OPEM
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Oriental Pharmacy and Experimental Medicine 2007 **7(4)**, 409-417
DOI 10.3742/OPEM.2007.7.4.409

Effect of a polyherbal formulation on anxiety and behaviour mediated *via* monoamine neurotransmitters

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SUMMARY

We investigated the effect of Arogh, a polyherbal formulation (PHF) on animal models of anxiety based on exploratory behavior. The anxiolytic activity of polyherbal formulation (30, 100, 300 and 500 mg/kg) was studied using various behavioural paradigms such as elevated plus maze (EPM), light/dark apparatus (LDA), open field apparatus (OFA), hole board apparatus (HBA). Diazepam (1 mg/kg) was used as a standard anxiolytic drug. The effect of PHF (100 and 300 mg/kg) on serotonin, dopamine and noradrenaline mediated behaviour was studied by lithium induced head twitches in rats, haloperidol induced catalepsy in mice and clonidine induced hypothermia in rats respectively. In EPM, PHF (100, 300 and 500 mg/kg) significantly ($P < 0.05$) increased the time spent in open arms and the number of entries in open arms. In LDA, PHF (100, 300 and 500 mg/kg) significantly ($P < 0.05$) increased the time spent in lit zone. In OFA, PHF (100, 300 and 500 mg/kg) significantly ($P < 0.05$) increased the number of assisted rearing and the number of squares traversed. In HBA, PHF (100, 300 and 500 mg/kg) significantly ($P < 0.05$) increased the number of head poking. In lithium induced head twitches, PHF (100 and 300 mg/kg) significantly ($P < 0.05$) decreased the number of head twitches. In haloperidol induced catalepsy, PHF (300 mg/kg) decreased the duration of catalepsy significantly ($P < 0.05$) at 60 min. In clonidine-induced hypothermia, PHF (300 mg/kg) did not modify the effect. Drugs must be carefully assessed on EPM test and therefore in the present study EPM is supported by other tests. Present study indicates that Arogh, a polyherbal formulation possess anxiolytic activity. It diminished serotonergic transmission and decreased the duration of catalepsy indicating potentiation of dopaminergic transmission. Thus, Arogh a polyherbal formulation contains bioactive principles which possess anxiolytic activity and modified 5-HT and DA mediated behaviour.

Key words: Arogh; Anxiolytic; Elevated plus maze; Open field apparatus; Monoamines

INTRODUCTION

Anxiety is an unpleasant emotional state characterized by apprehension and nervousness. It is a normal emotional behavior. However when it is severe or chronic, it becomes pathological and can precipitate or aggravate cardiovascular and psychiatric disorders. Although many drugs are available in allopathic medicine to treat anxiety disorders, they produce various systemic side effects or exhibit tolerance upon chronic use (Gopala *et al*., 2006). The etiology of most anxiety disorders although not fully understood, it has come into sharper focus into the recent past. *Bacopa monniera* (Bhattachyarya and

