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Acute and Chronic Effect of *Hibiscus rosa sinensis* Flower Extract on Anxiety Induced Exploratory and Locomotor Activity in Mice

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ABSTRACT

The present study was initiated with a view to establish the possible effects of *Hibiscus rosa sinensis* on anxiety induced locomotor activity and exploratory behaviour in mice. *Hibiscus rosa sinensis* is an acclaimed important medicinal plant of Indian system of medicine recommended for its refrigerant and calming effect for centuries. In this study, the effect of alcoholic and chloroform extracts *Hibiscus rosa sinensis* on latency and exploratory behavior was studied by using open field test. The results obtained were analyzed by student 't' test. The results obtained in the open field test shows statistically significant decrease in exploratory behaviour, latency and increased rearing and ambulatory behaviour. Both the vertical and horizontal movements increased significantly as compared to the control group ($p<0.05$). Ethanolic extract showed better results than the chloroform extract ($p<0.01$). Both the extracts showed no significant increase in urination and defecation of the animals. The results suggest that *Hibiscus rosa sinensis* has significant anti-anxiety associated psychobehaviour modulation.

Key words: Psychobehaviour, locomotor activity, exploratory behavior, ambulatory behavior, anxiety

INTRODUCTION

Anxiety has been considered as a normal physiological and emotional behavior if it is in reasonable amounts and at appropriate times. But it tends to be a pathological condition when it is either completely inappropriate to the situation or in excess of what the situation should call for, leading to precipitate or aggravate cardiovascular and psychiatric disorders (Krishna *et al*., 2006). Open field testing has very little or no impact on the animal's subsequent behavior. Rats and mice tend to avoid brightly illuminated, novel, open spaces, so the open field environment acts as an anxiogenic stimulus and allows for measurement of anxiety-induced locomotor activity and exploratory behaviors (Prut and Belzung, 2003). Indian system of medicine has several solutions for successful treatment of various psychiatric complications with less or no side effects; *Hibiscus rosa sinensis* flower is acclaimed to be one of them (Pandey and Chunekar, 2004; Pari and Maheshwari, 1999).

Hibiscus rosa sinensis Linn. is most commonly known as Jasvand, belongs to family: Malvaceae. It is an evergreen, woody, glabrous and showy shrub. Several phytochemical investigations on *Hibiscus rosa sinensis* Linn. flowers have shown the presence of several

medicinally important active constituents including steroids, flavonoids, tannins, reducing sugars, mucilages. It is also being known to contain an anthocyanin pigment, cyanodin diglucoside, carotene, thiamine, riboflavin, niacin and ascorbic acid (Anonymous, 2001). In ancient text of Indian system of medicine various parts of this plant have been recommended for different kind of biological activities (Nandkarni, 1998). The whole plant including leaves, flowers and roots have been known to posses hair growth, anti-fertility, anti-implantation, abortifacient, aphrodisiac, mennorhagic, oral contraceptive and laxative properties (Nivsarkar *et al*., 2005). Several studies have shown that the flowers of this plant are more significant and important medicinal properties than compared to other parts of this plant. The alcoholic extract showed cardio protective activity (Gauthaman *et al*., 2006), due to angiotensin converting enzyme inhibition and blood pressure lowering effect (Kate and Lucky, 2010). The flower preparation of *Hibiscus rosa sinensis* is being most commonly used in traditionally based herbal hair oil preparations due to its hair dyeing and growing properties and is also based on the hypothesis of its central refrigerant and cooling effects (Rao and Sujatha, 2008) However, no detailed scientific investigations had been carried out till the date to validate this claim. Hence, the present study was undertaken to find out its efficacy modulation of anxiety induced locomotor and exploratory behaviour.

MATERIALS AND METHODS

Drugs: Diazepam (compose) ampoules were purchased from local market, (Ranbaxy laboratories limited). The flowers of *Hibiscus rosa sinensis* were collected in the ayurvedic college Bagalkot after authentification by Prof. V.V. Sidlingappanavar, Head, Department of Botany, BVVS science college, Bagalkot by the studies including organoleptic tests, macroscopic and microscopic observations. The flowers were dried in shade until they were free from moisture. Finally the flowers were subjected to get fine powder and then passed through sieve No. 44 to get uniform powder.

Plant preparation: The powdered flower was subjected to continuous hot extraction with chloroform and ethanol, then the solvent was distilled off and excess solvent was completely removed by using a rotatory flash evaporator to get green colored semisolid chloroform extract (yield: 0.44%) and brown colored ethanolic extract (yield: 1%). The extracts obtained were dried in Mini Lyotrap.

