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PHYTOCHEMICAL SCREENING OF ANTIDEPRESSANT ACTIVITY OF *ACYRANTHES ASPERA* BY USING FORCED SWIMMING TEST IN RATS

Rizwana Bee* and Dr. K. K. Maheshwari

Department of Pharmacy, M.J.P. Rohilkhand University, Bareilly, Uttar Pradesh (243006) India.

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***Corresponding Author**
Rizwana Bee
Department of Pharmacy, M.J.P. Rohilkhand University, Bareilly, Uttar Pradesh (243006) India.

ABSTRACT

Present research work was performed to evaluate the effect of *Acyranthes aspera* extract against physically induced depression in rats. Antidepressant activity of *Acyranthes aspera* extract was compared with imipramine. *Acyranthes aspera* extract was prepared by using maceration method. Physically induced depression parameters showed various behavioral abnormalities in experimental animals. Behavioral abnormalities were studied in rats by using forced swimming test apparatus. Rats were divided into 4 groups of 6 animals in each group. In forced swimming test Group I- distilled water (10 ml/kg), Group II- imipramine (20 mg/kg), Group III- *Acyranthes aspera* (400 mg/kg) and Group IV- *Acyranthes aspera* (400 mg/kg) + imipramine (20 mg/kg). analyzed the animals for antidepressant effect of *Acyranthes aspera* extract by using forced swimming test. The results showed that *Acyranthes aspera* extract 400 mg/kg and imipramine 20 mg/kg + *Acyranhes aspera* extract 400 mg/kg significant decrease immobility in forced swimming test apparatus. Thus, *Acyranthes aspera* extract may be included in effective treatment strategy of depression disorders.

KEYWORDS: Depression, *Acyranthes aspera*, forced swimming test, Imipramine.

INTRODUCTION

Depression is one of the most common affective disorder (it is defined as disorder of mood rather than the thought disturbances). In the world, depression is the major cause of disability and premature death. Individuals, those suffer from depression are more likely to die from other causes, such as heart disease or cancer. There are two different types of depressive disorder, first is *unipolar disorder*, in which up and down of mood and second is *bipolar*

affective disorder, in which cyclization of mood. *Bipolar disorder*, which usually occurs in early adult life (Rang et al., 2007). Depression is a psychological disorder that mostly affects an individual's mood, physical health and behavior. Depressed patients have symptoms that occurs due to the changes in brain, monoamine neurotransmitters, specifically nor epinephrine, serotonin, and dopamine (Gold et al., 1988). Depression is identified by feeling of sadness, hopelessness, despair and discouragement. Depression is also characterized by reduction thinking, pleasure concentration and impairment of sleep (Maheshwari, 2015).

Synaptic Transmission

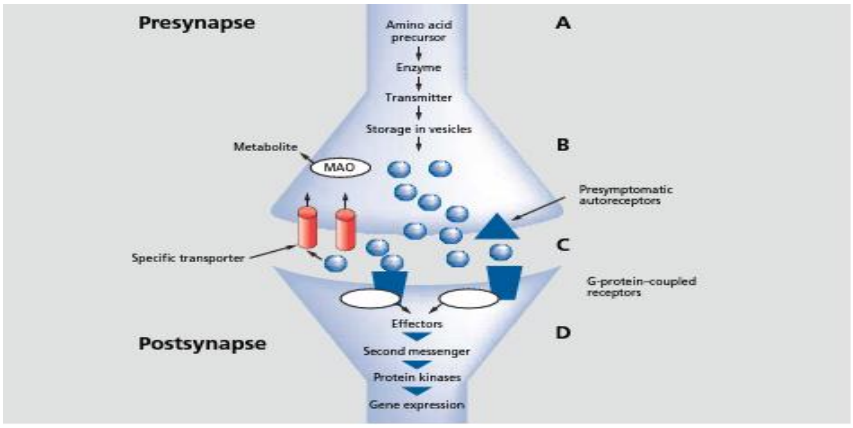

Fig. 1: Synaptic transmission (Bondy, 2002).

Diagrammatic representation of synaptic cleft, presynap, postsynap and neurotransmitter transmission.

