OPEM
www.opem.org

Oriental Pharmacy and Experimental Medicine 2005 **5(4)**, 331-335

KHUP

Neuropharmacological screening of the methanolic extract of *Hibiscus vitifolius* flowers

S Seethalakshmi[1], D Chamundeeswari[2,*], S Jamuna Rani[1], S Parvathavardhini[1] and J Vasantha[2]

[1]Department of Pharmacology, [2]Sri Ramachandra College of Pharmacy, Sri Ramachandra Medical College and Research Institute (Deemed University), Porur, Chennai 600 116, India

SUMMARY

The methanolic extract of *Hibiscus vitifolius* flowers (HVE), was evaluated for neuropharmacological activities by carrying out rota rod, locomotor activity and traction performance in mice and swim endurance activity in rats in different dosages (10, 30 and 100mg/kg body weight). HVE showed a significant effect on central nervous system by increasing the time taken for rota rod, traction performance and locomotor activity while swimming time was found to be decreased when compared to normal control animals. These results suggest that HVE possess significant anxiolytic and anti depressant activity which may be attributed to the presence of flavonoid in HVE.

Key words: *Hibiscus vitifolius* flowers; Rota rod; Traction performance; Locomotor activity; Swimming time

INTRODUCTION

The flowers of the plant *Hibiscus vitifolius* Linn. (Malvaceae) commonly called as Bharadvaji are used in the treatment of diabetes. The root extract has been traditionally used to kill head lice (Chopra *et al.*, 2002). The petals of *H.vitifolius* are rich in flavonoids (Mathews, 1947). Gossypin a bioflavonoid from the flowers was reported to have anti-inflammatory activity in carrageenan induced granuloma in rats and delayed the onset of cataract formation in galactosemic rats and produced a significant decrease in the accumulation of lens galactiol in the animals (Pardar and Ghosh, 1979). Also gossypin significantly reduced the initial as well as late phases of paw edema and protein extravasation (Pardar, 1980). Gossypin act via opiate and L-2 adrenergic receptors by delaying small intestinal transit (Viswanathan *et al.*, 1985). It was also found that the adrenergic effect of gossypin is antagonized by calcium administration (Viswanathan *et al.*, 1985). The pharmacognostic study on various parts viz. leaf, seed, stem and flowers of *Hibiscus vitifolius* were reported (Palanisamy *et al.*, 1988). Gossypin was reported to have hepatoprotective activity (Anon *et al.*, 1992). The flavonoids from the fresh petals of *H.vitifolius* reduces the toxicity of dermally induced lipid peroxidation (Vijayaragavan *et al.*, 1992). A new flavonoid bioside from the flowers of *H.vitifolius* was reported to have hypoglycemic activity (Ragunathan *et al.*, 1994).

The present investigation is undertaken to study the neuropharmacological activity of *Hibiscus vitifolius* flowers in albino mice and rats.

*Correspondence: D Chamundeeswari, Sri Ramachandra College of Pharmacy, Sri Ramachandra Medical College and Research Institute (Deemed University), Porur, Chennai 600 116, India. Tel: +91-44-2-4768403; E-mail: dvchamu@yahoo.co.in

Materials and Methods

Preparation of extract

H.vitifolius flowers (authenticated by Dr P Jayaraman, Director, Plant Anatomy Research center, Tambaram, Tamilnadu) were collected in July 2003 in and around Chennai and a voucher specimen (Pharma No. 04/03) has been retained in the herbarium of the College of Pharmacy, Sri Ramachandra Medical College and Research Institute, Chennai.

Shade dried and coarsely powdered flowers of *Hibiscus vitifolius* (500 g) were extracted with methanol in an aspirator bottle at room temperature for 72 h. Nearly 80% of the solvent was removed by distillation over boiling water bath at atmospheric pressure and the remaining under reduced pressure. This extract (HVE) (Yield 5.5% w/w) was dissolved in water and used for animal experiments. HVE when subjected to phytochemical screening revealed the presence of flavonoids, glycosides and terpenoids (Harborne, 1973).

Animal studies

Male albino mice wistar strain (25 - 30 g) and albino rats (150 - 200 g) maintained in the animal house of Sri Ramachandra Medical College at room temperature (25 ± 2°C), relative humidity of 75 ± 5% and 12 h, dark-light cycle, were used for experiments. The mice and rats were fed with commercial pelleted rat feed (M/s. Hindustan lever, Mumbai) and water was given *ad libitum*. The animals were maintained at room temperature during the entire period of observation.

