

EVALUATION OF ANTIDEPRESSANT ACTIVITY OF ACYRANTHES ASPERA BY USING OPEN FIELD 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. Extraction of *Acyranthes aspera* was carried out by using maceration. Physically induced depression parameters showed several behavioral abnormalities in experimental animals. Behavioral abnormalities were studied in rats by using open field test apparatus. Rats were divided into 4 groups of 6 animals in each group. In open field test 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). All the solutions were freshly prepared daily and animals were treated for two days on 22nd and 23rd day by oral route after 21 days depression inducing period. Examine the animals for antidepressant activity of *Acyranthes aspera* by using open field test apparatus. The results showed that *Acyranthes aspera* extract 400 mg/kg and imipramine 20 mg/kg + *Acyranthes aspera* extract 400 mg/kg significant decrease defecation and significant increase rearing and central square entries. Thus, *Acyranthes aspera* extract may be included in effective treatment strategy of depression disorders.

KEYWORDS: Depression, *Acyranthes aspera*, Open field test, Imipramine.

INTRODUCTION

Depression is the most common affective disorder (it is defined as disorders of mood rather than 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 reflect changes in brain, monoamine neurotransmitters, specifically nor epinephrine, serotonin, and dopamine (Gold et al., 1988). Depression is characterized by feeling of sadness, hopelessness, despair and discouragement. Depression is also characterized by reduction thinking, pleasure concentration and impairment of sleep (Maheshwari, 2015).

Plant profile

Acyranthes aspera (family-Amaranthaceae) is commonly known as Latjira in Hindi. The plant is used for the treatment of dysentery, fever and diabetes (Sutar et al., 2008). *Acyranthes aspera* is available as weed in whole India, Asia and many other parts of the world such as Mexico, Central America and Africa (Dey, 2011). It is described as bitter, pungent, purgative, heating, laxative, stomachic, carminative and digestive and is also used for the treatment of bronchitis, heart disease, piles, itching abdominal problems, ascites, rheumatism, abdominal enlargement, rabies and also for enlarged cervical gland (Dwivedi et al., 2008). It is use as folk medicine. It is also known as medicinal herb in different types of system of medicine in India. It is known by different names such as *Chirchita* (Hindi), *Apamarg* (Sanskrit), *Aghedi* (Gujrati), *Apang* (Bengali), *Nayurivi* (Tamil), *Kalalat* (Malyalam) (Dwivedi et al., 2008). This plant grows on road sides. It is also found as field boundaries and waste places as a weed throughout India up to an altitude of 2100 m and in South Andaman Islands (Gupta, 2010). The leaves extract of *Acyranthes aspera* having antifertility effect (Parmar and Sharma, 2015). Methanolic extract of *Acyranthes aspera* shows wide varieties of pharmacological activities however, little is known about its anti-depressant activity. Most of the researches are not found its antidepressant activity by using open field test so the aim of present study to assess the anti-depressant activity of *Acyranthes aspera* extract against physically induced depression in rats, using open field test (Gupta, 2010).

Depression is one of the most common mental disorders, which arise due to the imbalance of neurotransmitters release at the synaptic cleft. A large number of synthetic drugs are being used as standard treatment for depression, they have many adverse effect that a limit the

therapeutic treatment. Traditionally herbs are used for the treatment of depression which may offer advantage in terms of safety and tolerability, possibly by improvement in patient compliance.

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.

Authentication

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

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).

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 $25 \pm 3^\circ\text{C}$ and $50 \pm 20\%$ 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
- Open field apparatus

Open field apparatus

- The open field test apparatus is constructed of white plywood base and measured (38×38 cm) with 36 cm transparent walls. Walls of the open field test apparatus are clear Plexiglas, so rats could be visible in the apparatus. The floor of the apparatus is divided into sixteen (9×9 cm) squares via the blue marker. A central square (9×9 cm) was drawn in the middle of the floor of open field test apparatus with the red marker.
- The leaves of *Acyranthes aspera* will be expected to show antidepressant activity against physically induced depression in rats and this may possibly arise from modulation of neurotransmitter at the synaptic cleft, specially noradrenaline and serotonin.

