Antidepressant activity of ethanolic extract of *Hibiscus rosa sinenesis* Linn.

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Abstract: The present study was undertaken to assess the antidepressant effect of crude ethanolic extract of floral part of *Hibiscusrosa sinensis* (HRS) at doses 100mg/kg, 250mg/kg and 500mg/kg using three parameters which are forced induced swimming test (FST), tail suspension test (TST) and open field test(OFT). Flouxetine (15mg/kg, body weight) was used as standard. Significant dose dependent decline in immobility time was observed in all the three doses in FST and TST while in case of OFT none of the dose of HRS exhibited effectual results. To determine MAO"A" and MAO"B" activity HRS extract was used and the results revealed that each dose of this plant exhibited marked effect on MAO"A", while on MAO"B" only 250mg/kg dose was found significant.

Keywords: Antidepressants, monoamine oxidase, forced induced swimming test, tail suspension test, open field test.

INTRODUCTION

According to the World Health Organization report, approximately 450 million people suffer from mental or behavioral disorders, yet only a small minority of them receives even the most basic treatment. This amount is 12.3% of the global burden of disease and will rise to 15% by 2020 (WHO, 2001; Reynolds, 2003). It is considered to have its pessimistic impact on cognitive behavior and some patient may have suicidal tendency. There are a large number of aggravating factors for depression like stress, study load, relationship and socioeconomic problems. It is documented in literature that depression is mainly caused by insufficiency of neurotransmitters i.e. norepinephrine, serotonin, gamma amino butyric acid and glutamate. A number of proficient allopathic medicineslike selective serotonin reuptake inhibitors, tricyclic antidepressants and monoamine oxidase inhibitors are available which increase the level of such neurotransmitters and would be helpful in reducing depression but the main drawback of these drugsare their wide range of side effects. This perhaps wrenches the attention of researchers to search for new anti depressant drugs from the natural source. Many herbs have been reported for their anti depressant action like *Curcuma longa* and *Echium vulgare* etc (Yu *et al*, 2002; Seyed *et al*., 2007). Search for new therapeutic products for the treatment of neurological disorders from nature has progressed constantly, demonstrating the effectiveness of different plant species in a variety of animal models (Zhang, 2004).

Hibiscus rosa sinensis Linn. (Malvaceae) is an ornamental and ever blooming plant, commonly recognized as china rose. It is a native of tropical Asia cultivated in various regions of Pakistan. The flower of the plant is pendicillate, having five red petals, Fruits are typically capsular in structure and the leaves are simple, ovate and green in color (Gautam *et al*, 2013). It holds a large quantity of chemical entities likecyclopropanoids, methyl sterculate, methyl-2- hydroxysterculate, 2-hydroxysterculate, malvate, beta-rosasterol, anthocyanin, amino acids (aspartic acid and asparginin), fat, calcium, phosphorus, iron, thiamine, riboflavin, niacin, flavonoids ,fibers and vitamin C (Anonymous, 1995; Anonymous, 2000). This plant has great nutritional value along with an abortifacient**,** emollient, aphrodisiac, emmenagogue action and also used in heart disorders and pulmonary affections (Borhan *et al*, 2010; Reddy *et al*, 1997). The earlier studies had also proved that the flower extract of this plant have an effect on the male fertility (Singh *et al*, 1882). The aim of our study is to determine antidepressant activity of the plant and the mechanism by which it performs this action.

MATERIAL AND METHOD

Collection and identification of plant material

Fresh flowers of *Hibiscus rosa sinensis* Linn. (HRS) were collected from the botanical garden of University of Karachi and then authenticated by Prof. Dr. Ghazala H. Rizwani, Department of Pharmacognosy, Faculty of Pharmacy, University of Karachi and then sited in herbal museum with specimen number 001.

Preparation of ethanolic extract

Flowers HRS (2 kg) were broken, cleaned, into small pieces and then percolated in absolute ethanol (Merck, Germany) for the duration of 15 days at room temperature and shaken occasionally. Ethanolic percolate was filtered through Whatmann filter paper No. 1, then evaporated on

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rotary evaporator (Eyela, Japan) at reduced pressure and controlled temperature (40°C). The reddish brown semisolid residue (2.5 g) was obtained after complete evaporation of ethanolic flower percolate.

