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PHARMACOLOGICAL EVALUATION OF ANTI-DEPRESSANT ACTIVITY OF HIBISCUS LEAVES IN ANIMAL MODELS

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Abstract:

The objective was to investigate the antidepressant activity of methanolic extract of leaves of Hibiscus in mice. To study the effect of Hibiscus on anti depressant activity of brain. The results from the present study confirm the antidepressant activity of hibiscus, since it reduced the immobility in both FST and TST. In the present study, hibiscus is significantly increased the frequency of 5-HTP induced head twitches, Clonidine induced aggression and L-DOPA induced hyperactivity and aggressive behavior indicating its enhanced activity on serotonergic, noradrenergic and dopaminergic pathways respectively. Our results also confirm the involvement of serotonergic, noradrenergic and dopaminergic path ways in depression. Pre treatment with hibiscus, also significantly increased the levels of SOD and Catalase with simultaneous decrease in LPO levels in mice brain, suggesting its strong antioxidant activity. Since oxidative stress is reported to play an important role in depression, the antioxidant activity of hibiscus is might be a part of the mechanism for its antidepressant activity. Results from behavioral experiments indicate that the antidepressant activity of hibiscus is, might be due to the facilitatory effect on serotonergic, noradrenergic and dopaminergic systems apart from the anti depressant activity.

Keywords: *Depression, Hibiscus etc.*

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INTRODUCTION:

Depression is the most common of the affective disorders (disorders of mood rather than disturbances of thought or cognition); it may range from a very mild condition, bordering on normality, to severe (psychotic) depression accompanied by hallucinations and delusions. There are two types and they are Unipolar and Bipolar Unipolar depression is commonly (about 75% of cases) non-familial, clearly associated with stressful life-events and accompanied by symptoms of anxiety and agitation; this type is sometimes termed reactive depression. Other patients (about 25%, sometimes termed endogenous depression) show a familial pattern, unrelated to external stresses, and with a somewhat different symptomatology[2]. This distinction is made clinically, but there is little evidence that antidepressant drugs show significant selectivity between these conditions. Bipolar depression, which usually appears in early adult life, is less common and results in oscillating depression and mania over a period of a few weeks. There is a strong hereditary tendency, but no specific gene or genes have been identified either by genetic linkage studies of affected families, or by comparison of affected and non-affected individuals.[3,4]

Depression is one of several disorders affecting mood, along with mania, hypomania, and bipolar disorders. The present chapter focuses on behavioral assessment of antidepressant action in animals with a focus on simple tests performed in rodents. Many of the primary symptoms of depression (depressed mood, low self-esteem, guilt, difficulty in concentration, suicidal ideation, thoughts of death) are by their nature difficult to model in animals. This problem is further confounded by their unknown etiology. Several theories have been proposed but most theories of depression concur in suggesting that stressful life events play an important role. There is also a small genetic component, as demonstrated by substantially increased risk in families with heritability being estimated at between 40% and 70%, leading to a much greater incidence than observed in the general population, which is nevertheless very high at around 10%.[5,6]

If little is known about the etiology of depression, even less is known about mania and bipolar disorders. The genetic component appears to be greater than for unipolar depression. Modeling the cycling, recurrent nature of bipolar disorder in animals has not even been attempted. There are, however, some models for mania that present an interesting pharmacology, in particular the combined amphetamine-chlordiazepoxide hyperactivity model, although the few publications on these models and their lack of reproducibility from one laboratory to another make an overview of their utility difficult. They will not be further discussed in this chapter.[7,8]

The clinical diagnosis of depression requires the presence of several “core” symptoms (depressed mood, decreased pleasure) often accompanied by more variable symptoms such as irritability, changes in weight, sleep disturbance, feelings of guilt, poor concentration, thoughts of death, suicidal ideation, etc. It is clearly not possible to reproduce in animals all symptoms observed clinically. Below Table shows the principal symptoms observed in depressed patients and suggests analogous signs that can be observed in animals. These signs can be used as dependent variables (end point measures) allowing behavioral assessment in different animal models of depressive states.

Different animal models of depression are:

Learned helplessness (LH): The LH paradigm uses a stress-exposure period in which rats or mice are exposed to inescapable stress (e.g., electrical foot shock) in one or more sessions. In a subsequent session, the animals are tested for their performance in an active avoidance test. In a typical active avoidance test, animals are confined to one side of a shuttle box chamber where foot shocks are delivered but the animal has the opportunity of actively escaping the foot shock. Animals previously exposed to inescapable stress show reduced abilities to escape in this model. The reduced ability to escape is restored by different forms of antidepressant treatment, including tricyclic antidepressants, selective serotonin reuptake inhibitors, monoamine oxidase inhibitors, and electroconvulsive shock therapy This model has good validity for predicting antidepressant efficacy

Forced swim test (FST): The FST involves placing a rat or mouse in a cylinder with enough water so that it cannot touch the bottom with its hind paws. A normal animal will show an immediate burst of activity, try to escape, and then eventually adopt an ‘‘immobile’’ posture, where it will make only those movements necessary to keep its head above water. The development of immobility may be facilitated by prior exposure to the test and a 24-h prior pre exposure to the test is often used. Immobility is quantified during brief test periods and classical antidepressants such as the monoamine oxidase inhibitors, tricyclics, and atypical antidepressants all decrease the duration of immobility in rats and mice in a dose-dependent manner.

