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EVALUATION OF ANTIDEPRESSANT ACTIVITY OF AQUEOUS EXTRACT OF *HIBISCUS HIRTUS* LINN IN EXPERMENTAL RATS

D. Jeevan Mani Babu*, K. Adithya, T. Akshitha Mary Madhuri, S. Naga Sai Sruthi, M. Susmitha, K. Sruthi, Amasy Derar Osman Saad

Department of Pharmacology, Nova College of Pharmaceutical Education and Research, Jupudi, Andhra Pradesh, India

ABSTRACT

Aim is to study the antidepressant activity of aqueous leaf extract of *Hibiscus hirtus Linn* in male Sprague dawley rats. The Preliminary Phytochemical Analysis of aqueous extract of leaves of *Hibiscus hirtus Linn* showed the presence of carbohydrates, sugars, proteins, aminoacids, flavanoids, triterpenoids, steroids, tannins, phenols, fixed oils and fats and absence of alkaloid, glycosides, saponins, gums and mucilages. The present study revealed the significant anti-depressant effect of aqueous extract of *Hibiscus hirtus Linn* leaves in experimentally induced depression by Forced swim test and Tail suspension test models. The aqueous extract of *Hibiscus hirtus Linn* leaves significantly decreased the immobility time in dose dependent manner which is an indicator of antidepressant activity.

Key Words: *Hibiscus hirtus* Linn, antidepressant activity

Author for correspondence
D. Jeevan Mani Babu,
Department of Pharmacology, Nova College of Pharmaceutical Education and Research,
Jupudi, Andhra Pradesh, India.
Email: drjeevanbabu@gmail.com

INTRODUCTION

The human being is multifaceted in both its organization and function. Disease, decay and death have always co-existed with life, the study of diseases, disorder and their treatment must also have been contemporary with the dawn of the human intelligence. Abnormalities in any normal functioning of the body to maintain the homeostasis result in disease or disorder affecting individual's health. Among all the diseases and disorders, the most Complex and disturbing all age groups are the psychological and neurological disorders. These disorders not only influence the individual's health but also the mental status of person. Everybody feels sad during his life for a temporary period that passes away with time but depression disturbs the daily life and causes pain for himself and caring person. Depression is more than just feeling "down". It is the most prevalent mental disorder and is recognized to be symptomatically, psychologically and biologically heterogenous. Depression involves low mood or loss of interest or pleasure in usual activities and is caused by changes in brain chemistry. It is charecterized by emotional and physical manifestations, such as feelings of worthlessness, helplessness, hopelessness, gloominess, guilt or indecision, change in appetite, change in sleep habits, loss of concentration, loss of energy, loss of interest, loss of pleasure, retardation of

thinking and activity, agitation, mental and motar slowing, despair, suicidal ideation and social withdrawl. Symptoms are experienced most days and last for at least two weeks. The symptoms interfere with all areas of a person's life, including work and social relationships. Other factors contribute to the onset of depression, including genetics, changes in hormone levels, certain medical conditions, stress and difficult life circumstances. According to world health report, approximately 450 million people suffer from a mental disorder or behavioural disorder. This amounts to 12.3% of the global burden of disease, and will rise to 15% by 2020. Psychiatric illness is also often associated with suicide and there are between 10 and 20 million suicide attempts every year. Depression is treated by anti-depressant drugs.

Antidepressants are third the most commonly sold group of therapeutic agents worldwide. Most of these treatments are based on molecules that target a single protein in the brain, the serotonin (5-HT) transporter. These agents, the selective serotonin reuptake inhibitors (SSRIs), which inhibit 5-HT reuptake, account for about 80% of all antidepressants on the market. Other antidepressant drugs such as the serotonin and noradrenaline reuptake inhibitors (SNRIs) or the classic tricyclic antidepressants (e.g., amitryptyline, clomipramine, imipramine) inhibit the reuptake of noradrenaline as well. Some of these old drugs, such as clomipramine, have a complex pharmacology and have been proved to be the best antidepressant treatments for severe depression, although the presence of their many severe side effects is a very serious limitation to their use. Inspite of availability of these drugs also depression continues to be a major problem Psychopharmacology as well as other medical disciplines still require a great research effort for the achievement of new therapeutic strategies for the management of psychiatric diseases. Basic neuroscience offers the promise of improving our understanding of disease pathophysiology, identifying novel mechanisms that can be targeted by more effective pharmacotherapies and screening of herbal sources of drugs. These considerations implicate the search for new antidepressant agents that have a fast onset of action, with lesser side effects and a wider safety margin. Nature is a great treasure of resources for humankind. The plant kingdom is one of the well explored and cost effective resources available which offer the biggest advantage of being source of the medicinal remedies that is easily available in the local surroundings, having no side effects, cost effective and easy to use. Various plants are being used in complementary and alternative medicines for management of mood disorders to improve compliance and quality of life of the patient suffering from depression (1-3).