Animal selection: Male Swiss Albino mice weighing between 18-25 g were used for the study. To prevent any hormonal variations only male mice were selected. The colony was maintained under controlled conditions of light (12:12, light and dark), temperature (25±2°C) and humidity (50±10%). The animals were housed in sterile polypropylene cages containing autoclaved paddy husk as bedding material. Animals were provided with pellet diet and filtered acidified water ad libitum. All the pharmacological protocols were approved by the Institutional animal ethical committee, (HSKCP/IAEC.Clear/2004-05) HSK College of Pharmacy, Bagalkot, Karnataka.

The animals were divided into nine groups of seven animals each for open field test.

Group 1: Normal
Group 2: Solvent control (2% Tween 80, 5 mL kg^{-1}, p.o.)
Group 3: Standard (Diazepam 2 mg kg^{-1}, i.p.)
Group 4: Chloroform extract (50 mg kg^{-1}, p.o.)
Group 5: Chloroform extract (100 mg kg^{-1}, p.o.)

Group 6: Chloroform extract (200 mg kg^{-1}, p.o.)
Group 7: Ethanolic extract (50 mg kg^{-1}, p.o.)
Group 8: Ethanolic extract (100 mg kg^{-1}, p.o.)
Group 9: Ethanolic extract (200 mg kg^{-1}, p.o.)

Open-field test: Open field test is used to assess exploratory behavior of animals during a period of five minutes. Animals were allowed to fast overnight with water *ad libitum* and kept under laboratory conditions one hour prior to open field test. The floor of field was made by polyvinyl tiles with a black grid dividing the open field into 64 squares (5×5 cm, for mice). Illumination was provided by a bulb (60 Watts) placed above the field in the center of the field, while the rest of the room was in darkness. The mouse was placed in the center of the field and observed for 5 min. During this period the following observations were recorded manually: Latency to explore, rearings (vertical movement), lines crossed (horizontal movement), grooming, defecation and urination. Animals were given single daily dosing of various treatments for a period of fourteen days. Study was carried on first day for acute study and seventh and fourteenth day for chronic study (Vogel, 2002).

Statistical analysis: Results were expressed as Mean±SEM and were analyzed by student 't' test by comparing control group with normal mice and extracts treated with control group. Graph pad prism software was used for analysis of the experimentally generated data.

RESULTS

All the treatment groups have shown no significant effect on anxiety induced defecation and urination in open field test, throughout the study. Significant reduction in exploration latency was observed on first, seventh and fourteenth days of observation when treated with 50, 100 and 200 mg kg^{-1} dose of *Hibiscus rosa sinensis* ethanolic extract. Whereas small and moderate dose of chloroform extract have only shown reduction in exploratory latency. On first day of treatment chloroform extract (50 mg kg^{-1}) significantly improved latency, rearing and ambulatory behavior ($p<0.01$) when compared to control group, with ethanolic extract on day 1 it has variable effects with most significant results in highest dose (Table 1). Both horizontal and vertical movements were significantly increased in mice treated with chloroform extract (50 and 100 mg kg^{-1}) on

Table 1: Effect of *Hibiscus rosa sinensis* on locomotion activity of male swiss albino mice in open field test (acute study)

Drug treatment	Latency to explore (Sec)	Vertical movement rearing	Horizontal movement	Grooming	Defecation	Urination
Normal	22.43±6.5	4.43±1.2	68.14±10	1.71±0.42	1.57±0.37	0.43±0.20
2%Tween 80 (5 mL kg^{-1})	12.42±1.3	4.00±0.69	70.14±6.3	2.57±0.57	0.71±0.29	0.43±0.20
Diazepam 2 mg kg^{-1}	10.42±1.4	7.57±1.0*	107.57±14*	1.57±0.48	0.71±0.29	0.29±0.28
Chloroform extract 50 mg kg^{-1}	7.00±1.4*	9.60±1.3**	137.00±17**	2.40±0.53	1.10±0.53	0.00±0.40
Chloroform extract 100 mg kg^{-1}	6.60±1.3**	7.40±1.3	109.00±13*	2.70±0.57	0.86±0.26	0.00±0.00
Chloroform extract 200 mg kg^{-1}	14.00±2.6	6.00±0.87	76.00±11	2.40±0.53	1.00±0.38	0.14±0.14
Ethanolic extract 50 mg kg^{-1}	6.57±2.10*	5.86±1.06	81.90±12.8	4.00±0.69	1.14±0.26	0.286±0.184
Ethanolic extract 100 mg kg^{-1}	9.29±1.38	5.14±0.80	61.30±6.99	3.43±0.751	1.14±0.261	0.286±0.184
Ethanolic extract 200 mg kg^{-1}	5.29±1.13**	11.70±3.97	123.00±18.5*	6.00±0.85**	0.571±0.291	0.143±0.143