A- Precursors that are transported from blood into brain.

B- Precursors converted into neurotransmitter via enzymatic processes.

C- The neurotransmitters are released into the synaptic cleft. Neurotransmitters that released into the synaptic cleft they either react with presynaptic autoreceptors to regulate synthesis and release, or interact with postsynaptic receptors to induce the events of the downstream signal transduction cascade.

D- Monoamine oxidase (MAO).

Chemical transmission is the major tool through which nerves communicate with each other. Now it is well known that the presynaptic and postsynaptic events are responsible for the plasticity and learning within the Central nervous system. Chemical transmission requires different types of steps including synthesis of the neurotransmitters, their storage in secretary vesicles, and their release into the synaptic cleft between presynaptic and postsynaptic cleft. The initial step of the synthesis of neurotransmitters is the facilitated transport of amino acids

from blood to the brain, in the brain precursors are converted into neurotransmitters enzymatically. These are stored in the synaptic vesicles, and finally released into the synaptic cleft via calcium dependent process. The release rate of neurotransmitters determined the rate of firing of neurons which means that the drug alter the firing rate of neurons. This modification of neurons carried out the alteration of release of neurotransmitters. After this released neurotransmitters bind with somatodendritic autoreceptors. Thus binding of neurotransmitter to auto-receptors is responsible for reducing the synthesis of neurotransmitters or additional release from the presynapes. The synaptic results of neurotransmitter are ceased via binding with specific receptors and reuptake into the pre-synapse. Neurotransmitters metabolized by monoamine oxidase enzymes in the presynapse (Kuhar et al., 2001).

Plant Description

Acyranthes aspera is a medicinal plant. It is found throughout India as an annual herb. It is also found in Asia and many parts of world such as Mexico, Central America and Africa. *Acyranthes aspera* consist of many antioxidants like alkaloids, terpenoids and saponins, which have various pharmacological properties. Different types of chemical constituents have been isolated from this plant by various techniques. All pharmacological properties and chemical constituents are used for the treatment of various human diseases (Sharma and Chaudhary, 2014). *Acyranthes aspera* globally available as a medicinal weed in Baluchistan, Ceylon, Tropical Asia, Africa, Australia and America. It is also present Shivbari and Himachal Pradesh. In India it is found in field boundaries and also present at the road sides (Sharma and Chaudhary, 2014). The plant grows to a height of 0.2 to 2.0 m. root is cylindrical, 0.1-1.0 cm in thickness. Root is yellowish- brown in color, which is of two types secondary and tertiary. Stem is square in shape and yellowish-brown in color. Leaves are simple, slightly acuminate, petiolate and ovate. Leaves contain anomocytic type of stomata on the lower epidermis. Flower is 8-30 cm long and 3-7 mm wide. It is bisexual greenish-white. It consist of 5 perianth segment, 5 stamens, short filament, 7 gynoecium bicarpellary, syncarpous, ovary superior, single ovule, style and single stigma. It is found in two different color red and white flower. Seeds are round at the base, which are brown in color (Sharma and Chadhary, 2014). The whole plant and seeds of *Acyranthes aspera* consist of an alkaline substance known as potash (Sanjay, 2015). It is common herbal drug in Ayurvedic, Unani-Tibbi, Siddha, Allopathic, Homeopathic, Naturopathic and Home remedies (Dhale and Bhoi,

2013). It is useful for the treatment of cough, renal dropsy, fistula, scrofula, skin rash, nasal, infection, chronic malaria, impotence, fever, asthma, piles and snake bites (Hasan, 2014).

Quantitative analysis of the leaves of *Acyranthes aspera*

Table 1: Quantitative analysis table (Dhale and Bhoi, 2013).

S. No.	Parameter	Range	Mean
1	Stomatal frequency (upper surface)	73-113	93
2	Stomatal frequency (lower surface)	162-190	176
3	Stomatal index (upper surface)	7.3-11.3	9.3
4	Stomatal index (lower surface)	16.2-19.0	17.65
5	Veinislet number	10.1-13.1	11.1
6	Vein termination number	8.5-13.1	10.8
7	Palisade ratio	1.13-1.18	1.16

MATERIALS AND METHODS

Plant material

The leaves of *Acyranthes aspera* plant were collected during the month of November-December, 2017 from the medicinal garden of the M.J.P. Rohilkhand University, Bareilly.