Tail suspension test (TST): The TST is conceptually similar to the FST and is suggested to have greater sensitivity. A mouse is suspended by the tail in this test and observed for the extent of immobility versus active movement. Similar to the FST, the TST is also based on the adoption of a passive response in a stress situation. Acute antidepressant treatment given prior to the test reduces immobility time in the TST and it is considered to have good predictive validity

Hypo neophagia paradigms: Examples of hyponeophagia tests that are used in rats and mice are novelty induced hypophagia (NIH) and novelty suppressed feeding (NSF) paradigms. They are anxiety based and compare feeding behavior in an anxiogenic versus a non anxiogenic environment. The stress employed in these models is very mild relative to most other tests for antidepressant action, and consists of placing the experimental animal in a novel environment to induce anxiety during testing. The animal experiences conflict between the desire to approach and feed or drink, and the anxiety-induced avoidance of the novel environment.

Chronic unpredictable mild stress: In comparison to LH and FST/TST procedures that rely on relatively short term aversive stress exposure, the chronic unpredictable mild stress (CUS) paradigm was developed to study neural changes that result from stress of a more chronic nature. CUS paradigms aim to model a chronic depressive like state that develops gradually over time in response to stress, and is thus considered more naturalistic in the induction. Rats or mice are exposed to a series of different stress conditions over a period of several weeks. Several stressors (6–8) are applied (1 or 2 per day) for several hours each day. Typical stressors include overnight illumination, periods of food or water restriction, cage tilt, and isolation or crowded housing. The sequential and unpredictable stress exposure decreases the likelihood of the animals habituating to any one reoccurring condition

Hedonic sensitivity: Methods for quantifying hedonic sensitivity include conditioned place preference procedures in which animals learn to associate a particular environment with reward experience, brain stimulation reward (BSR) paradigms, and quantifying consumption of sweet solutions. Quantifying consumption of sweetened fluids (sucrose or saccharin) is the most commonly employed endpoint for assessing CUS effectiveness. Rats previously habituated to sucrose are typically given a choice of drinking sucrose versus water in a two-bottle test. While control rats typically show a preference for drinking weak sucrose solutions, rats exposed to CUS loose this preference. The development of this effect can be demonstrated by repeated sucrose preference testing during the course of CUS exposure. The time-dependent reversal of this effect with chronic antidepressant treatment can also be demonstrated by repeated testing.

Early life stress (ELS): Early life adverse experience is an important predisposing factor for psychopathology in humans. Several human studies indicate that exposure to stress or adversity early in life increases the risk for depression, and that stress exposure may interact with genetic risk factors The ELS models typically employ stress exposure during critical periods of development and result in stable phenotypic changes. ELS- induced changes that have been particularly replicable involve alterations in neural systems that regulate or respond to stress such as the hypothalamus pituitary axis and include endocrine, neurochemical, and behavioral alterations.
Social defeat: Social stress represents a significant type of adversity in many species and is thought to play a role in the development of depression and other psychopathology in humans The use of social conflict as a stressor and the use of social interaction as a quantifiable endpoint both have validity for depression. Experimental models in rodents frequently utilize a conflict situation that results in one animal becoming or retaining dominant status and another ending up subordinate or ‘‘defeated’’. A phenotypic trait produced in these models is social avoidance, which can be quantified and is suggested to model social withdrawal in human depression.

MATERIALS AND METHODS:

Materials

Drugs and Chemicals

Thiobarbituric acid and DTNB reagent (Hi Media Laboratories Ltd., Mumbai), Trichloro acetic acid (Qualigens Fine Chemicals, Mumbai), Riboflavin (Astra IDL, Bangalore), Sodium dihydrogen phosphate and Disodium hydrogen phosphate (S.D. Fine Chemicals, Mumbai), Lorazepam (Ranbaxy, India), 1,1,3,3,-Tetraethoxy propane, O-Dianisidine, Imipramine hydrochloride, 5-Hydroxy Tryptophan (5-HTP), Clonidine and L-DOPA (Sigma, St. Louis, USA) were used in the study. The other chemicals and solvents used were of analytical grade and purchased from commercial suppliers. Imipramine (IMP), 5-HTP, clonidine, L-DOPA, Lorazepam was administered intraperitoneal by dissolving in normal saline.