Aim is to study the antidepressant activity of aqueous leaf extract of *Hibiscus hirtus Linn* in male Sprague dawley rats.

MATERIALS AND METHOD

Plant Material

The fresh leaves of Hibiscus hirtus Linn belonging to the family Malvaceae was collected from Hanuman junction, Krishna District, Andhra Pradesh. The plant was identified and authenticated.

Experimental Animals (4-8)

Male albino sprague dawley rats (180 - 220 gm) were obtained from Mahaveer Enterprises, Hyderabad, which were maintained at Vijaya Institute of Pharmaceutical Sciences for Women, Enikepadu, Vijayawada at standard conditions. They were housed in well ventilated cages, maintained at 25 ± 2^0C with 12 hours dark cycle/ light cycle. They were fed with standard pellet diet supplied by Sai Durga Feeds and Foods, Bangalore and had free access to water. The animals were maintained in these conditions for one week before the start of experimental session.

Standard drug

Fluoxetin was used as a standard drug which was given in a dose of 10 mg/kg body weight of animal and was obtained from local market.

Test drug

Hibiscus hirtus Linn aqueous leaf extract solutions were used as test drug which was administered at a dose of 100 mg/kg and 500 mg/kg orally to the experimental animals according to their body weight.

Collection and Drying

The fresh leaves of Hibiscus hirtus Linn were washed and left for shade drying for ten days. Then the leaves were dried in hot air oven at 40˚C for an hour just before starting the extraction process to remove the equilibrium moisture content.

Procedure

After drying, the leaves were made into coarse powder with the use of a mixer grinder. The dried powdered material was weighed and subjected to extraction by maceration using distilled water for 5 days with occasional stirring. Then the extracts were filtered by Whatmann filter paper and filtrate was concentrated to obtain crude extracts. The extract obtained was kept for drying and stored in vacuum desiccators. The percentage yield of the extract was 7.34 %. The required amount of extract was dissolved in sterile water and used for in vivo pharmacological studies.

Dosing and grouping of Animals

The animals were dosed and grouped as shown in Table-1

Table-1 Dosing and grouping of Animals

Group	Name	Treatment	Duration	No. of Animals
I	Normal	Saline	7 days	6
II	Standard treatment	Fluoxetine (10 mg/kg)	7 days	6
III	Low dose treatment	HHAE (100 mg/kg)	7 days	6
IV	High dose treatment	HHAE (500 mg/kg)	7 days	6

Forced Swimming Test (FST)

Rats of same sex were divided into four different groups each containing six animals (n=6), the animals were marked individually. The animals were weighed and numbered appropriately. The test and standard drugs were given orally for 7 days. Rats were forced to swim individually in an open cylindrical glass container of 10cm diameter, 25cm height containing fresh water up to a height of 19cm, at a temperature of 25±1ºc for 6 min. This test session was conducted after 60 min of the drug treatment on day 1 and day 7. The rat was considered immobile when it floats motionlessely or made only those movements necessary to keep its head above water surface. The total duration of the immobility during the last 4 min of the 6min test was recorded for each animal.

Tail Suspension Test (TST)

Tail suspension test is behavioural test useful in the screening of potential antidepressant drugs. The animals were placed in a testing room for a period of acclimation (generally at least one hour). Rats were suspended by tail from a height of 75cm. The rat made attempts to regain upright posture, but continued in motion less state (immobility phase). This test session was conducted after 60 min of the drug treatment on day 1 and day 7. The duration of baseline immobility was measured for a period of 6min.

Statistical Analysis

The data of pharmacological experiments obtained by various parameters were expressed as mean ± standard error mean (SEM). Statistical analysis was performed using Graph pad prism software. Data of immobility time in forced swim test and tail suspension test on rats was analyzed by one-way analysis of variance (ANOVA) followed by Post-hoc Tukey's multiple comparision test. $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Phytochemical Investigation

The Preliminary Phytochemical Analysis of aqueous extract of leaves of *Hibiscus hirtus Linn* showed the presence of carbohydrates, sugars, proteins, aminoacids, flavanoids, triterpenoids, steroids, tannins, phenols, fixed oils and fats and absence of alkaloid, glycosides, saponins, gums and mucilages. The results can be showed in the table-2.