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Ghosal, 1998), *Albizzia lebbeck* (Une et *al*., 2001), *Sesbania grandiflora* (Kasture *et al*., 2002), Zingiber officinale (Vishwakarma *et al*., 2002), *Stachys lavanduifolia* (Rabbani *et al*., 2003), *Echium amoenum* (Rabbani *et al*., 2004), *Coriandrum sativum* (Emamghoreishi *et al*., 2005), *Korean ginseng* (Mohan *et al*., 2005), *Salvia reuterana* (Rabbani *et al*., 2005), *ginger* (Mohan *et al*., 2006), *Saussurea lappa* (Ambavade *et al*., 2006), *Sphaeranthus indicus* (Bodhankar *et al*., 2006), *Aniba riparia* (Carla *et al*., 2006), *Passiflora edulis* (Miguel *et al*., 2006), *Nepeta persica* (Rabbani *et al*., 2007) are some of the plants reported to posses anxiolytic activity. Arogh is a polyherbal formulation (PHF) and has been studied for its antioxidant property (Suchalatha *et al*., 2004) and in myocardial infarction (Shyamala and Suchalatha, 2004). It is composed of nine plant ingredients- *Nelumbo nucifera, Hibiscus rosasinensis, Hemidesmus indicus, Rosa damascena, Zingiber officinale, Eclipta alba, Terminalia chebula, Glycyrrhiza glabra* and *Quercus infectoria.* There are no published reports of Arogh on its anxiolytic profile. This prompted us to explore its anxiolytic activity. Thus objective of the present article is to explore the potential of PHF for its anxiolytic effects using various behavioural paradigms such as elevated plus maze (Lister, 1987), light/dark apparatus (Belzung *et al*., 1990), open field apparatus (Turner, 1972), hole board apparatus (Clark *et al*., 1971). These models are pharmacologically validated and diazepam shows a strong anxiolytic profile on these models (Green *et al*., 2001). The discovery of benzodiazepine in the early 60's and their considerable commercial success in the treatment of anxiety has led to the development of numerous animal models of anxiety mostly based on pharmacological action of benzodiazepine (Belzung and Griebel, 2001). Hence diazepam was used as a positive control to compare the results of the test drug. Studies on behavior influenced by noradrenaline (NA), dopamine (DA), serotonin (5-HT) were also carried out to explain the observed actions.

MATERIALS AND METHODS

Animals

Male albino mice (22 - 25 g) and albino rats (150 - 200 g) were obtained from Serum Institute, Pune. Animals were housed in groups of five at ambient temperature of 25 ± 1ºC. Animals had free access to water and food (Hindustan Lever, India). They were deprived of food but not water 4 h before the experiment. The experiments were carried out between 9:00 and 14:00 h. The Institutional Animal Ethical Committee approved the protocol of this study.

Drugs and chemicals

Diazepam (Calmpose, Ranbaxy) was used as a standard anxiolytic drug. Diazepam and PHF were dissolved in distilled water and administered orally. Haloperidol (Searle, Mumbai), clonidine (German Remedies, Mumbai) and lithium sulphate (Glenmark, Mumbai) were administered intraperitoneally. The PHF, Arogh was provided by Rumi Herbals Pvt. Ltd; Chennai. The phytochemical analysis of PHF showed the presence of flavonoids, glycosides, saponins, tannins and steroids (Trease and Evans, 1983).

Elevated plus maze (EPM)

The EPM consisted of two open arms (25 × 5 cm) crossed with two closed arms (25 × 5 × 20 cm). The arms were connected together with a central square of 5 × 5 cm.The Maze was elevated to a height of 50 cm and placed inside a light and sound attenuated room (Lister, 1987). Groups of mice in five each were treated with vehicle, diazepam (1 mg/kg i. p.), PHF (30, 100, 300 and 500 mg/kg p. o.) 1 h before placing individually in the EPM. The time spent in open arms, entries in open and closed arms were recorded for a period of 5 min.

Open field apparatus test

The apparatus consisted of a wooden box (96 × 96 × 5 cm). The floor of the box was divided into 16

squares (Turner, 1972). Groups of mice in five each were treated with vehicle, diazepam (1 mg/kg i. p.) or PHF (30, 100, 300 and 500 mg/kg p. o.) 1 h before placing individually in one corner of the square. The parameters like number of rearing and number of squares crossed were recorded for 5 min.

Hole Board apparatus

The apparatus consisted of a wooden box (40 × 40 × 25 cm) with 16 holes (diameter 3 cm) evenly distributed on the floor. The apparatus was elevated to a height of 25 cm (Clark *et al*., 1971). Groups of mice in five each were treated with vehicle, diazepam (1mg/kg i. p.) or PHF (30, 100, 300 and 500 mg/kg p. o.) 1 h before placing individually in the apparatus and number of head poking were recorded for 5 min.