Drugs and chemicals

Flouxetine (Merck Pharmaceutical, Pakistan) was taken as standard drug, diluted in 10% Dimethyl sulfoxide solution (DMSO). For rat's and mice's all the treatments were given in a volume of 5ml/kg and 10ml/kg respectively.

Animals

Male Swiss Albino mice (24-30gm) and Wistar rats (120-200gm) were provided by the animal house of HEJ Research Institute of Chemistry, International Centre for Chemical Sciences, University of Karachi. Animals were kept in a noise free area in a plastic cage maintained on standard light cycle (12 hours light/dark) and supplied with standard food and water.

Forced induced swimming (FST)

This test was performed in two parts, first was known as pre test, which took place one day before the experiment for the selection of animals for further studies (Porsolt *et al*, 1977). The selected rats were divided into five groups i.e. control, standard (Flouxetine), HRS (100mg/kg, 250mg/kg and 500mg/kg). Each group contained ten animals. All the drugs were given by means of intra peritoneal route. After one hour of drug administration rats were again forced to swim in a glass tank (height 45cm, width 12cm) in which water level should not increases from 15cm for the duration of 6min. In this period immobility time have to be counted (when rats stop struggling) (Porsolt *et al.*, 1979; Selvi *et al.,* 2012).

Tail suspension test (TST)

In this experiment male mice's were divided into five groups with ten members in each. Mice's were administered with control, standard (Flouxetine 15mg/kg), HRS (100mg/kg, 250mg/kg, and 500mg/kg) respectively by intra peritoneal route. One hour later mice's were suspended with their tails to the edge of the table, 35cm away from the bottom for 5min interval. Immobility time was counted when mice's hang submissively (Steru *et al.,* 1985).

Open field test (OFT)

This test was carried out on mice's to evaluate the effects of investigational drug on mobility of animal. Open field equipment was made of plywood which is white in colour and measured 72 by 72 and wall is 36cm long In this test mice's were treated individually with DMSO, standard drug Flouxetine (15mg/kg) and testing drugs ethanolic extracts of *Hibiscus rosa sinensis* L. (100mg/ml, 250mg/ml and 500mg/ml). Then placed them independently in the middle of the open field for 5 minutes to count Total Locomotion (TL) i.e. the total number of square crossed both outer and inner ones, Peripheral Locomotion (PL), and Central Locomotion (CL) respectively. The other factors, which were also evaluated, are number of rearing, leaning, grooming and defecation (Walsh *et al*, 1976; Seyed *et al*, 2007).

MAO Assay

As explained before with some alterations (Veronika *et al*, 2003), rats brain mitochondrial fraction had been prepared then suspended this fraction into 10 volume of (0.2M, pH 7.4 cold sodium phosphate buffer .Centrifuged the mixture at 1000× g for the duration of 10 min at 0°C, the resulting supernatant was repeatedly centrifuged at 17 000 × g for the time period of 30min at 4°C.Supernatent was discarded and re suspended the pallet in the same buffer and it was store up for 72 hours for MAO investigation at -30°C .Protein concentration was anticipated via the process (Lowry *et al*, 1951) by means of bovine serum albumin as the standard and attuned to 1mg/ml. The assay combinations contained 0.3ml of 4mmol 5-HT or 8 mmol Benzylamine (as exact substrates for MAO-A and MAO-B, respectively), mitochondrial fraction 0.5ml and at last volume adjusted to 3ml by sodium phosphate buffer. The reaction was permitted to proceed at 37°C for 60 min, and clogged by adding 0.6ml of 1 M hydrochloric acid. The product of reaction was extracted with 4ml of butylacetate for MAO-A assay and cyclohexane for MAO-B assay respectively. After separation of organic phases of each reaction, measured them on spectrophotometer at wavelength of 280nm for MAO-A and at 242 nm for MAO-B assay. Blank samples were assembled in a same manner like assay combination without using mitochondrial fraction (Benhong *et al*, 2011).