Table-2 Phytochemical Investigation of *Hibiscus hirtus Linn* (Leaves)

S. No.	Phytochemical Constituents	Aqueous Extract
1.	Alkaloids	-
2.	Carbohydrates	+
3.	Sugars	+
4.	Cardiac Glycosides	-
5.	Anthraquinone Glycosides	-
6.	Protein & Free Amino Acid	+
7.	Tannins & Phenolic compounds	+
8.	Flavonoids	+
9.	Steroids & Triterpenoids	+
10	Saponins	-
11	Fixed oil & Fats	+
12	Gums & Mucilages	-

Note-(+) indicate positive means present (-) indicate negative means absent

Effects of Extracts on Immobility Time in Forced Swimming Test (FST)

The results of acute model of FST with rats were displayed in Table-3 & Fig-1. In this test, animals of all the test groups showed significant results. The extract (100 & 500mg/kg body weight) treated groups exhibited significant delay in the onset of immobility and also significantly reduced time of immobility in the forced swimming test after 7 days of treatment. Post-hoc Tukey's multiple comparison tests analysis demonstrated that the test treatment significantly reduced the immobility time in comparison to the control group ($p<0.001$). Group IV showed significantly reduced immobility and increase in the normal behaviour of rats in water filled apparatus and also exhibited antidepressant activity comparable to the standard drug fluoxetine (10mg/kg body weight) i.e. the standard group. On day-1 of the test the immobility time was 135.2± 1.07, 129.6± 2.49 seconds in test groups respectively. The results were statistically significant in test groups when compared to control in which the immobility time was 158.5± 3.87 seconds. However with subsequent drug administration the immobility time was significantly reduced in all test groups to 130.7± 0.8 and 121.67± 4.41 seconds (test groups respectively) when compared to control group which was 152.7± 3.89 seconds on day 7 of the test.. However, the results of the standard drugs were significantly better on all the test days at 96.5± 9.0 and 80± 4.46 seconds on day 1 and 7 respectively.

Table-3 Effects of extracts on immobility time in forced swimming test (FST)

Groups	Dose	Immobility Time (s)	
		Day 1	Day 7
I	Saline	158.5±3.87	152.7±3.89
II	Fluoxetine (10mg/kg)	96.5± 9.0***	80±4.46***
III	Extract (100 mg/kg)	135.2±1.07***	130.7±0.80***
IV	Extract (500 mg/kg)	129.6±2.49***	121.67±4.41***

Values are expressed as mean ± SEM; n=6 animals in each group. Comparison between control v/s all the other groups. Statistical test done by one-way ANOVA followed by Post- hoc Tukey's multiple comparison test, *$p<0.05$, **$p<0.01$; *** $p<0.001$

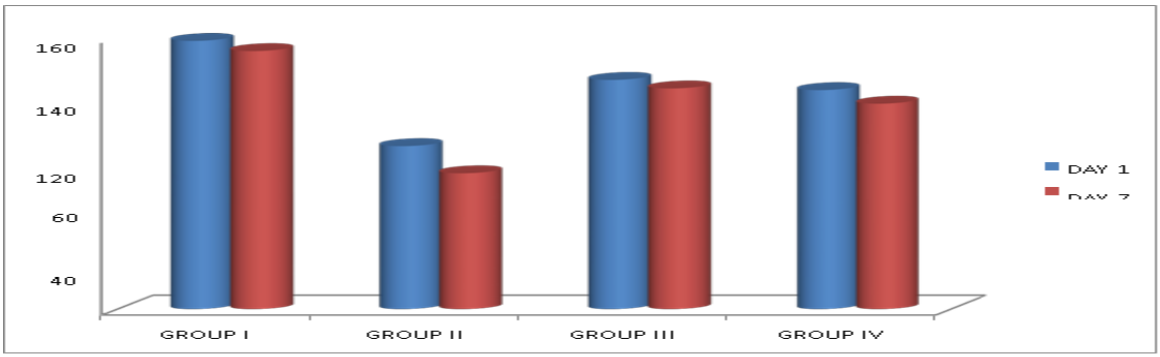

Fig-1 Effects of extracts on immobility time in forced swimming test (FST)

Effects of extracts on immobility time in Tail suspension test (TST)

The results of the antidepressant effect of Ethanolic extract of Hibiscus hirtus Linn were presented in Table-4 and Fig-2. The extracts showed slight reduction in immobility on 1 day treatment, but significantly reduced the immobility time after 7 days of treatment. On day 1 of the test the immobility time in test groups was 232.3± 5.39 and 201.7± 3.45 seconds respectively which was statistically significant when compared to the control group in which the immobility time was 257± 13.92 seconds. On day 7 the of the test the immobility time in test groups was 234.8± 2.91 and 194.2± 3.96 seconds respectively which was statistically significant when compared to the control group in which the immobility time was 264.8± seconds. The standard drug was far superior in reducing the immobility time on all the test days at 112.3± 2.52 and 103.8± 2.04 seconds respectively on days 1 and 7 of the test.