Light/dark apparatus test

Two equal sized boxes (20 × 20 × 14 cm, one dark and other lit) were connected with tunnel (5 × 7 ×10 cm) (Belzung *et al*., 1990). Groups of mice in five each were treated with vehicle, diazepam (1 mg/kg i. p.) or PHF (30, 100, 300 and 500 mg/kg p.o.) 1 h before placing individually in the lit area. The number of transitions and the time spent in the lit box were recorded for 5 min.

Lithium induced head twitches (5 HT mediated behaviour)

Rats were divided into four groups of five animals each. Rats received lithium sulphate (200 mg/kg i. p.) 1 h after vehicle or PHF (30, 100, and 300 mg/kg, p. o.) treatment. The number of head twitches was recorded for 1 h after Lithium sulphate administration (Wielosz, 1979).

Haloperidol induced catalepsy (DA mediated behaviour)

The mice in groups of five each were treated with vehicle, PHF (100, 300 mg/kg, p. o.) 1 h before administration of haloperidol (1 mg/kg, i. p.). Mice were gently removed from their home cages and their forepaws placed over a glass horizontal bar, 0.5 cm in diameter and 30 cm long, which was fixed at a height of 4 cm above the working surface. Duration of catalepsy was recorded from the time all animals were placed over the bar till the time they removed both forepaws from the bar or climbed over the bar (Sanberg, 1980).

Clonidine induced hypothermia (NA mediated behaviour)

Rats were divided into three groups of five animals each. Rats were treated with vehicle, PHF (100 and 300 mg/kg p. o.) 1 h before administration of clonidine (100 μg/kg, i. p). Rectal temperature was recorded every 30 min after clonidine (100 μg/kg) treatment till 180 min (Drew *et al*., 1977).

Behavioral assessment

To investigate the central actions of the PHF the method described by Irwin *et al*. (1968) was employed. The procedure involved an initial phase of undisturbed observations and later a manipulative phase during which the animals were subjected to the least provoking stimuli. In the initial phase the animals were observed for body position, locomotion, rearing, respiration, tremors, gait and in the later phase righting reflex and lacrimation was observed. The animals were observed for 2 h after treatment.

Neurotoxicity test

In this test a knurled rod (2.5 cm in diameter) was rotated at a speed of 15 rpm. All animals were trained to remain on the rotating rod for 5 min. A normal mouse could maintain its equilibrium for long periods. In a drug treated mouse, the neurological deficit was indicated by the inability of the mouse to maintain equilibrium for 3 min in each of the 3 trials as described earlier (Dunham and Miya, 1957). PHF was administered at doses of 100, 300 mg/kg doses and the animals were tested for neurological deficit 30 min after the drug treatment. The control group received diazepam at a dose of 1 mg/kg.

Statistics

All data are shown as mean ± S.E.M. Statistical analysis was performed with one way ANOVA followed by Dunnett's test. Differences of $P < 0.05$ was considered statistically significant.

RESULTS

Elevated Plus Maze

The vehicle treated mice spent 45.8 ± 1.65 s in the open arm, whereas animals treated with PHF (100, 300 and 500 mg/kg) spent significantly ($P < 0.05$) more time in the open arms and also increased the entries in both open arms significantly (Table 1).

Light/dark apparatus

The vehicle treated group spent 56.6 ± 2.61 s in the lit box and showed as 10 ± 0.74 as number of transitions, whereas animals treated with PHF (100, 300 and 500 mg/kg) spent significantly more time in the lit box and also showed a significant ($P < 0.05$) increase in the number of transitions (Table 2).

Open field apparatus

The vehicle treated mice traversed 142.8 ± 2.08 squares during the observation interval of 5min. PHF (100, 300 and 500 mg/kg) significantly ($P < 0.05$) increased the numbers of squares traversed and assisted rearing (Table 3).

Hole board apparatus

The vehicle treated mice showed 12.4 ±1.20 head dips. PHF (100, 300 and 500 mg/kg) significantly ($P < 0.05$) increased the number of head dips (Table 4).