RESULTS

Three different doses of ethanolic extract (100, 250 and 500mg/kg, i.p.) of *Hibiscus rosa sinensis* were used to investigate the antidepressant effect of this plant showed in table 1and 2. Injection of DMSO (control) did not exhibit significant effect on immobility time and swimming time in the forced swimming test compared to pre injection status. Therefore, all experimental groups were compared with saline as the control group. The administration of, Flouxetine (15mg/kg) as a positive control, in rats significantly decreased immobility time (99.0±3.78) respectively compared to the control group. While HRS extract (100, 250 and 500mg/kg) significantly decreased immobility time 99.0±1.73, 63.6±5.84, 49.3±3.17 respectively.

HRS extract and standard drug (Flouxetine 15 mg/kg) induced significant diminution of immobility time in tail suspension test (Control, 164.33±8.37; HRS 100mg/kg, 155.6±4.6; HRS 250mg/kg, 105.3±13.2; HRS 500mg/kg,

Table 1: Effects of *Hibiscus rosa sinensis* on duration of immobility time in forced swim test (FST)

Treatment Groups	Concentration (mg/kg)	Length of immobility Mean SEM (sec)	% Change
Control	-	128.66±8.45	-
Flouxetine	15	99.0±3.78↓	23.1**
Hibiscus rosasinensis L.	100	99.0±1.73↓	23.1**
	250	63.6±5.84↓	50.6***
	500	49.3±3.17↓	61.7***

Table 2: Effects of *Hibiscus rosa sinensis* on duration of immobility time in tail suspension test (TST)

Treatment Groups	Concentration (mg/kg)	Length of immobility Mean SEM (sec)	% Change
Control	-	164.33±8.37	-
Flouxetine	15	96.6±6.3↓	39.4***
Hibiscus rosasinensis L.	100	155.6±4.6↓	5.4
	250	105.3±13.2↓	36***
	500	89.6±7.8↓	45.5***

Table 3: Effects of *Hibiscus rosa sinensis* on duration of immobility time in open field test (OFT)

Treatments	TL	CL	PL	L	G	D
Control	143.3±6.5	31.0±4	112.3±2.51	9.0±2	1.6±0.57	0.33±0.57
Flouxetine (15mg/kg)	152.6±2.72	36±2.08	116.6±2.33	9.0±1.52	0.33±0.32	0.0±0.0
Hibiscus rosa sinensis (100mg/kg)	119.3±5.24	24.3±2.4	95.0±5.5	11.3±2.02	1.33±0.32	0.0±0.0
Hibiscus rosa sinensis (250mg/kg)	135.6±13.5	27.3±5.04	108.3±8.95	10.3±1.20	2.33±1.20	0.0±0.0
Hibiscus rosa sinensis (500mg/kg)	149.6±17.87	27.3±5.2	120.3±17.2	11.0±3.21	1.33±0.32	0.0±0.0

Values are expressed as Mean ± S.E.M (n=10). **P* <0.05, ***P*<0.01, ****P*<0.001 when compared with control groups. TL: Total Locomotion, PL: Peripheral Locomotion, CL: Central Locomotion (CL), L: leaning, G: grooming, D: defecation

Table 4: Effects of *Hibiscus rosa sinensis* on activity of MAO-A in the mouse whole brain

Treatment	Concentration (mg/kg)	MAO A activity (µmol/mg protein. h) Mean ±SEM	% Change
Control	-	40.06±1.80	-
Flouxetine	15	23.0±0.49	42.6***
Hibiscus rosasinensis L.	100	23.1±1.78	42.4***
	250	29.8±0.99	25.7***
	500	32.5±0.84	19**

Table 5: Effects of *Hibiscus rosa sinensis* on activity of MAO-B in the mouse whole brain

Treatment	Concentration (mg/kg)	MAO B activity (µmol/mg protein. h) Mean ±SEM	% Change
Control	-	16.63±1.15	-
Flouxetine	15	13.3±0.23	20.1*
Hibiscus rosasinensis L.	100	13.3±0.71	20.1
	250	10.7±0.34	35.7**
	500	15.1±1.99	9.3

Values are expressed as Mean ± S.E.M (n=10). **P*<0.05, ***P*<0.01, ****P*<0.001 when compared with control groups

89.6±7.8; and Flouxetine 15 mg/kg, 96.6±6.3; compared with the control. The results obtained were shown in table 2.