Preparation of *Acyranthes aspera* leaves extract

Fresh leaves of *Acyranthes aspera* were cleaned and washed thoroughly under running water. Washed fresh leaves were dried under the shade in clean and dust free environment. Dehydrate leaves were powdered with the help of grinder and stored in air tight container. About 250 gm of powdered leaves were extracted with 1000ml of 95% of methanol for 72 hours in beaker. This mixture was stirred every 18 hrs by using a sterile glass rod. The solvent (95% methanol) was filtered on 3rd day by using whatman filter paper no 1 and thus, the filtrate was obtained. The obtained filtrate of *Acyranthes aspera* was transferred to a petri dish and kept over the water bath (50°C) until the solvent gets completely evaporated. It was stored in air tight container at 4°C for further use. Recovery was 4.98% (w/w) (Barua et al., 2010).

Authentication

The collected leaves and *Acyranthes aspera* plant was authenticated by Department of Plant Science, M.J.P. Rohilkhand University, Bareiily. Authentication number is RU/PS/2016/415.

Identification tests of *Acyranthes aspera* extract

- **Dragendorff's test:** In this test dragendorff's reagent mixed with *Acyranthes aspera* extract. After some time this mixture produced an orange color precipitate.

- **Mayer's test:** In this test mayer's reagent mixed with *Acyranthes aspera* extract. After some time this mixture produced a cream color precipitate.
- **Froth formation test:** In this test placed 2 ml of solution of *Acyranthes aspera* in water in a test tube, shake well, stable foam is formed.

Animals

Albino wistar rats (150-200 gm) were selected from the animal house of Department of Pharmacy, M.J.P. Rohilkhand University, Bareilly. Animals were housed 2-4 rats per cage and fed on standard pellet diet and water ad libitum and kept in environmental controlled room at 25 ± 3°C and 50 ± 20% humidity with 12 hrs light/dark cycle. Animals are allowed 2-3 weeks to acclimate to the housing environment.

Drugs, chemicals and materials

The following drugs, chemicals and materials were used in the present research work.

- Imipramine
- *Acyranthes aspera* extract
- Distilled water
- 70% Ethanol
- 95% Methanol (solvent for extraction)
- Stopwatch without beepers
- Whatman filter paper no 1
- Cloth towel
- Standard rat group housing cage
- Forced swimming apparatus

Forced swimming apparatus

To measure the antidepressant activity in rats mainly forced swimming test is used. It is performed by using three transparent plastic cylinders each of having 15 cm diameter × 30 cm height, containing water (25°C ±2°C) up to depth of 10 cm.

Experimental Protocols

Treatment

Male rats were divided into four groups of six animals in each group as mentioned below. All the solutions were freshly prepared and administered in animals by intra-peritoneal and oral route.

Experiment protocol for forced swimming test

Table 2: Animal groups table (Forced swimming test).

GROUPS	TREATMENT
Group I	Distilled water (10 ml/kg, i.p.)
Group II	Imipramine (20 mg/kg, i.p.)
Group III	*Acyranthes aspera* (400 mg/kg, p.o.)
Group IV	*Acyranthes aspera* (400 mg/kg, p.o.) + imipramine (20 mg/kg, i.p.)

Distilled water Group (Group I, n=6)

Rats were administered distilled water (10 ml/kg) 24 hours and 30 min before forced swimming test on day 1 and day 2 respectively.

Imipramine treated Group (Group II, n=6)

Rats were administered imipramine (20 mg/kg) 24 hours and 30 min before forced swimming test on day 1 and day 2 respectively.

***Acyranthes aspera* treated Group (Group III, n=6)**

Rats were administered *Acyranthes aspera* extract (400 mg/kg) 24 hours and 30 min before forced swimming test on day 1 and day 2 respectively.