METHODOLOGY:

Collection and Authentification of Plant Material: The Aerial Parts of *Hibiscus* were collected and authenticated

Extraction of Plant Material: The plant leaves are grinded in to a coarse powder with the help of suitable grinder.

Cold Extraction (Ethanol Extraction): In this work the cold extraction process was done with the help of ethanol. About 45-60gms of powdered material was taken in a clean,flat bottomed glass container and soaked in 750ml of ethanol.The container with its contents were sealed and kept for period of 7days accompanied by continuous shaking with the shaker.The whole mixture then went under a coarse filtration by apiece of a clean,white cotton wool.

Evaporation of Solvent: The filtrates (ethanolextract) obtained were evaporated using Rotary evaporatorin a porcelain dish. They rendered a gummy concentrate of greenish black. The extract was kept in vacuum desiccators for 7 days.

Preliminary Phytochemical Screening

Preliminary phytochemical screening of the *Hibiscus* extract was carried out for the analysis of Alkaloids, Carbohydrates, Tannins, Saponins, Steroids, Phenols, Flavonoids.as per the standard methods[40].

1. Detection of Alkaloids: Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

a).Mayer'sTest: Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide).Formation of a yellow coloured precipitate indicates the presence of alkaloids.

b).Wagner's Test: Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

c).Dragendroff's Test: Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

d).Hager's Test: Filtrates were treated with Hager's reagent (saturated picric acid solution).Presence of alkaloids confirmed by the formation of yellow coloured precipitate.

2.Detection of Carbohydrates: Extracts were dissolved individually in 5ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

a).Molisch's Test: Filtrates were treated with 2 drops of alcoholic α-naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of Carbohydrates.

b).Benedict's Test: Filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

c).Fehling's Test: Filtrates were hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A&B solutions. Formation of red precipitate indicates the presence of reducing sugars.

3. Detection of saponins

a). Froth Test: Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for15 minutes. Formation of 1cm layer off a am indicates the presence of saponins.

b).FoamTest:0.5gm of extract was shaken with 2ml of water. If foam produced persists forten minutes it indicates the presence of saponins.

4.Detectionofsteroids.

a).Salkowski's Test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of Conc. Sulphuri cacid, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpenes.

b).Libermann Burchard's test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride,boiled and cooled. Conc. Sulphuric acid was added. Formation of brown ring at the junction indicates the presence of phytosterols.

5.Detection of Phenols

Ferric Chloride Test: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

6. Detection of Tannins

GelatinTest: To the extract,1 %gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

7. Detection of Flavonoids

Alkaline ReagentTest: Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid,indicates the presence of flavonoids.

Lead acetate Test: Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

Animals: Healthy Adult Male mice of 5 weeks old with Average weight in the range of 20-25gms were selected. Animals are housed 4 percage in temperature controlled ($27^0C\pm3^0c$) room with light/dark cycle In a ratio of 12:12hrs is to be maintained. The Animals are allowed to a climatize to the environment for seven

days and are supplied with a standard diet and water *adlibitum*. The prior permission was sought from the Institutional Animal Ethics Committee (IAEC) for conducting the study.

Acute toxicity studies: Acute toxicity studies will be performed for Ethanolic extract according to the acute toxic classic method as per OECD guidelines. Male mice were used for acute toxicity study. The animals were kept fasting for overnight providing only water, after which the extract will be administered orally at the dose of 300mg/kg and observed for14days. If mortality was observed in two animals out of three animals, then the dose administered was assigned as toxic dose. If the mortality was observed in one animal, then the same dose was repeated to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher doses such 50,200 &2000mg/kg body weight.The animals were observed for toxic symptoms for 72h.

***IN VIVO* MODELS OF DEPRESSION EMPLOYED IN THE STUDY**

1. Forced swimming test (FST)
2. Tail suspension test (TST)
3. 5-HTPinduced head twitches in mice
4. Clonidine-inducedaggressioninmice
5. L-DOPA-inducedhyperactivityandaggressivebehaviorinmice(LHA)

1. Forced swimming test(FST)

Principle : Behavioral despair was proposed as a model to test for anti depressant activity. It was suggested that mice or mice forced to swim in a restricted space from which they can not escape are induced to a characteristic behavior of immobility. This behavior reflects a state of despair which can be reduced by several agents which are therapeutically effective in human depression.

Advantages of the method are the relative simplicity and the fact that no interaction with other drugs is necessary. Like in other behavioral tests, e.g.the catalepsy test in chicken, not only antidepressants and monoamine oxidase inhibitors but also central stimulants give positive results.