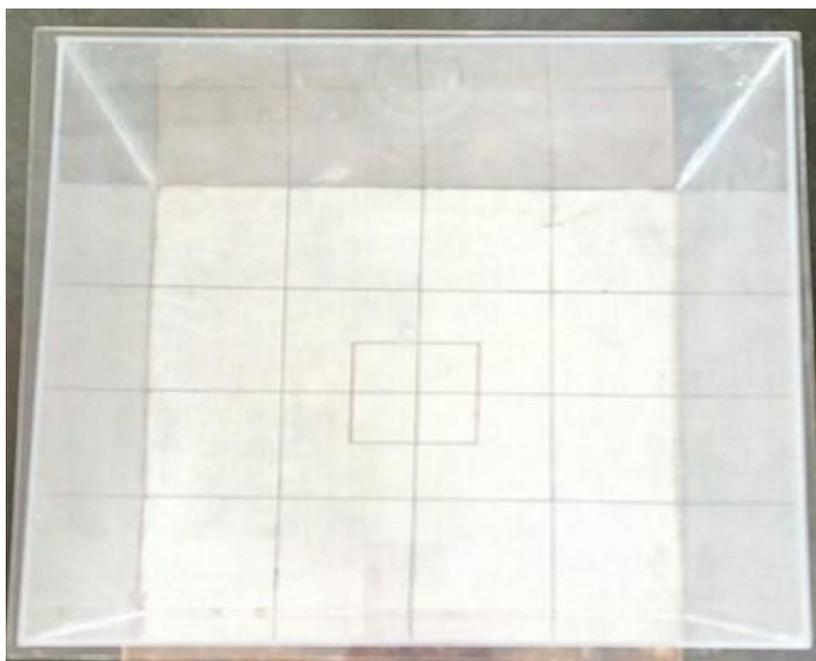


Fig. 1: Open field apparatus (Overview of open field apparatus).

Depression induced physically by following parameters.

Table.1 Physically depression induced parameters.

Day	Treatment
1	Tail pinch
2	Food deprivation (46 hrs)
3	Blank
4	Cold swim
5	Water deprivation (46 hrs)
6	Blank
7	Tail pinch
8	Shaker (160 displacement)
9	Cold swim
10	Tail pinch
11	Switch cage mates
12	Tail pinch
13	Food deprivation (46 hrs)
14	Blank
15	Isolation of housing (24 hrs)
16	Switch cage mates
17	Cold swim
18	Tail pinch
19	Shaker (160 displacement)
20	Water deprivation (46 hrs)
21	Blank
22	Testing
23	Testing

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.

Experimental protocol for open field test

Table 2: Animal groups table (Open field test).

GROUPS	TREATMENT
Group I	Distilled water (10 ml/kg, i.p.)
Group II	Imipramine (20 mg/kg, i.p.)
Group III	<i>Acyranthes aspera</i> (400 mg/kg, p.o.)
Group IV	<i>Acyranthes aspera</i> (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) 30 min before open field test on day 22 and day 23 respectively.

Imipramine treated Group (Group II, n=6)

Rats were administered imipramine (20 mg/kg) 30 min before open field test on day 22 and day 23 respectively.

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

Rats were administered *Acyranthes aspera* (400 mg/kg) 30 min before open field test on day 22 and day 23 respectively.

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

Rats were administered imipramine (20 mg/kg) and *Acyranthes aspera* (400 mg/kg) 30 min before open field test on day 22 and day 23 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 open field test apparatus for the experimentation.

Open field test

Rats were placed either into the center or one of the four corners of the open field test and allowed to move in the apparatus for 5 minutes. Rats were returned in their home cages after

5 minutes and the apparatus was cleaned with 70% ethyl alcohol and permitted to dry between tests. It is done for 5 minutes on 2 consecutive days to acclimate the rats to the test situation (Brown *et al.*, 1999). Behaviour scored-Central square entries: Frequency with which rats crossed one of the red lines with all four paws into the central squares. Rearing: Frequency with which rats stood on their hind legs in the apparatus. Defecation: Number of fecal boli produced in the apparatus during test (Brown *et al.*, 1999).