Results were expressed as Mean±SEM and were analyzed by student 't' test by comparing control group with normal mice and extracts treated with control group. p-value<0.05 was considered significant. *p<0.05, **p<0.01, ***p<0.001

Table 2: Effect of *Hibiscus rosa sinensis* on locomotor activity of male swiss albino mice in open field test (chronic study, Day-7)

Drug treatment	Latency to explore (Sec)	Vertical movement rearing	Horizontal movement	Grooming	Defecation	Urination
Normal	22.30±6.29	5.86±1.49	69.60±9.29	2.71±0.471	2.00±0.309	0.857±0.350
2% Tween 80 (5 mL kg^{-1})	15.10±1.43	4.57±0.84	67.14±5.57	3.29±0.778	1.14±0.26	0.571±0.202
Diazepam 2 mg kg^{-1}	7.43±1.54**	9.14±1.18**	111.00±10.6**	3.71±0.421	0.49±0.202	0.49±0.202
Chloroform extract 50 mg kg^{-1}	5.43±0.812***	11.40±1.32***	137.00±15.5**	5.00±0.420	0.286±0.184	0.143±0.143
Chloroform extract 100 mg kg^{-1}	7.29±1.03***	8.57±1.3*	109.00±12.7*	5.29±0.274*	0.714±0.36	0.00±0.00
Chloroform extract 200 mg kg^{-1}	14.90±3.62	8.40±1.9*	74.40±9.56	3.86±0.705	1.29±0.286	0.286±0.184
Ethanolic extract 50 mg kg^{-1}	6.29±1.38***	8.71±1.34*	85.30±10.8	6.43±0.972*	0.714±0.286	0.286±0.184
Ethanolic extract 100 mg kg^{-1}	9.00±1.40*	8.00±0.816*	64.60±7.44	5.57±0.812	1.00±0.378	0.429±0.297
Ethanolic extract 200 mg kg^{-1}	4.57±1.07***	14.10±3.00**	127.00±16.6**	11.10±1.10***	0.286±0.184	0.143±0.143

Results were expressed as Mean±SEM and were analyzed by student't' test by comparing control group with normal mice and extracts treated with control group. p-value<0.05 was considered significant. *p<0.05, **p<0.01, ***p<0.001

Table 3: Effect of *Hibiscus rosa sinensis* on locomotor activity of male swiss albino mice in open field test (chronic study, Day-14)

Drug treatment	Latency to explore (Sec)	Vertical movement rearing	Horizontal movement	Grooming	Defecation	Urination
Normal	20.30±5.28	7.14±1.35	67.1±9.24	5.43±0.71	1.57±0.20	0.857±0.261
2% Tween 80 (5 mL kg^{-1})	17.40±1.69	5.57±0.97	66.0±4.94	5.43±1.11	1.00±0.30	0.714±0.286
Diazepam 2 mg kg^{-1}	6.71±1.21***	12.40±1.07***	118.0±11.4**	6.86±0.88	0.429±0.20	0.286±0.184
Chloroform extract 50 mg kg^{-1}	4.86±0.80***	13.40±1.41***	144.0±13.7***	7.57±0.75	0.286±0.18	0.286±0.184
Chloroform extract 100 mg kg^{-1}	6.14±1.10***	11.90±1.39**	115.0±10.4**	10.40±1.15**	0.571±0.29	0.143±0.143
Chloroform extract 200 mg kg^{-1}	11.90±1.70*	11.70±1.11**	70.3±7.79	5.43±0.94	1.140±0.34	0.571±0.202
Ethanolic extract 50 mg kg^{-1}	7.00±1.15***	9.86±1.03*	89.4±10.1	6.57±0.84	0.714±0.28	0.429±0.202
Ethanolic extract 100 mg kg^{-1}	8.00±0.97***	9.29±0.89*	62.4±5.73	6.71±0.56	0.571±0.20	0.429±0.202
Ethanolic extract 200 mg kg^{-1}	4.86±0.67***	14.90±3.31*	133.0±15.6**	11.70±0.75***	0.429±0.20	0.0±0.0