Oral toxicity test

Groups of six mice were given graded doses of HVE (10 - 100 mg/kg, i.p.) and continuously observed for 2 h to detect changes in various anatomical and behavioural response. Any mortality during the experiment and the following 7 days was also recorded. A group of animals treated with the vehicle (water) served as control (Turner, 1965).

Neuropharmacological screening

The animals were divided into four groups six in each group. Three groups were given HVE (10, 30, 100 mg/kg, i.p.) whereas one group was kept as control. All the experiments were carried out between 9 a.m - 12 noon to minimize the circadian variability. The neuro pharmacological activities were determined by the following methods.

Rota rod method

The procedure of Dunham and Miya (1957) was adopted for this study. Male albino mice which have the ability to remain on the revolving rod for at least 1 minute were selected for the test . The retention time of animals on the revolving rod was noted after 10, 20, 30, and 60 minutes of HVE administration. The same was noted for other animals also.

Traction performance test

The method of Turner (1965) was followed for this experiment. Mice were divided into 4 groups each containing 6 animals. They were suspended separately by means of their forepaws to a metallic wire stretched horizontally. The time taken for the animals to touch the wire at least with one of the hind paws was noted 10, 20, 30, and 60 minutes after HVE treatment in 3 doses. The first group served as control.

Locomotor activity

The method of Kuhn and Van Manen (1961) was followed for this study. Mice were housed in individual cages for 1 or 2 hours prior to intraperitoneal dosing. After 15 minutes the mice were placed in groups in counting boxes. The different groups were given HVE intra peritoneally. Interruption of a light beam passing through the box activates a photo cell relay and a summation counter. Counting was carried out at 10, 20, 30 and 60 minutes interval to assess locomotor activity in both HVE treated and control mice.

Table 1. Effect of *Hibiscus vitifolius* flower extract on central nervous system in rodents

Treatment Time in minutes	Normal control	HVE treated 10 mg/kg	HVE treated 30 mg/kg	HVE treated 100 mg/kg	*F*-ratio	*P*-value
Rota rod						
10	26.5 ± 1.76	32 ± 2.1	34.5 ± 2.17	34.67 ± 1.21	25.512	$P < 0.001$
20	26.0 ± 1.97	33.33 ± 1.97	37.5 ± 1.87	42.17 ± 1.72	85.973	$P < 0.001$
30	30.83 ± 1.17	38.33 ± 1.86	42.67 ± 1.75	49.83 ± 1.47	150.927	$P < 0.001$
60	26 ± 1.41	30.67 ± 2.16	34.67 ± 0.82	41.5 ± 1.52	107.376	$P < 0.001$
Traction performance						
10	7 ± 0.63	6 ± 0.63	7 ± 0.63	7.83 ± 0.75	7.642	$P < 0.001$
20	7 ± 0.63	10 ± 0.89	8 ± 0.89	11.67 ± 0.82	39.00	$P < 0.001$
30	7 ± 0.63	10 ± 0.89	9.33 ± 0.82	13 ± 0.89	55.00	$P < 0.001$
60	7 ± 0.63	8.17 ± 0.75	8 ± 0.89	10 ± 0.89	14.610	$P < 0.001$
Locomotor activity						
10	95 ± 1.87	98.17 ± 2.8	105.77 ± 2.7	110.0 ± 1.41	280.3	$P < 0.001$
20	122.33 ± 1.47	125.17 ± 1.47	141.5 ± 2.43	153 ± 1.41	959.616	$P < 0.001$
30	320 ± 1.41	323 ± 2.37	335.83 ± 2.86	354.33 ± 1.03	10565.668	$P < 0.001$
60	421.67 ± 2.16	426.33 ± 3.61	450 ± 1.41	527.33 ± 4.27	1673.907	$P < 0.001$
Swim endurance test						
10	30.33 ± 1.03	30.5 ± 1.52	28.5 ± 1.05	20.33 ± 1.03	100.281	$P < 0.001$
20	30 ± 0.63	30 ± 1.41	28 ± 1.41	19.83 ± 1.33	91.27	$P < 0.001$
30	30.17 ± 1.33	30 ± 1.41	26 ± 1.41	19.83 ± 1.33	74.749	$P < 0.001$
60	30.29 ± 2.36	29.67 ± 2.16	24 ± 1.41	20 ± 1.41	40.908	$P < 0.001$