STATISTICAL ANALYSIS

All results were expressed as mean \pm 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 open field test apparatus for depression. The following parameters are noted down i.e. rearing, defecation and central square entries during the time session of 5 min. Each value of rearing, defecation and central square entries is the mean value obtained from open field test and expressed as mean \pm SEM. According to these parameters graphs were plotted and their activities were evaluated.

EFFECT OF DRUG ON PHYSICALLY INDUCED DEPRESSION USING OPEN FIELD TEST

Effect of drug on rearing using open field test

Table 3: Effect of Drug on Rearing (Distilled Water).

S. No	Treatments	Rearing
1	Distilled water (C)	10.00 \pm 0.579
2	Imipramine (S)	20.25 \pm 0.645****
3	<i>Acyranthes aspera</i> (T)	19.50 \pm 0.809****
4	<i>Acyranthes aspera</i> + Imipramine (T+S)	12.41 \pm 0.645

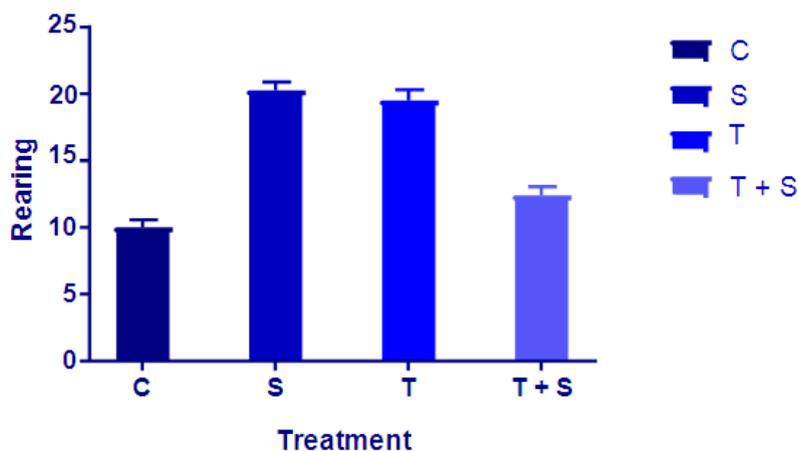


Fig. 2: Effect of drug on rearing (Distilled water).

Acyranthes aspera (400 mg/kg, p.o.) when compared to imipramine (10 mg/kg, i.p.), *Acyranthes aspera* extract (400 mg/kg, p.o.) + imipramine (20 mg/kg, i.p.) and distilled water (10 mg/kg, i.p.) administered groups. There was found to be significant increase ($p < 0.0001$) rearing in *Acyranthes aspera* (400 mg/kg, p.o.) group as compared to distilled water (10 mg/kg, i.p.) and *Acyranthes aspera* extract (400 mg/kg, p.o.) + imipramine (20 mg/kg, i.p.) while decrease rearing in *Acyranthes aspera* (400 mg/kg, p.o.) as compared to imipramine (20 mg/kg, i.p.) group.

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 (10 mg/kg, i.p.) administered groups. There was found to be decrease rearing in *Acyranthes aspera* (400 mg/kg, p.o.) + imipramine (20 mg/kg, i.p.) group as compare to *Acyranthes aspera* extract (400 mg/kg, p.o.) and imipramine (20 mg/kg, i.p.) groups.

Effect of drug on defecation using open field test

Table. 4: Effect of drug on defecation (Ditilled water).

