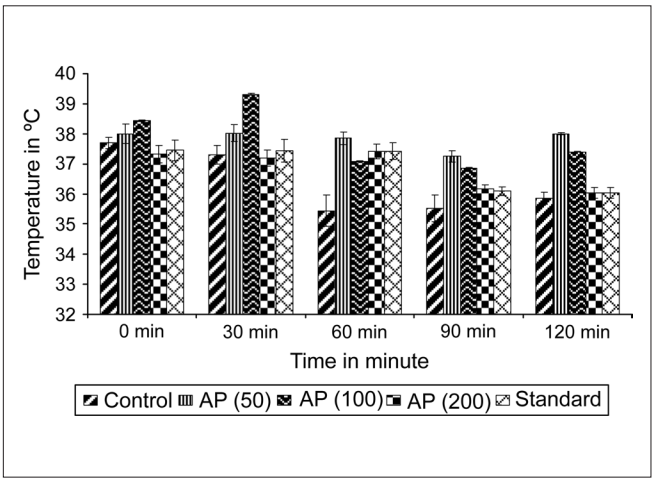

Figure 2: Effect of *Anacyclus pyrethrum* root extract on Clonidine-induced hypothermia. Control vehicle; AP 50 - AP root extract (50 mg/kg p.o); AP 100 - AP root extract (100 mg/kg p.o) AP 200 - AP root extract (200 mg/kg p.o); Standard- standard Amitriptyline (16 mg/kg i.p.)

Table 1: Effect of *Anacyclus pyrethrum* root extract on locomotor activity

Treatments mg/kg	Locomotor activity
Vehicle	293.6±2.581
AP (50)	325.2±3.555*
AP (100)	322.4±0.928*
AP (200)	325±1.859*
Amphetamine (1 mg/kg)	326±4.061

n=5; *$P<0.05$ (One way ANOVA followed by Dunnett's test)

Table 2: Effect of *Anacyclus pyrethrum* root extract on Haloperidol-induced catalepsy

Treatment mg/kg	Duration of catalepsy at different time					
	5 minutes	15 minutes	30 minutes	60 minutes	90 minutes	120 minutes
Vehicle	133.56±4.05	161.86±1.59	399.48±3.68	459.1±3.57	280.44±2.75	329.48±2.94
AP (50)	18.32±1.006*	36.22±2.06*	282.56±2.30*	277.28±3.40*	252.28±2.44*	318.24±3.33
AP (100)	17.74±2.81*	29.72±1.19*	219.61±2.06*	243.11±2.16*	215.88±1.63*	212.16±2.70
Zendopa (100)	23.74±1.05*	160.45±2.27	259.14±2.54*	457.71±2.33	245.12±2.32*	322.81±3.765

n=5; *$P<0.001$ compared with control (vehicle treated) group; AP = *Anacyclus pyrethrum;* (ANOVA followed by Dunnett's test)

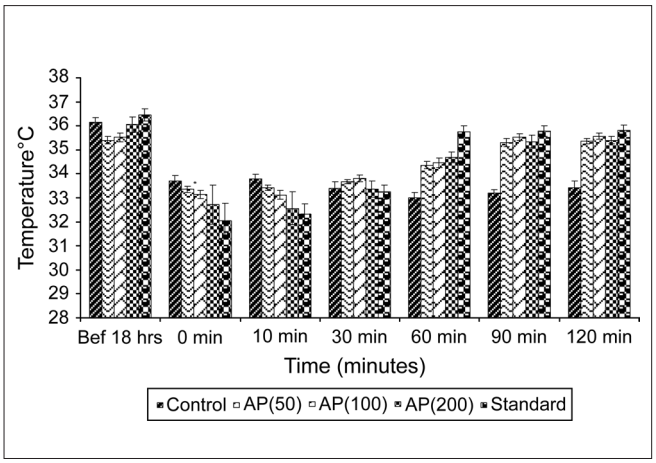

Figure 3: Effect of *Anacyclus pyrethrum* root extract on Reserpine- induced hypothermia. Control vehicle: AP 50 - AP root extract (50 mg/kg p.o): AP 100 - AP root extract (100 mg/kg p.o) AP 200 - AP root extract (200 mg/kg p.o), standard Amitriptyline (16 mg/kg i.p.)

antidepressant activity. The increase in ambulatory behaviour indicates a stimulant effect and AP root extract has shown stimulant activity in the photoactometer. In our study, we found that AP root extract inhibited haloperidol-induced catalepsy, and haloperidol induces catalepsy due to blockade of dopamine neurotransmission. In forced swim test (FST) and tail suspension test (TST), a normal animal submitted to a non-soluble aversive situation alternate between agitation and immobility. The reason of agitation is searching, it is highly energy consuming, while the purpose of immobility is energy conservation. Animals after antidepressant treatment struggle more even in desperate situation, and they spend less time with immobility. AP root extract produces significant antidepressant like effect in both FST and TST, as they reduced the immobility period. Clonidine is $alpha_2$ adrenoreceptor agonist. It has specificity towards the presynaptic $alpha_2$ receptors in the vasomotor center in the CNS. This binding inhibits the production of NE, thus decreasing sympathetic outflow, this result in fall in body temperature. While Reserpine produces hypothermia due to peripheral neuronal depletion of catecholamine, AP root extract was found to be effective in reversing hypothermia produced by clonidine and reserpine. From the discussion it is suggested that AP root extract might produce antidepressant effect either by interaction with adrenergic or dopamine receptor thereby increasing the level of noradrenaline and dopamine in brains of mice or by decreasing biomarkers of inflammation. Further detail investigation is underway to determine exact mechanism of antidepressant action of AP root extract.

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