Table 1. Effect of PHF on time spent in open arms, entries in open and closed arms and head dips in elevated plus maze

Groups	Dose (mg/kg)	Elevated Plus Maze			
		Time spent in O.A (s)	Entries in O.A.	Entries in C.A.	Head dips
Control	-	45.8 ± 1.65	3.0 ± 0.31	5.2 ± 0.37	9.6 ± 1.03
Diazepam	1	118.4 ± 2.94*	7.8 ± 0.58*	8.0 ± 0.31*	15.2 ± 1.35*
PHF	30	51 ± 1.87	4.4 ± 0.24	5.8 ± 0.37	12.4 ± 0.74
PHF	100	89.4 ± 2.22*	5.6 ± 0.50*	6.4 ± 0.24*	14.8 ± 0.37*
PHF	300	116 ± 2.16*	8.4 ± 0.67*	8.4 ± 0.50*	18.6 ± 0.4*
PHF	500	138.2 ± 3.77*	10.8 ± 0.37*	10.8 ± 0.37*	20.4 ± 0.81*
Diazepam + PHF	1 + 300	164.4 ± 2.58*	11.0 ± 0.44*	9.8 ± 0.37*	21.6 ± 1.36*
	F (6, 28)	297.06	42.77	31.09	21.07

The observations are mean ± S.E.M (n = 5). *$P < 0.05$, as compared to vehicle (ANOVA followed by Dunnett's test). PHF: polyherbal formulation; O.A: open arms; C.A: closed arms

Table 2. Effect of PHF on time spent in lit zone and number of transitions in light/dark apparatus

Groups	Dose (mg/kg)	Time spent in lit zone (s)	Number of transitions
Control	-	56.6 ± 2.61	10 ± 0.74
Diazepam	1	117.4 ± 4.55*	19 ± 0.31*
PHF	30	65.0 ± 2.02	13.8 ± 0.66
PHF	100	92.6 ± 2.61*	16.2 ± 0.58*
PHF	300	112.4 ± 2.65*	19.4 ± 0.67*
PHF	500	145.4 ± 2.11*	21.4 ± 1.20*
Diazepam + PHF	1 + 300	163.6 ± 2.89*	26.4 ± 0.92*
	F (6, 28)	186.09	65.59

The observations are mean ± S.E.M (n = 5). *$P < 0.05$, as compared to vehicle (ANOVA followed by Dunnett's test). PHF: polyherbal formulation.

Table 3. Effect of PHF on locomotion and rearing in open field apparatus

Groups	Dose (mg/kg)	Number of squares traversed	Rearing	
			Assisted rearing	Self rearing
Control	-	142.8 ± 2.08	26.2 ± 2.57	15.6 ± 0.50
Diazepam	1	264.2 ± 4.93*	44.4 ± 2.42*	13.8 ± 1.42
PHF	30	156.4 ± 3.54	33.4 ± 0.67	14.2 ± 0.37
PHF	100	198.4 ± 3.50*	36.4 ± 0.50*	12.6 ± 0.74
PHF	300	239.4 ± 2.76*	44.2 ± 2.13*	12 ± 0.83
PHF	500	260 ± 3.05*	55.4 ± 2.20*	17.6 ± 0.67
Diazepam + PHF	1 + 300	304.8 ± 4.88*	55.2 ± 1.74*	13.4 ± 0.97
	F (6, 28)	265.94	32.70	4.95

The observations are mean ± S.E.M (n = 5). $^{*}P < 0.05$, as compared to vehicle (ANOVA followed by Dunnett's test). PHF: polyherbal formulation.

Table 4. Effect of PHF on number of head poking in hole board apparatus

Groups	Dose (mg/kg)	Number of head poking
Control	-	12.4 ± 1.20
Diazepam	1	34.4 ± 0.50*
PHF	30	16.4 ± 0.67
PHF	100	26.4 ± 1.20*
PHF	300	37.2 ± 0.96*
PHF	500	52.6 ± 1.86*
Diazepam + PHF	1 + 300	56.4 ± 2.31*
	F (6, 28)	147.27

The observations are mean ± S.E.M (n = 5). $^{*}P < 0.05$, as compared to vehicle (ANOVA followed by Dunnett's test). PHF: polyherbal formulation.