S. No	Treatments	Defecation
1	Distilled water (C)	3.75 ± 0.354
2	<i>Acyranthes aspera</i> (T)	1.33 ± 0.211***
3	<i>Acyranthes aspera</i> + Imipramine (T+S)	1.33 ± 0.211***
4	Imipramine (S)	1.33 ± 0.211***

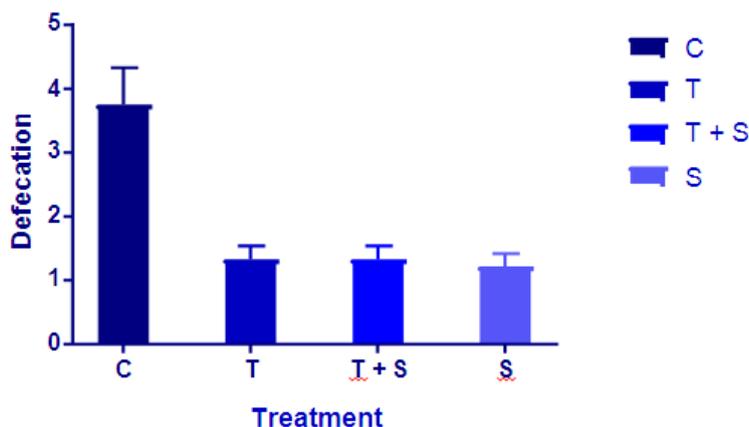


Fig. 3: Effect of drug on defecation (Distilled water).

Acyranthes aspera (400 mg/kg, p.o.) when compared to imipramine (10 mg/kg, i.p.), *Acyranthes aspera* extract (400 mg/kg, p.o.) + imipramine (20 mg/kg, i.p.) and distilled water (10 mg/kg, i.p.) administered groups. There was found to be significant decrease ($p < 0.0001$) defecation in *Acyranthes aspera* (400 mg/kg, p.o.) group as compared to distilled water (10 mg/kg, i.p.) but there is no significant difference in *Acyranthes aspera* extract (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.).

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 (10 mg/kg, i.p.) administered groups. There is no significant difference ($p < 0.0001$) in defecation in *Acyranthes aspera* (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.

Effect of drug on central square entries using open field test

Table 5: Effect of drug on central square entries (Distilled water).

S. No	Treatments	Central square entries
1	Distilled water (C)	1.00 ± 0.183
2	Imipramine (S)	2.25 ± 0.254**
3	<i>Acyranthes aspera</i> (T)	1.91 ± 0.253*
4	<i>Acyranthes aspera</i> + Imipramine(T+S)	2.20 ± 0.274**

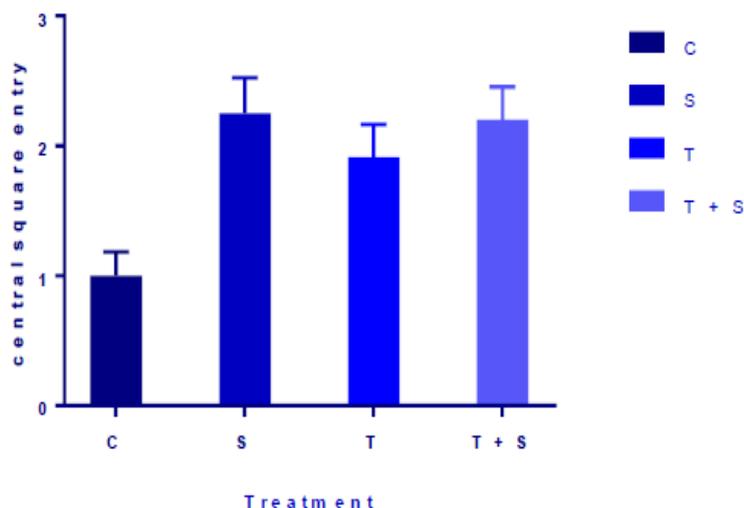


Fig 4: Effect of drug on central square entries (Distilled water).