Results were expressed as Mean±SEM and were analyzed by student't' test by comparing control group with normal mice and extracts treated with control group. p-value<0.05 was considered significant. *p<0.05, **p<0.01, ***p<0.001

seventh and fourteenth day of observation. Ethanolic extract produced significant increase only in vertical movements without affecting horizontal movements at 50 and 100 mg kg^{-1} dose. The maximum dose of ethanolic extract (200 mg kg^{-1}) significantly increased both the vertical and the horizontal movements throughout the study. On the seventh day of the study chloroform extract (50 and 100 mg kg^{-1}) significantly reduced the latency to explore with values of 5.43 and 7.29 seconds, respectively (p,0.001), lowest dose of chloroform extract and highest dose of alcoholic extract significantly increased vertical as well as horizontal movements (Table 2) Chloroform extract (50 mg kg^{-1}) and ethanolic extract (200 mg kg^{-1}) significantly improved latency and ambulatory behavior on fourteenth day of study with a value of 4.86 seconds for both the extracts (p<0.001), both the vertical and horizontal movements were increased by all the doses of both the extracts however most significant response was produced by chloroform extract (13.4, 144, p<0.001) at dose of 50 mg kg^{-1} Table 3. Significant effect in grooming behaviour was observed only with 200 mg kg^{-1} ethanolic extract on first seventh and fourteenth days of observation.

DISCUSSION

Anxiety may be associated with depression in the so called anxiodepressive syndrome (Do-Rego *et al*., 2005; Aderibigbe *et al*., 2010). This has prompted us to investigate the effect of *Hibiscus rosa sinensis* on open field test which is classically used for testing mood levels, locomotor

activity, grooming, defecation and to measure spontaneous activity (latency to explore, lines crossed and rearings) (Kasture *et al*., 2000). Normally animals when exposed to open field test show high defecation and urination scores, due to increase in colonic spike burst activity by involving cholinergic mechanism (Taiwe *et al*., 2010). However, treatment with chloroform and ethanolic extracts at all the doses produced no significant effect on total defecation and urination score indicating that the effect of *Hibiscus rosa sinensis* does not involve cholinergic mechanism in its activity.

The grooming behaviour is related to dearousal following exposure to stress. On acute and chronic administration with moderate dose of chloroform extract and high dose of ethanolic extract has pronounced grooming behaviour in the open field which could be viewed as high expression post stress behaviour. This grooming behaviour is believed to be elicited from paraventricular nucleus of the hypothalamus due to high activation of HPA (hypothalamic-pituitary-adrenocorticalaxis) (Do-Rego *et al*., 2005). This shows that the chloroform extract and ethanolic extract of *Hibiscus rosa sinensis* at these doses may be acting through HPA whereas other doses of both the extracts does not have any significant effect on grooming behaviour of acute and chronic treatment. The effect of *Hibiscus rosa sinensis* on spontaneous activity was studied by considering latency to explore, rearings and lines crossed. The prolonged latency time is reflective of immobility and depression whereas increased number of lines crossed (horizontal movement) is an indicative of CNS stimulant properties. Similarly the rearing (vertical movements) is an indicative of tendency of locomotion (Vogel, 2002). On acute and chronic treatment with diazepam, all these parameters significantly decreased throughout the study similar to previous results (Woode *et al*., 2009). On first day of treatment chloroform extract (50 mg kg^{-1}) significantly improved latency, rearing and ambulatory behavior ($p<0.01$) when compared to control group, with ethanolic extract on day 1 it has variable effects with most significant results in highest dose. *Hibiscus rosa sinensis* chloroform and alcoholic extract treatments significantly improved all the parameters on chronic treatment. These results indicate that the lower dose of chloroform extract and high dose of ethanolic extract are effective even after acute treatment better than diazepam. Hibiscus rosa sinensis flower extracts have decreased the latency of animals to move, the treatments have significantly increased the ambulatory behavior of the animals and did not show signs of depression by not affecting defecation and urination. Thus as a consequence of these observations it can be predicted that *Hibiscus rosa sinensis* possess anxiolytic properties without producing CNS depression.

CONCLUSION

Based on the results, it can be concluded that chloroform and ethanolic extracts of *Hibiscus rosa sinensis* flowers have significant effects on anxiety induced defects in ambulatory behavior. However, more promising results were obtained with alcoholic extract than the chloroform extract when compared to the control group. Most of the anti anxiety agents like Diazepam show increased incidence of urination and defecation in animals when brought to open field however no such incidences have been reported with *Hibiscus rosa sinensis*. Thus we conclude that chronic use of *Hibiscus rosa sinensis* has only antianxiety effect and no depression is produced.

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