Procedure: The procedure was described by Porsoltetal. (1978) was used. Swimming sessions were conducted by placing mice in individual glass cylinders (45cm high×20cm in diameter) containing (25±2°C) water 38cm deep, so mice could not support themselves by touching the bottom with their feet. Two swimming sessions were performed between12:00 hand 19:00h,an initial 15 min pre test followed 24 h later by a 6 min test.

Doses were given once daily for 7days. On the7th day mice were subjected to15min pretest. After15min,in the water the mice were removed and allowed to dry in a heated enclosure (32°C) before being returned to their home cages. They were again placed in the cylinder 24h later and the total duration of immobility was measured during a 6min test. Floating behavior during this 6min period had been found to be reproducible in different groups of mice. An animal was judged to be immobile when ever it remains floating passively in the water in a slightly hunched but upright position, its nose just above the surface. The total immobility time for the period of 6min was recorded with the help of stop watch.

2. Tail suspension test(TST)

Principle: The "tail suspension test" has been described by Steruetal. (1985) as a facile means of evaluating potential antidepressants. The immobility displayed by rodents when subjected to an unavoidable and in escapable stress has been hypothesized to reflect behavioral despair which in turn may reflect depressive disorders in humans. Clinically effective antidepressants reduce the immobility that mice display after active and un successful attempts to escape when suspended by the tail.

Doses are given once daily for 7days. On the 7thday, 1hr after the administration of the test and standard drugs, mice were suspended on the edge of a table 50cm above the floor by the adhesive tape placed approximately1cm from the tip of the tail. Immobility time was recorded during a 6min period. Animal was considered to be immobile when it did not show any movement of body and hanged passively.

3.5-HTP induced head twitches in mice

Principle: According to the monoamine hypothesis of depression compounds exertanti depression activity because they are capable of enhancing central noradrenergic and/or serotoninergic functions. Several antidepressant agents potential to serotonin effects by a block of there –up take of serotonin. DL-5-Hydroxy trypto phan is used as the precurs or of serotonin. Enzymatic break down is inhibited by the MAO-inhibit or pargyline. In mice the characteristic symptom of head twitches is observed.

Doses were given once daily for7 days. On the 7thday,1 hr after the administration of the test and standard drugs, mice were treated with 5-HTP(100mg/kgi.p.)and the numbers of head twitches performed by each mice was counted by staggering method using three 2 min periods (19–21min), (23–

25min), (27–29min) after 5-HTP administration and number of head twitches were scored live by a blind observer.

4. Clonidine-induced aggression in mice

The method of Morpurgo (1968)was used. Mice were divided in to 5 groups of 8 each (n=8),each group contain 4 pairs of mice, two pairs from each sex(each pair contained same sex of mice). Doses were given once daily for 7days. On the7thday, Clonidine was given 1h after the administration of the test and standard drugs. The animals were then caged in bell shaped glass jar with a floor are a of approximate 16cm^2. The biting/fighting episodes were recorded live by a blind observer over a period of 30min,in each pair.

5. L-DOPA induced hyperactivity and aggressive behavior in mice (LHA)

Mice were treated with L-DOPA (100mg/kgi.p.) and the experiment was performed according to the method of Mice were divided in to 5 groups of 8 each (n=8), each group contain 4pairs of mice, two pairs from each sex (each pair contained same sex of mice). Doses were given once daily for 7days. On the 7thday, L-DOPA was given 1h after the administration of the test and standard drugs, Stages of activity and aggressive behavior were recorded live every 10min for 30 min after L-DOPA administration by the blind observer. The different parameters of observation were piloerection, salivation, increase in motor activity, irritability, reactivity, jumpings queaking, and aggressive fighting. The scores were graded in the following manner:

0—No effect;1—Piloerection, slight salivation, slight increase in motor activity;2—Piloerection, salivation, marked increase in motor activity and irritability;3—Piloerection, profuse salivation, marked increase in motor activity, reactivity, jumping, squeak in g and aggressive fighting.

STATISTICAL ANALYSIS

Results were expressed as mean±S.E.M. Statistical analysis was performed using one-way analysis of variance (ANOVA). If the overall *P*-value was found statistically significant ($P<0.05$)

RESULTS:

Preliminary Phytochemical Screening

Investigation revealed the presence of steroid, Alkaloid, saponins, Tannins, phenols & Flavonoid in Ethanolic Extract of *Hibiscus.*

Acute toxicity studies

As per (OECD) draft guidelines 423 Female albino mice were administered *Hibiscus* and doses was be selected in the sequence (1.75- 5000) using the default dose progression factor, for the purpose of toxicity study. Animals are observed individually at least once during the first 30 minutes after dosing, periodically during the first 24 hours and daily thereafter, for a total of 14 days,. In all the cases, no death was observed within 14 days. Attention was also given to observation of tremors and convulsions, salivation, diarrhoea, lethargy, sleep and coma. Overall results suggested the LD_{50} value as 2000 mg/kg. Hence therapeutic dose was calculated as 1/10th and 1/20th i.e. 100mg/kg and 200 mg/kg of the lethal dose for the purpose anti depressant investigations.