Imipramine + *Acyranthes aspera* treated Group (Group IV, n=6)

Rats were administered imipramine (20 mg/kg) and *Acyranthes aspera* (400 mg/kg) 24 hours and 30 min before forced swimming test on day 1 and day 2 respectively.

Perform pretest preparations

Setup the room environments, adjust the illumination. Before testing the apparatus will be clean with paper towel moistened with 70% ethanol and water. After this prepare each animal naïve to forced swimming test and open field test apparatus for the experimentation.

Forced swimming test

It is performed into two sessions, first session was performed for 15 minutes to the familiar of animals. The second test was performed for 5 minutes after 24 hrs of first test. Each and individual animal was transferred in the plastic cylinder from which they do not escape (Castagne et al., 2010).

STATISTICAL ANALYSIS

All results were expressed as mean ± SEM. Data was analyzed using on-way ANOVA followed by Dunnett's multiple comparison test using Graph pad prism. $P < 0.05$ was considered to be statistical significance.

RESULTS

Following groups are evaluated by using forced swimming test apparatus for depression. The immobility is noted down during the time session of 5 min. Each value of immobility is the mean value obtained from forced swimming test and expressed as mean ± SEM. According to the immobility parameter graphs were plotted and their activities were evaluated.

EFFECT OF DRUG ON PHYSICALLY INDUCED DEPRESSION USING FORCED SWIMMING TEST

- **Effect of drug on immobility**

Table 3: Effect of drug on immobility (Distilled water).

S. No	Treatments	Immobility
1	Distilled water (C)	173.33 ± 2.409
2	*Acyranthes aspera* (T)	75.66 ± 1.590****
3	*Acyrnthes aspera* + Imipramine (T+S)	77.83 ± 1.614****
4	Imipramine (S)	67.50 ± 1.500****

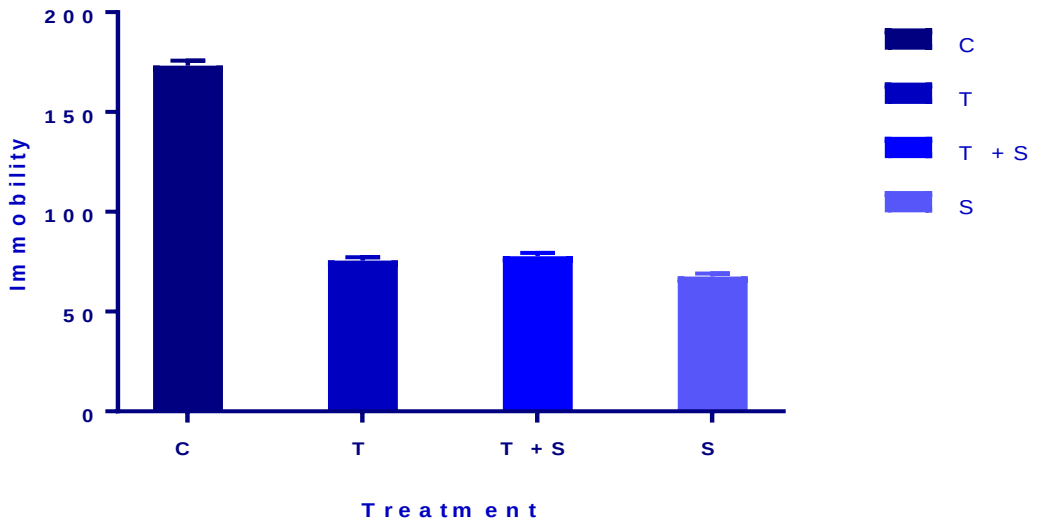

Fig. 2: Effect of drug on immobility (Distilled water).

Acyranthes aspera (400 mg/kg, p.o.) when compared to Distilled water (10 ml/kg, i.p.), *Acyranthes aspera* (400 mg/kg, p.o.) + imipramine (20 mg/kg, i.p.) and imipramine (20 mg/kg, i.p.) administered groups. The result was found to be significant decrease ($P < 0.0001$) immobility in *Acyranthes aspera* (400 mg/kg, p.o.) as compared to distilled water (20 mg/kg, i.p.) while there is no significant ($P < 0.0001$) difference in immobility in *Acyranthes aspera* (400 mg/kg, p.o.), *Acyranthes aspera* (400 mg/kg, p.o.) + imipramine (20 mg/kg, i.p.) and imipramine (20 mg/kg, i.p.) groups.