Table 5. Effect of PHF on lithium sulphate (200 mg/kg) induced head twitches in rats

Groups	Dose (mg/kg)	Head twitches
Control	200	35 ± 1.95
PHF	30	29.75 ± 1.88
PHF	100	16.75 ± 1.25*
PHF	300	11.25 ± 0.75*
	F (3, 16)	51.33

The observations are mean ± S.E.M (n = 5). $^{*}P < 0.05$, as compared to vehicle (ANOVA followed by Dunnett's test). PHF: polyherbal formulation.

Lithium induced head twitches

In vehicle treated rats, lithium sulphate produced 35 ± 1.95 head twitches. PHF (100 and 300 mg/kg) significantly ($P < 0.05$) decreased the number of head twitches to 16.75 ± 1.25 and 11.25 ± 0.75 respectively (Table 5).

Haloperidol induced catalepsy

In vehicle treated rats, haloperidol produced maximum catalepsy after 90 min. PHF (300 mg/kg) significantly ($P < 0.05$) decreased the duration of catalepsy after 60 and 90 min (Table 6).

Table 6. Effect of PHF on haloperidol (1 mg/kg) induced catalepsy in mice

Groups	Dose (mg/kg)	Duration of catalepsy time at (s)				
		15 min	30 min	60 min	90 min	120 min
Control	1	32.2 ± 2.4	81.8 ± 4.21	154.8 ± 5.43	172.8 ± 3.78	157.8 ± 1.96
PHF	100	29.8 ± 1.59	77.8 ± 4.51	159.6 ± 5.06	172 ± 2.9	157 ± 2.55
PHF	300	21.4 ± 0.81	61.6 ± 4.54*	126.4 ± 3.90*	164 ± 4.29	149 ± 2.53
	F (2, 12)	10.50	6.38	13.71	2.41	3.45

The observations are mean ± S.E.M (n = 5). $^{*}P < 0.05$, as compared to vehicle (ANOVA followed by Dunnett's test). PHF: polyherbal formulation.

Table 7. Effect of PHF on rectal temperature in ºC in clonidine (100 µg/kg) treated rats

Groups	Dose (mg/kg)	Rectal temperature in ºC at time (min)						
		0	30	60	90	120	150	180
Control	0.1	30.12 ± 0.073	29.58 ± 0.037	28.58 ± 0.030	28.08 ± 0.037	28.36 ± 0.050	28.88 ± 0.030	29.36 ± 0.070
PHF	100	30.14 ± 0.040	29.74 ± 0.030	29.18 ± 0. 030	28.9 ± 0.031	28.52 ± 0.058	29.10 ± 0.050	29.66 ± 0. 050
PHF	300	30.08 ± 0.080	29.86 ± 0.025	29.74 ± 0.020	29.14 ± 0.060	29.60 ± 0.031	29.84 ± 0.020	29.94 ± 0.020

The observations are mean ± S.E.M (n = 5). $^{*}P$ <0.05 as compared to vehicle (Mann -Whitney U test)

Clonidine induced hypothermia

In vehicle treated rats, clonidine produced a fall in the rectal temperature and the peak effect was observed at 90 min. PHF (300 mg/kg) did not modify hypothermia produced by clonidine (Table 7).

Behavioral assessment

The animals did not exhibit any abnormal signs. In the initial phase, body position, locomotion, rearing, respirations were normal. They were no tremors and gait was normal. The grip strength, pain response and righting reflex were not affected.

Neurotoxicity test

Mice treated with PHF (100 and 300 mg/kg) were able to maintain equilibrium on the rotating rod for more than 3 min, whereas the animal treated with diazepam exhibited incordination and fall off time was significantly (P < 0.05) reduced to 100 ± 2.5 s.