1. Forced Swim Test (FST)

The results (Table. 1) showed that both EEHRS (100, 200 and 400 mg/kg, p.o.) and imipramine (15 mg/kg, i.p.) significantly decreased the duration of immobility time in a dose dependent manner in FST model. Post-hoc analysis showed that the EEHRS (100, 200 and 400 mg/kg) and Imipramine (IMP) treated groups were significantly different ($p<0.001$) from the vehicle treated group (Fig. 1).

Table. 1. Effect of *Hibiscus* and imipramine (IMP) on forced swim test (FST) in mice.

Group no.	Treatment (dose in mg/kg)	Immobility period (sec) Mean ± SEM
I	Control (0.3% CMC) + FST	135.1±5.1
II	*EEH* (100 mg/kg, p.o.) + FST	122.5±8.5
III	*EEH* (200 mg/kg, p.o.) + FST	96.3±2.7*
IV	*EEH* (400 mg/kg, p.o.) + FST	80.1±5.2*
V	Imipramine (15 mg/kg, i.p.) + FST	73.2±8.1*

Each column represents mean ± S.E.M. of immobility period (sec), n = 6. * = p<0.001 compared to control

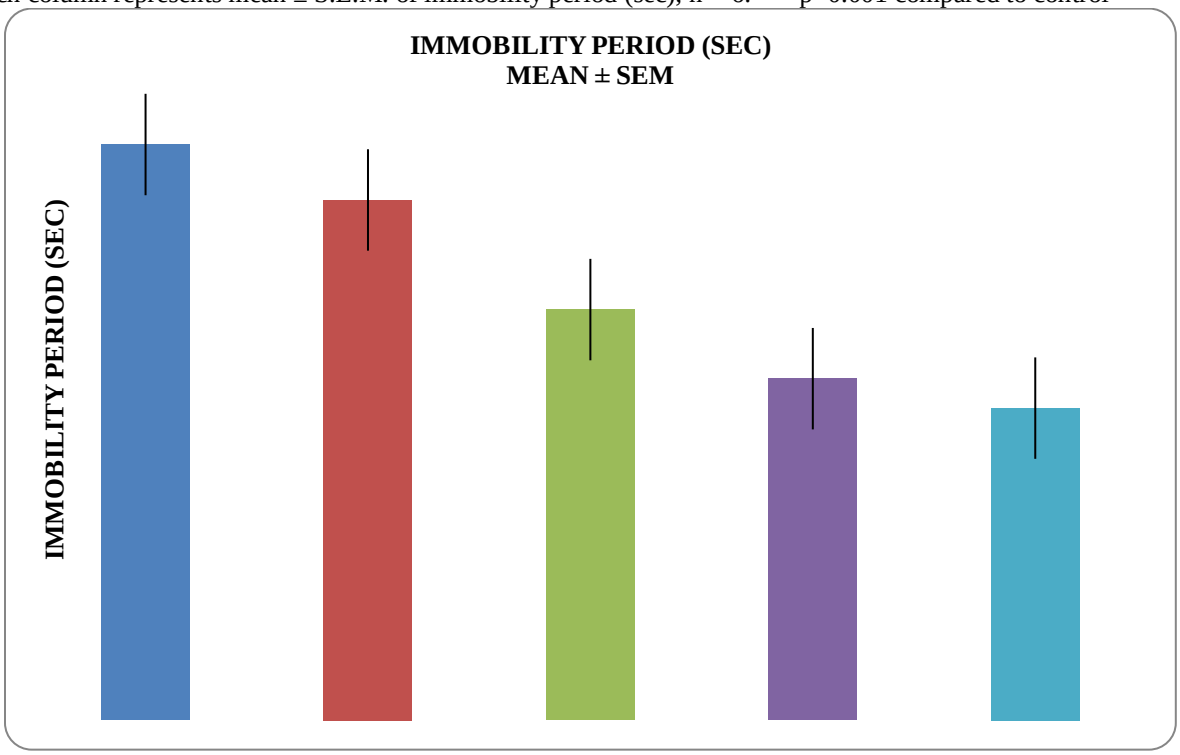

Figure. 2. Effect of *Hibiscus* (100, 200 and 400 mg/kg, p.o.) and Imipramine (IMP; 15 mg/kg) on forced swim test (FST) in mice. Each column represents mean ± S.E.M. of immobility period (sec), n = 6. *= p<0.001 compared to control.

2) Tail Suspension Test (TST)

The results (Table. 2) showed that both EEHRS (100,200,400 mg/kg, p.o.) and imipramine (15 mg/kg, i.p.) significantly decreased the duration of immobility time in a dose dependent manner in TST model. Post-hoc analysis showed that the EEHRS (100, 200 and 400 mg/kg) and IMP treated groups were significantly different (p<0.001) from the vehicle treated group.