Table-4 Effects of extracts on immobility time in Tail suspension test

Groups	Dose	Immobility Time (s)	
		Day 1	Day 7
I	Vehicle Control (10ml/kg)	257±13.91	264.8±4.43
II	Fluoxetine(10mg/kg)	112.3±2.525***	103.8±2.04***
III	Extract (100 mg/kg)	232 .3±5.391**	234.8±2.921*
IV	Extract (500 mg/kg)	201.7±3.451***	194.2±3.962***

Values are expressed as mean ± SEM; n=6 animals in each group. Comparison between control v/s all the other groups. Statistical test done by one-way ANOVA followed by Post- hoc Tukey's multiple comparison test, *p<0.05, **p<0.01; *** p<0.001

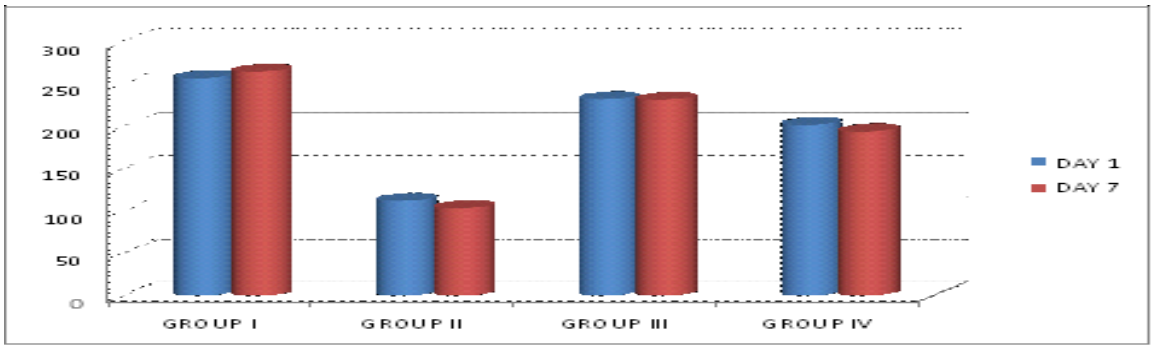

Fig-2 Effects of extracts on immobility time in Tail suspension test

CONCLUSION

The present study was aimed to study the antidepressant activity of aqueous extract of *Hibiscus hirtus Linn* leaves on experimental animals. The Preliminary Phytochemical Analysis of aqueous extract of leaves of *Hibiscus hirtus Linn* showed the presence of carbohydrates, sugars, proteins, aminoacids, flavanoids, triterpenoids, steroids, tannins, phenols, fixed oils and fats and absence of alkaloid, glycosides, saponins, gums and mucilages. The present study revealed the significant anti-depressant effect of aqueous extract of *Hibiscus hirtus Linn* leaves in experimentally induced depression by Forced swim test and Tail suspension test models. The aqueous extract of *Hibiscus hirtus Linn* leaves significantly decreased the immobility time in dose dependent manner which is an indicator of antidepressant activity. The extract (100 & 500mg/kg body weight) treated groups exhibited significant delay in the onset of immobility and also significantly reduced time of immobility in the forced swimming test after 7 days of treatment. The extract showed slight reduction in immobility on 1 day treatment tail suspension test, but significantly reduced the immobility time after 7 days of treatment. On day 1 of the test the immobility time in test groups was 232.3± 5.39 and 201.7± 3.45 seconds respectively which was statistically significant when compared to the control group in which the immobility time was 257± 13.92 seconds. Though the *Hibiscus hirtus Linn* extract have a modest effect when compared to standard it can serve as an add-on drug to current regimens or may be used along with current regimens in lower dose. The reduction in dose of these standard drugs is always a welcome change and may help in reducing the adverse effect profile which becomes obvious at higher doses. Further isolation and identification of the bioactive ingredient responsible for anti depressant activity is necessary. However, further studies are needed to elicit its exact mechanism of action and to identify the active ingredient as a potent and efficacious antidepressant agent.

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