DISCUSSION

The Elevated plus maze is based on the premise that exposure to open arm evoked an approach avoidance conflict that was considerably stronger than that evoked by the exposure to an enclosed arm (Montgomery, 1955). The reduction in entry, time spent, head dips are the indication of high level of fear or anxiety. Anxiolytics drugs increases the proportion of entries, time spent and head dips. Imaizumi *et al.*(1994) have shown that diazepam (4 and 8 mg/kg) increased the open arm entries and time spent in open arm without changes in total arm entries. Researchers have reported different effects of buspirone on EPM i. e anxiolytic (Dunn *et al*., 1989; Kshama *et al*., 1990; Lee *et al*., 1991) non-effective (Moulton *et al*., 1990) anxiogenic (Pellow *et al*., 1987; Moser, 1989; Klint, 1991; Critcheley *et al*., 1992). A drug may have both anxiolytic and anxiogenic activities and either of the activites may be dependent on experimental conditions (Handley and Mcblane, 1993). Drugs must be carefully assessed on EPM test and therefore in the present study EPM is supported by other tests. In our study, we observed that PHF (100, 300, and 500 mg/kg) significantly (P < 0.05) increased the time spent in open arms and number of entries in open arms.

In the light and dark box paradigm, the brightly lit environment is a noxious environment stressor that inhibits the exploratory behaviour of rodents. Reduction of number of entries, time spent in the lit chamber are regarded as markers of anxiety (Costall *et al*., 1988). It is interesting that many studies in the light dark test have been conducted using mice rather than rats. Crawley reported that rats were not respondent to treatment with diazepam in this paradigm (Crawley, 1985) and that their exploratory tendencies appeared considerably lower in mice, suggesting that rats were not useful in this test. Anxiolytics increase light to dark transitions and time spent in lit area. In our study PHF (100, 300 and 500 mg/kg) increased the time spent in lit area and the number of transitions.

In open field apparatus, when animals were taken from their home cages and placed in a novel environment they express their anxiety and fear by decreasing ambulation and exploration. These

behavioural changes were attenuated by anxiolytic drugs (Kumar *et al*., 2000). In open field test, PHF (100, 300 and 500 mg/kg) showed a significant ($P < 0.05$) increase in squares traversed and an increased tendency to reach to the walls and rear rather rearing without support. Decrease in locomotion is indicative of diminished dopaminergic transmission, which may be secondary to the rise in 5-HT level caused by anxiogenic agents (Kahn *et al*., 1988; Jones *et al*., 1992). File and Wardil (1975) have assessed the anxiogenic and anxiolytic activity of some agents using the hole board test. We observed a significant ($P < 0.05$) increase in head poking with PHF (100, 300 and 500 mg/kg).

Despite of extensive research the neurological basis of anxiety is still not clear. Although the involvement of the central serotonergic system is well established, the role of other neurotransmitters cannot be ignored. Lithium produces head twitches by increasing the synaptic concentration of serotonin (Wielosz and Kleinark, 1979). The observed reduction in the number of head twitches may be due to direct and indirect action of PHF on the 5-HT system. A reciprocal relationship between 5-HT and DA has been demonstrated in many brain areas (Kuczenski *et al*., 1985). We noted such relationship with the concentration of DA and 5-HT. In halpoperidol induced catalepsy, PHF (300 mg/kg) decreased the duration of catalepsy at 60 and 90 min. These changes can be correlated with a increase in brain levels of dopamine. PHF (100 and 300 mg/kg) significantly decreased the number of head twitches indicating a decrease in brain 5-HT levels. Clonidine reduces noradrenaline release (Drew *et al*., 1977) and thereby induces hypothermia. PHF (100 and 300 mg/kg) did not modify clonidine induced hypothermia. Thus, noradrenergic mechanism does not seem to play an important role in anxiolytic effect of PHF.

Thus, Arogh a polyherbal formulation possess anxiolytic activity as evident in various models of anxiety. It diminished serotonergic transmission and delayed the onset of catalepsy indicating potentiation of dopaminergic transmission.