Acyranthes aspera extract (400 mg/kg, p.o.) + imipramine (20 mg/kg, i.p.) when compared to *Acyranthes aspera* (400 mg/kg, p.o.) and imipramine (20 mg/kg, i.p.). There was found to be no significant ($P < 0.0001$) difference in immobility in *Acyranthes aspera* extract (400 mg/kg, p.o.) + imipramine (20 mg/kg, i.p.), *Acyranthes aspera* (400 mg/kg, p.o.) and imipramine (20 mg/kg, i.p.) groups.

DISCUSSION

In the present study, the antidepressant activity of *Acyranthes aspera* extract against physically induced depression in rats was evaluated by forced swimming test apparatus forced swimming test apparatus standardized by Porsolt et al (1977a-b, 1978). Forced swimming test demonstrated antidepressant activity of *Acyranthes aspera* extract by decreasing the time period of immobility of rats using forced swimming test apparatus in dose dependent manners as compare to control group (Barua et al., 2010). It has been cleared

from the analysis of mechanism of action of antidepressant drugs that they act by increasing the neurotransmission at monoaminergic synapse by blocking the reuptake of 5-hydroxytryptamine and nor-adrenaline. Models of depression have various neurobiological effects. These effects reversed by the treatment of antidepressant drugs. It has been cleared from the study that antagonist of 5-hydroxytryptamine (5-HT) and noradrenaline (NA) at the synapse which is responsible for inducing the depression because these antagonize the specific components of antidepressant effects. This effect was firstly applied to the model in the context of dopaminergic effect of antidepressant drugs. After long treatment of antidepressant drugs of all categories increase the expression and functional sensitivity of dopamine receptors in the nucleus accumbens. It is described that antidepressant drugs act through the noradrenergic and 5-HT1A/5-HT2B receptors. These antidepressants increase the intracellular secondary messengers and protein kinase, leading to enhanced expression of CREB (cAMP response element binding protein) which is responsible for the expression of BDNF (brain derived neurotrophin factor) and other neurotrophins that prompt neurogenesis in the hippocampus and synaptogenesis in the hippocampus and PFC (prefrontal cortex). Neurogenesis is responsible for the renovation of damaged projections and again balanced the information processing in the forebrain (Wiilner et al., 2013, 2014). *Acyranthes aspera* extract act as antidepressant drug due to the presence of its chemical constituents like saponins, flavonoids and terpenoids. *Acyranthes aspera* extract act as antidepressant by increasing the serotonin release at the synaptic cleft (Goel, 2018). Different types of antidepressant models used for the evaluation of antidepressant drugs (Vogel, 2002). But there is no evaluation study of *Acyranthes aspera* extract as antidepressant drug by using forced swimming test apparatus so I decided to evaluate the antidepressant effect of *Acyranthes aspera* extract by using forced swimming test.

From the results, the following notable findings may be possible:

The following findings are summarized on the basis of results obtained in the present antidepressant activity of *Acyranthes aspera* extract by using forced swimming test apparatus.

- Imipramine, imipramine + *Acyranthes aspera* extract and *Acyranthes aspera* extract administered groups showed no significant difference in immobility means immobility decrease in all groups as compare to distilled water administered group. This result shows that *Acyranthes aspera* extract improve depression in rats.

- *Acyranthes aspera* extract administered groups showed significant decrease in immobility as compare to *Acyranthes aspera* + imipramine administered group. This result shows that the *Acyranthes aspera* extract improve the depression in rats.

CONCLUSION

Finally it may be concluded that, *Acyrantes aspera* extract exert a protective effect against physically induced depression in rats and may be asigned to its antidepressant effect. *Acyranthes aspera* extract 400 mg/kg significantly decrease the duration of immobility by using forced swimming test apparatus. Thus, *Acyranthes aspera* extract at 400 mg/kg dose may be included in effective treatment strategy for the depression.

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