Table.3. Effect of *Hibiscus* and Imipramine (IMP) on tail suspension test (TST) in mice

Group no.	Treatment (dose in mg/kg)	Immobility period (sec)
I	Control (0.3% CMC) + TST	137.1±8.1
II	*EEH* (100 mg/kg, p.o.) + TST	113.2±02.1[a]
III	*EEH* (200 mg/kg, p.o.) + TST	96.2±7.2[a]
IV	*EEH* (400 mg/kg, p.o.) + TST	82.1±5.2 [a]
V	Imipramine (15 mg/kg, i.p.) + TST	62.5±1.4 [a]

Each column represents mean ± S.E.M. of immobility period (sec), n = 6. a = p<0.001 compared to control

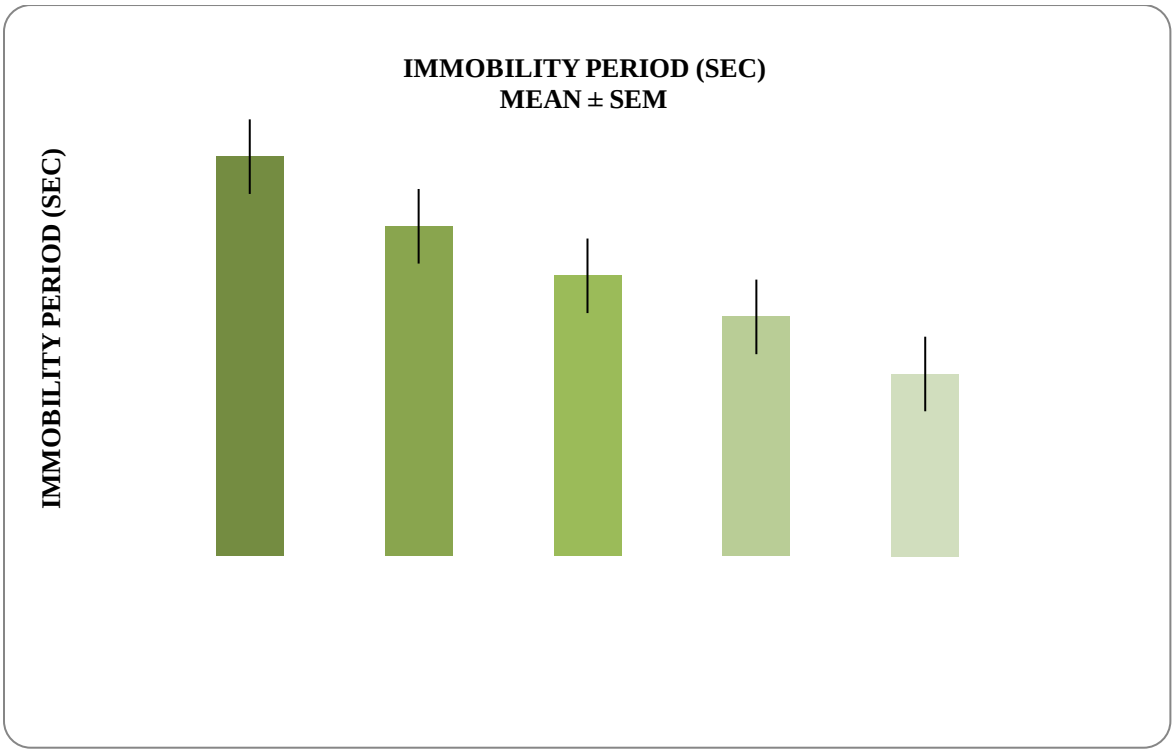

Figure. 3. Effect of *Hibiscus* (100, 200 and 400 mg/kg, p.o.) and Imipramine (IMP; 15 mg/kg) on tail suspension test (TST) in mice. Each column represents mean ± S.E.M. of immobility period (sec), n = 6. a = p<0.001 compared to control.

3) 5-HTP induced head twitches in mice

Table.3. Illustrates the effect of *Hibiscus* and IMP on 5-HTP-induced head twitches in mice. Post-hoc analysis revealed that three doses of *Hibiscus* (100, 200 and 400 mg/kg, p<0.01, p<0.001) significantly increased the 5-HTP-induced head twitches in comparison to control group. Further, the dose of 400 mg/kg was more effective than 100, 200 mg/kg. Similarly, IMP treated group showed significant increase (p<0.001) in the 5-HTP-induced head twitches compared to control. However, the effect of 400 mg/kg of *Hibiscus* was significantly higher than IMP (p<0.001) (Fig. 3).

Table. 4. Effect of *Hibiscus* on 5-HTP-induced head twitches in mice.