ACKNOWLEDGEMENTS

The authors are grateful to Dr SB Kasture and Dr VS Kasture for their continuous support and encouragement.

REFERENCES

Ambavade SD, Mhetre NA, Patil KM**,** Bodhankar SL. (2006) Anxiolytic activity of root extract of *Saussurea lappa* Clark. in mice. *J. Nat. Remedies* **6**, 103-108.

Belzung C, Misslin R, Vogel E. (1990) Anxiogenic effects of methyl Beta -Carboline -3 Carboxylate in a light/dark choice situation. *Pharmacol. Biochem. Behav*. **28**, 29-33.

Belzung C, Griebel C. (2001) Measuring normal and pathological anxiety like behaviour in mice: a review. *Behav. Brain Res*. **125,** 141-149.

Bhattacharya SK and Ghosal S. (1998) Anxiolytic activity of a standardized extract of *Bacopa monniera*-an experimental study. *Phytomedicine* **5**, 77-82.

Bhattacharya SK, Bhattacharya A, Sairam K, Ghosal S. (2000) Anxiolytic antidepressant activity of *Withania somnifera* glycowithanolides: An experimental study. *Phytomedicine* **7**, 463-469.

Bodhankar SL, Ambavede SD, Mhetre NA, Tate VD. (2006) Pharmacological evaluation of the extracts of *Sphaernthus indicus* flowers on anxiolytic activity in mice. *Indian J. Pharmacol.* **38**, 254-259.

Carla TV, Andreisa PM, Caroline PL, Fernando LO, Vera TM, Marta M F. (2006) Anxiolytic-Like Effects of (*O*-Methyl)-*N*-2, 6-dihydroxybenzoyl-tyramine (Riparin III) from *Aniba riparia* (NEES) MEZ (Lauraceae) in Mice. *Biol. Pharm. Bull.* **29**, 451-454.

Clark G, Koster AG, Person D W. (1971) Exploratory behaviour in chronic disulfotan poisoning in mice. *Psychopharmacology* **20**, 169-171.

Costall B, Domeney AM, Kellym (1988) Zacopride: anxiolytic profile in rodent and primate models of anxiety. *J. Pharm. Pharmacol*. **40,** 302-305**.**

Crawley J, Goodwin FK. (1980) Prelimnary report of a simple animal behaviour for the anxiolytic effect of benzodiazepines. *Pharmacol. Biochem. Behav.* **13,** 167-170.

Crawley JN. (1985) Exploratory behavior models of

anxiety in mice. *Neurosci. Biobehav. Rev*. **9**, 37-44.

Critchley MAE, Njung'e K, Handley SL. (1972) Actions and some interactions of 5-HT_{1A} ligands in the elevated X-maze and effects of dorsal raphe lesions. *Psychopharmacology* **106,** 484-490.

Drew GM, Gower AJ, Marriot AS. (1977) Pharmacological characterisation of alpha adrenoceptor which mediates clonidine induced sedation. *Br. J. Pharmacol*. *63*, 468-469.

Dunham NW, Miya TS. (1957) A note on a simple apparatus for detecting neurological deficit in rats and mice. *J. Am. Pharm. Assoc.* **46**, 208-209.

Dunn RW, Corbett R, Fielding S. (1989) Effects of 5-HT_{1A} receptor agonists and NMDA receptor antagonists in the social interaction test and the elevated plus maze. *Eur. J. Pharmacol*. **169**, 1-10.

Emamghoreishi M, Khasaki M, Fath AM. (2005) *Coriandrum sativum*: evaluation of its anxiolytic effect in the elevated plus-maze. *J. Ethnopharmacol.* **96**, 365-370.

File SE, Wardil AG. (1975) Validity of head dipping as a measure of exploration in a modified hole board. *Psychopharmacology* **44**, 53-59.

Gopala HN, Sangha RB, Misra N. (2006) Antianxiety activity of NR-ANX-C, a polyherbal preparation in Rats. *Indian J Pharmacol* **38**, 330-335.