The Open field test provides simultaneous measures of locomotion, exploration and anxiety. The results of open field test were demonstrated in table 3.

The effects of *Hibiscus rosa sinensis* extract on the MAO A and B activities in mouse, whole brain were shown in tables 4 and 5. The MAO A and B activities in normal group were 40.06±1.06 µmol/mg protein. h and 16.63±1.15 µmol/mg protein. h respectively. Oral administration of HRS extract at the doses of 100 mg/kg, 250 mg/kg and 500 mg/kg provided 23.1±1.78, 29.8± 0.99, 32.5±0.84µmol/mg protein. (MAO-A) and 13.3±0.71, 10.7±0.34, 15.1±1.99µmol/mg protein. (MAO-B) activity respectively.

DISCUSSION

The widespread use of FST is mainly due to its ability to detect a broad spectrum of antidepressant agents. The test is based on the observation that animals following initial escape-oriented movements develop an immobile posture when placed inside an inescapable cylinder filled with water. The immobility is thought to reflect either a failure of persistence in escape-directed behavior (i.e., despair behavior) or the development of a passive behavior, meaning the loss of the animal's ability to cope with stressful stimuli (Cryan *et al.*, 2005; Detke *et al.*, 1997). The agents that decrease this behavior are presumed to have antidepressant effects (Porsolt *et al.*, 1997).

There was no significant difference between the effect of the various doses of the HRS extracts and that observed with Flouxetine group on the immobility time when the mice were exposed to the TST. Markedly showed a significant (P<0.001) decrease in the time spent immobile by animals. By performing tail suspension test, the reduced immobility time directed the antidepressant effect of extract (Onasanwo *et al.*, 2010).The antidepressant effect of methanolic extract of *Hibiscus rosa sinensis* was also performed previously but its mechanism of action was not cleared (Shewale *et al.,* 2012). In this study MAO inhibitory activity of this plant was also performed to confirm its mechanism by which it produced its antidepressant effect, which was not reported earlier.

For the open field test number of line crosses and the frequency of rearing are usually used as measures of locomotor activity, but are also measures of exploration and anxiety. A high frequency of these behaviors indicates increased locomotion and exploration and/or a lower level of anxiety. The number of central square entries and the duration of time spent in the central square are measures of exploratory behavior and anxiety. A high frequency/duration of these behaviors indicates high exploratory behavior and low anxiety levels (Walsh and Cummins, 1976).

Monoamine oxidase (MAO A and B), are most important enzymes in the metabolism of a wide range of monoamine neurotransmitters, including nor adrenaline, dopamine, and5-hydroxytryptamine (5-HT), plays a crucial role in depression and age-related disorders. MAO-A is more important than MAO-B in the metabolism of the major neurotransmitter monoamines therefore MAO-A inhibitors have been accepted to treat depression more precisely (Knoll, 1997; Wouters, 1998). Inhibition on MAOs could increase monoamine neurotransmitters in brain and leading towards moderate depressive symptoms (Guangting *et al.*, 2008). A number of drugs are available for the treatment of depression, but clinical evaluation of these drugs have shown incidence of relapses, side effects, and drug interactions. MAO inhibitors can induce sedation or behavioral excitation and have a high risk of inducing postural hypotension, sometimes with sustained mild elevations of diastolic blood pressure (Hamilton and Opler, 1992).

CONCLUSION

From the results it was concluded that all the doses of ethanolic HRS extract (100mg/kg, 250mg/kg and 500mg/kg) showed significant antidepressant activity.

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