Acyranthes aspera (400 mg/kg, p.o.) when compared to imipramine (10 mg/kg, i.p.), *Acyranthes aspera* extract (400 mg/kg, p.o.) + imipramine (20 mg/kg, i.p.) and distilled water (10 mg/kg, i.p.) administered groups. There was found to be significant increase ($p < 0.0001$) central square entries in *Acyranthes aspera* (400 mg/kg, p.o.) group as compared to distilled water (10 mg/kg, i.p.) while decrease central square entries in *Acyranthes aspera* (400 mg/kg, p.o.) as compared to imipramine (20 mg/kg, i.p.) and *Acyranthes aspera* extract (400 mg/kg, p.o.) + 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 (10 mg/kg, i.p.) administered groups. There was found to be significant increase ($p < 0.0001$) central square entries in *Acyranthes aspera* (400 mg/kg, p.o.) + imipramine (20 mg/kg, i.p.) as compared to *Acyranthes aspera* (400 mg/kg, p.o.) group while there was no significant difference in *Acyranthes aspera* extract (400 mg/kg, p.o.) + imipramine (20 mg/kg, i.p.) 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 using open field test. Open field test apparatus was standardized by Brown et al (1999). Open field test demonstrated antidepressant activity of *Acyranthes aspera* extract by increasing the rearing activity, decreasing the defecation and also increasing the central square entries of rats into the open

field test. Defecation is frequently used as the indication of depression although it has been questioned that the defecation is an indication of depression by Lister (1990). After that Hall (1934) also demonstrated that defecation is symptom of depression. 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 D₂ dopamine receptors in the nucleus accumbens (Willner, 2017). It is described that antidepressant drugs act through the noradrenergic and 5-HT_{1A}/5-HT_{2B} 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 (Willner et al., 2013). 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 open field test apparatus so I decided to evaluate the antidepressant effect of *Acyranthes aspera* extract by using open field test.

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

Imipramine and *Acyranthes aspera* extract administered groups showed significant increase in rearing. This result shows that both *Acyranthes aspera* extract and imipramine have antidepressant effect for physically induced depression in rats. Imipramine and *Acyranthes aspera* extract administered groups showed significant decrease in defecation. This result shows that both imipramine and *Acyranthes aspera* extract used as antidepressant drugs for the physically induced depressive rats. Imipramine and *Acyranthes aspera* extract administered groups showed significant increase in central square entries. This result shows that the imipramine and *Acyranthes aspera* extract used for the treatment of physically

induced depression in rats. This result shows that both imipramine and *Acyranthes aspera* extract have antidepressant activity in physically induced rats.

The effect of drug in increasing order of imipramine > *Acyranthes aspera* extract > *Acyranthes aspera* extract + imipramine according to data given in **table no 3** and shown in **figure no 2** increase rearing activity of rats. Thus imipramine is a standard drug and it is more potent which shows maximum increase rearing. It is a parameter for measuring the effect of antidepressant drug just because of number of rearing decreased in depressive rats.

The effect of drug in increasing order of imipramine > *Acyranthes aspera* extract > *Acyranthes aspera* extract + imipramine according to data given in **table no 4** and shown in **figure no 3** decrease defecation of rats. Thus imipramine is a standard drug and it is more potent which shows maximum decrease defecation. It is a parameter for measuring the effect of antidepressant drug just because of total counts of defecation increased in depressive rats.

The effect of drug in increasing order of imipramine > *Acyranthes aspera* extract + imipramine > *Acyranthes aspera* extract according to data given in **table no 5** and shown in **figure no 4** increase central square entries of rats. Thus imipramine is a standard drug and it is more potent which shows maximum increase central square entries. It is a parameter for measuring the effect of antidepressant drug just because of number of central square entries decreased in depressive rats.

Finally it has been demonstrated that, *Acyranthes aspera* extract exert a protective effect against physically induced depression in rats. *Acyranthes aspera* extract 400 mg/kg significantly increase rearing, central square entries and also significantly decrease defecation by using open field test apparatus. Hence *Acyranthes aspera* extract may be used for the treatment of depression.

CONCLUSION

Finally it may be concluded that, *Acyranthes aspera* extract exert a protective effect against physically induced depression in rats and may be attribute to its antidepressant activity. *Acyranthes aspera* extract 400 mg/kg have significant effect in physically induced depression. Thus, *Acyranthes aspera* may be included in effective treatment strategy of disorders which is related to depression.

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