Group no.	Treatment (dose in mg/kg)	Head twitches Mean ± SEM
I	Control (0.3% CMC)	11.9±2.1
II	*EEH* (100 mg/kg, p.o.)	28.5±1.2[a]
III	*EEH* (200 mg/kg, p.o.)	26.1±3.2[b]
IV	*EEH* (400 mg/kg, p.o.)	35.5±2.1[b]
V	Imipramine (15 mg/kg, i.p.)	23.1±1.1[b]

Each column represents mean ± S.E.M. of number of head twitches, n = 6. a = p<0.01, b = p<0.001 compared to control.

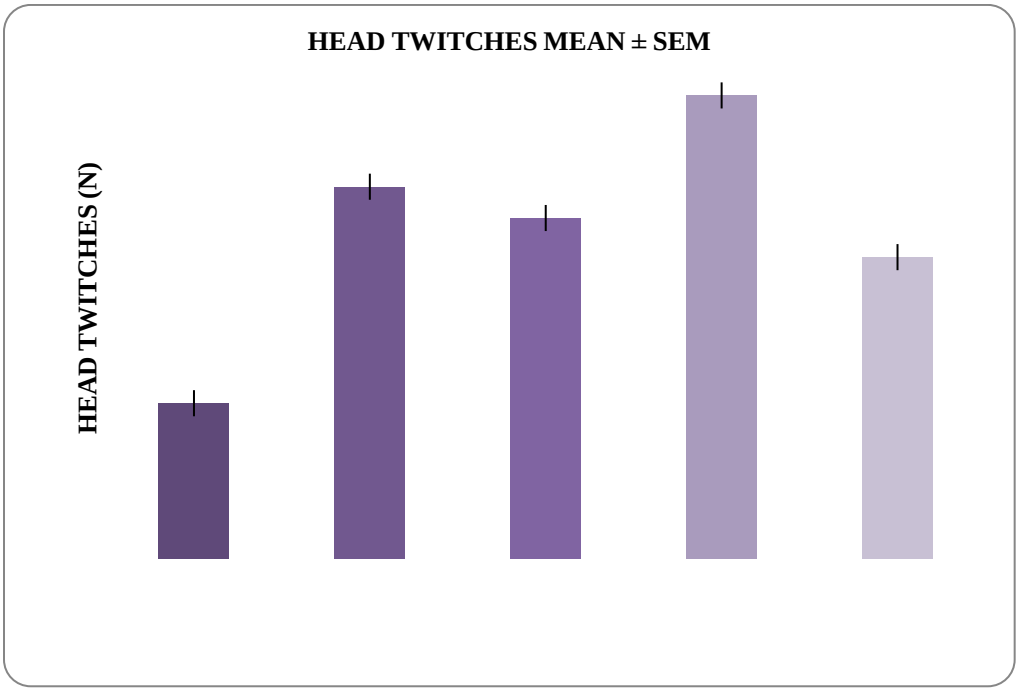

Figure. 4. Effect of *Hibiscus* (100, 200 and 400 mg/kg, p.o.) and Imipramine (IMP; 15 mg/kg) on 5-HTP-induced head twitches in mice. Each column represents mean ± S.E.M. of number of head twitches, n = 6. a = p<0.01, b = p<0.001, compared to control

4) L-DOPA induced hyperactivity and aggressive behavior in mice

The effect of *Hibiscus* and lorazepam on L-DOPA-induced hyperactivity and aggressive behavior is shown in Table 4. Post-hoc analysis revealed that three doses of *Hibiscus* (100,200 and 400 mg/kg, p<0.001) significantly increased the L-DOPA-induced hyperactivity and aggressive behavior (LHA) in comparison to control group.

Table.5. Effect of *EEH* and Lorazepam on L-DOPA-induced hyperactivity and aggressive behavior in mice.

Group o.	Treatment (dose in mg/kg)	Behavioral score
I	Control (0.3% CMC)	1
II	*EEH* (100 mg/kg, p.o.)	2.1 ± 0.2[a]
III	*EEH* (200 mg/kg, p.o.)	2.3 ± 0.2 [a]
IV	*EEH* (400 mg/kg, p.o.)	2.2 ± 0.2 [a]
V	Lorazepam (2.5 mg/kg, i.p.)	2.2 ± 0.2 [a]

Each column represents mean ± S.E.M. of number of head twitches, n = 6. a = p<0.001, compared to control