The value represents mean±S.D. of six rats/mice

Swim endurance test

The swim endurance test was conducted as per the method of Thompson (1990). The rats were forced to swim in a plastic tub (60 × 40 cm) containing water at room temperature. Water depth was always maintained at 30 cm. The forced swimming test was studied at 10, 20, 30, 60 minutes after HVE treated and control rats. The escape latency time was noted periodically for each rats.

Statistical analysis

The results were analysed by the application of one-way analysis of variance (ANOVA). Values of $P < 0.05$ were considered statistically significant.

RESULTS

Effect of HVE on toxicity study

HVE did not produce any perceptible changes in the autonomic and behavioral responses in mice. There was no mortality up to the dose of 1.6 g/kg body weight even after 7 days.

Effect of HVE on rota rod

A statistically significant ($P < 0.001$), dose dependant increase in rota rod activity was observed in HVE treated mice compared to normal control. HVE treated animals showed increased activity until 30 minutes time after which it decreases. However the effect was more when compared to normal control group at all time intervals.

Effect of HVE on traction performance

A significant increase in traction performance was observed in the HVE treated mice compared to normal control group. It was found that the effect was maximum till 30 minutes after the drug administration but reduced after 30 minutes.

Time-dependent effect of HVE on locomotor activity

HVE showed a significant increase in locomotor

activity. The activity increases with time when compared to normal control group.

Effect of HVE on swim endurance activity

The decrease in swimming time was dose dependant compared to normal control animals.

DISCUSSION

Anxiety, depression and mental health problems in general and senile neurological disorders in particular are widely prevalent in the modern life with a multitude of stressful conditions. It was well established that the plant *H.vitifolius* possesses anti-inflammatory and anti-diabetic properties. In the present study administration of various doses of the alcoholic extract of the flowers of the plant *H.vitifolius* (HVE) did not show any toxicity thereby confirming the safety of *H.vitifolius* flowers.

The preliminary basis to assess whether a drug has CNS stimulant or depression effect is by rota rod test. In the present study HVE treated mice prolonged retention on the revolving rod until 30 minutes after drug treatment. But after 60 minute there was a decrease in motor activity thereby showing the biphasic effect of HVE. The effect may be due to the presence of bioflavonoid present in the *H.vitifolius* flowers. According to Raj Narayana *et al.* (2001), bioflavonoid possess anxiolytic activity like properties and the same property observed with *H.vitifolius*. In alternative system of medicine the anxiolytic effect is well documented in *Withania somnifera* (Salil *et al.*, 1997).

The ability of the animal to hold the metallic wire determines the traction performance study. HVE treated mice also showed increase in traction performance until 30 minutes but the effect was decreased in the later period (i.e. after 60 minute).

The locomotor activity is assessed by the movement of animal that crosses the light beam. In our present study HVE treated animals showed a dose-dependent increase in locomotor action with time. According to Ganesh *et al.* (2001) anxiolytic agents like diazepam increased locomotor activity. The anxiolytic activity may be due to the bioflavonoid present in HVE, that may acts on GABA and produces anxiolytic effect as given by synthetic flavonoids (Griebel *et al.*, 1999).

Swim endurance test is a proper model to test behaviour activity in animals. It was suggested that mice or rats forced to swim in a restricted space from which they cannot escape are induced to a characteristic behaviour of immobility. This behaviour reflects a state of despair which can be reduced by several agents that are therapeutically effective in depression.. In our study HVE treated rats showed a dose dependant decrease in swimming time i.e. reduced duration of immobility thereby showing its antidepressant effect . This activity is similar to activity of *Bacopa monnieri,* Ginseng and *Withania somnifera* (Sairam, 2002; Bhattacharya and Muruganandham, 2003).

Thus it is concluded that the flowers of *H.vitifolius* produces anti-stress and anti depressant effect which may be due to the presence of bioflavonoids and may acts on GABA receptors thereby produces its effects. Further studies are required to isolate the bioflavonoids and finding its efficacy as anxiolytic and antidepressant activity.