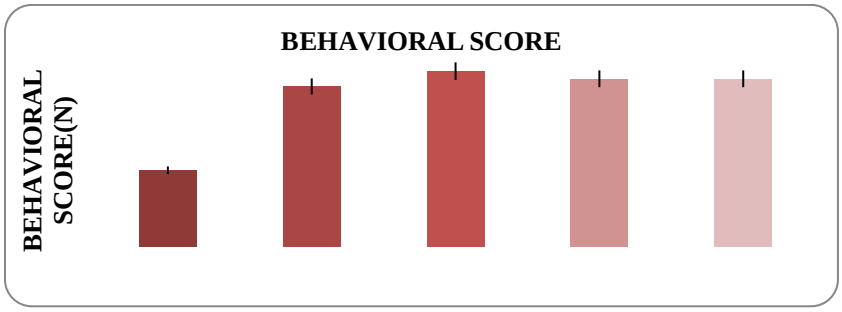

Figure. 5. Effect of *Hibiscus* (100, 200 and 400 mg/kg, p.o.) and Lorazepam (2.5 mg/kg) on L-DOPA-induced hyperactivity and aggressive behavior in mice. Each column represents mean ± S.E.M. of number of head twitches, n = 6. a = p<0.001, compared to control.

5) Clonidine induced aggression in mice

Table. 5. Indicates the effect of *Hibiscus* (100, 200 and 400 mg/kg, p.o.) and lorazepam (LA; 2.5 mg/kg) on the latency to first attack and the number of bouts in the clonidine induced aggressive behavior in mice. Post-hoc analysis showed that *Hibiscus* (p<0.001) significantly increased the latency to first attack and decrease the no. of bouts compared to control.

Table 6: effect of *Hibiscus* on clonidine induced aggression in mice.

Group no.	Treatment (dose in mg/kg)	% Response(MEAN ± SEM)	
		Latency to 1st attack	Fighting response
I	Control (0.3% CMC)	101.2 ± 8.2	98.9 ± 5.1
II	*EEH* (100 mg/kg, p.o.)	120.2 ± 10.2 [a]	86.2 ± 1.5 [a]
III	*EEH* (200 mg/kg, p.o.)	132.3 ± 15.1 [b]	65.2± 3.4 [b]
IV	*EEH* (400 mg/kg, p.o.)	132.1 ± 8.9 [b]	63.2 ± 4.1 [b]
V	Lorazepam (2.5 mg/kg, i.p.)	141.2 ± 6.1 [b]	41.3± 2.5 [b]

Each column represents mean ± S.E.M, n = 6. a = p<0.01, b = p<0.001 compared to control

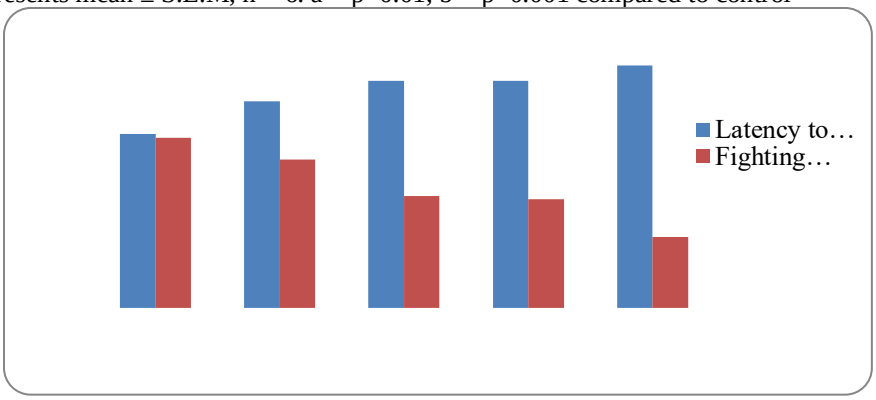

Figure. 6. Effect of *Hibiscus* (100, 200 and 400 mg/kg, p.o.) and Lorazepam (2.5 mg/kg) on clonidine induced aggression in mice. Each column represents mean ± S.E.M, n = 6. a = p<0.01, b = p<0.001 compared to control

CONCLUSION:

- ✓ The results from the present study confirm the antidepressant activity of hibiscus since it reduced the immobility in both FST and TST.
- ✓ In the present study, hibiscus significantly increased the frequency of 5-HTP induced head twitches, Clonidine induced aggression and L-DOPA induced hyperactivity and aggressive behavior indicating its enhanced activity on serotonergic, noradrenergic and dopaminergic pathways respectively. Our results also confirm the involvement of serotonergic, noradrenergic and dopaminergic pathways in depression.
- ✓ Pretreatment with hibiscus, also significantly increased the levels of SOD and Catalase with simultaneous decrease in LPO levels in mice brain, suggesting its strong antioxidant activity. Since oxidative stress is reported to play an important role in depression, the antioxidant activity of hibiscus might be a part of the mechanism for its antidepressant activity.
- ✓ Results from behavioral experiments indicate that the antidepressant activity of hibiscus, might be due to the facilitatory effect on serotonergic, noradrenergic and dopaminergic systems apart from the antioxidant activity.

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