ACKNOWLEDGEMENTS

The authors thank Dr E Sukumar, Scientist, Central Research Institute for Siddha (CCRAS), Arumbakkam, Chennai for selection of plant material and the management of SRMC & RI (Deemed University) for providing facilities.

REFERENCES

Anon MT, Ubeda A, Alearaz MJ. (1992) Protective effect of phenolic compounds like flavones. *J. Fultad De Farmacia.* **47**, 275-279.

Bhattacharya SK, Muruganandam AV. (2003) Adaptogenic activity of *Withania somnifera*: an experimental study using a rat model of chronic stress. *Pharmacol. Biochem. Behav.* **75**, 547-555.

Chopra RN, Nayar SL, Chopra IC. (2002) Glossary of Indian Medicinal Plants, pp. 134. National Institute of Science Communication and Information Resources, CSIR, New Delhi.

Dunham NW, Miya TS. (1957) A note on a simple apparatus for detecting neurological deficit in rats and mice. *J. Am. Pharm. Assoc. Am. Pharm. Assoc.* **46**, 208-210.

Ganesh Chandra Sonavane, Vikram Sarveiya, Veena Kasture, Sanjay B Kasture. (2001) Behavioural actions of Myristica fragrans seeds. *Indian J. Pharmacol.* **33**, 417-224.

Griebel G, Perrault G, Tan S, Schoemaker H, Sanger DJ. (1999) Pharmacological studies on synthetic flavonoids, comparison with diazepam. *Neuro Pharmacol.* **38**, 965-977.

Harborne JB. (1973) *Phytochemical Methods*, pp. 52-105, Chapman & Hall, London.

Kuhn and Van Mannen. (1961) Depressants of the Central Nervous System. In: Screening Methods in Pharmacology, edited by Turner RA, Peter H, p. 78, Academic Press, New York.

Mathews M, Parmer NS, Ghosh MN. (1947) Tinctorial properties of flavonols of *Hibiscus vitifolius*. Important Institute London, **45**, 347-348.

Pardar NS, Ghosh MN. (1979) Delayed onset of cataract formation by gossypin. *J. Exp. Eye Res.* **29**, 224-232.

Pardar N.S, Ghosh MN. (1980) Reduction of paw edema by gossypin. *J. Pharmacol.* **12**, 201-202.

Palanichamy S, Nagarajan S. (1988) Pharmacognostic studies on Bharadwaji-*Hibiscusvitifolius*. *Bull Medico Ethnobot Res.* **9**, 148-152.

Raghunathan V, Sulochana K. (1994) hypoglycemic activity of flavonol bioside from *Hibiscus vitifolius*. *J. Indian Chem. Soc.* **71**, 705-706.

Raj Narayana K, Sripal Reddy M, Chaluvadi MR, Krishna DR. (2001) Bioflavonoids classification, Pharmacological, biochemical effects and therapeutic potential. *Indian J. Pharmacol.* **33**, 2-16.

Sairam K, Dorababu M, Goel RK, Bhattacharya SK. (2002) Antidepressant activity of standardized extract of Bacopa monniera in experimental models of depression in rats. *Phytomedicine* **9**, 207-211.

Salil K Bhattacharya, Kalkunte S Satyan, Shibnath Ghosal. (1997) Antioxidant activity of glycowithanolides from *Withania somnifera*. *Indian J. Exp. Biol.* **35**, 236-239.

Turner RA. (1965) Screening Methods in Pharmacology, Vol I, pp 26-27, Academic Press, New York.

Thompson EB. (1990) Drug bioscreening, Drug evaluation techniques in Pharmacology, pp. 33-34, VCH Publishers Inc., New York.

Vijayaraghavan R, Surendran K, Pant SC, Hussain K, Malhotra RC. (1991) Reduction of toxicity by flavones. *J. Toxicol.* **69**, 35-42.

Viswanathan S, Thirugnanasambantham P, Bapha JS, Kameswaran L. (1984) Mediation of action of gossypin. *Indian J. Pharmacol.* **270**, 151-157.

Viswanathan S, Sambandam P, Ramasamy R, Kameswaran L. (1985) Studies on gossypin tolerance and mechanism of action. *Indian J. Exp. Biol.* **23**, 525-526.