Green S. (1991) Benzodiazepines, putative anxiolytics and animal models of anxiety. *Trends Neurosci.* **14,** 101-104.

Handley SL, McBlane JW. (1993) 5-HT dugs in animal models of anxiety. *Psychopharmacology* **112**, 13-20.

Imaizumi M, Suzuki T, Machida H, Onodera K. (1994) A fully automated apparatus for a light/ dark test measuring anxiolytic or anxiogenic effects of drugs in mice. *Nihon Shinkei Seishin Yakurigaku Zasshi* **14**, 83-91.

Irwin S, Taber RI, Fox JA, Roth FE. (1968) Comparison of perfenazine and fluphenazine enanthates in rats. *Psychopharmacologia* **12**, 441-447.

Iyer M, Sherikar O, Belapurkar H, Kasture SB. (2004) Anxiolytic activity of *Trigonella foenum- graecum* seeds. *J. Nat. Remedies* **4**, 61-65.

Jones GH, Hernanadez TD, Kendall DA. (1992) Dopaminergic and serotonergic function following rearing in rats. *Pharmacol. Biochem. Behav*. **43,** 17-35.

Kahn RS, Van Praag HM, Wizlaer S, Asnis GM, Barr G. (1988) Serotonin and anxiety revisited. *Biol. Psychiatry* **23,** 189-208.

Kasture VS, Deshmukh VK, Chopde CT. (2002) Anxiolytic and anticonvulsive activity of *Sesbania grandiflora* leaves in experimental animals. *Phytother. Res.* **16**, 445-460.

Klint T. (1991) Effects of 8-OH-DPAT and buspirone in a passive avoidance test and in the elevated plus maze test in rats. *Behav. Pharmacol.* **2**, 481-489.

Kshama D, Hrishikeshavan I, Shanbhogue R, Munonyedi US. (1990) Modulation of baseline behaviour in rats by putative serotonergic agents in three ethoexperimental paradigms. *Behav. Neural Biol*. **54**, 234-253.

Kumar V, Jaiswal AK, Singh PN, Bhattacharya SK. (2000) Anxiolytic activity of Indian *hypericum peraratum* linn: an experimental study. *Indian J. Exp. Biol*. **38,** 36-41.

Lee C, Roders RJ. (1991) Effects of buspirone on antinociceptive and behavioral responses to the elevated plus maze in mice. *Behav. Pharmacol.* **2,** 491-496.

Lister RG. (1987) The use of plus maze to measure anxiety in mouse. *Psychopharmacology* **92**, 180-185.

Miguel C, Maria TB, Maria GC, Rui C, Maria DC. (2006) Neuropharmacological evaluation of the putative anxiolytic effects of *Passiflora edulis* Sims, its sub-fractions and flavonoid constituents. *Phytother. Res.* **20**, 1067-1073.

Mohan M, Kasture S B, (2005) Anxiolytic activity of standardized extract of *Korean ginseng* – a study on exploratory behavior. *Orient. Pharm. Exp. Med.* **5**, 301-307.

Mohan M, Kasture S B, Balaraman R. (2006) Anxiolytic effect of chronic ginger treatment using elevated T-maze in mice. *Orient. Pharm. Exp. Med*. **6**, 179-185.

Montgomery KC. (1955) The relation between fear induced by novel and exploratory behavior. *J. Comp. Physiol. Psychol.* **48**, 254-260.

Moser PC. (1989) An evaluation of the elevated plus maze test using the novel anxiolytic buspirone. *Psychopharmacology* **99,** 48-53.

Moulton B, Morinan A. (1990) The effects of RS-30199 on anxiety and hippocampal monoaminooxidase activity in the rat. *Br. J. pharmacol.* **101**, 516.

Pal BC, Achari B, Yoshikawa K, Arihara S. (1995) Saponins from *Alizzia lebbeck. Phytochemistry* **38,**

1287-1291.

Pellow S, Chopin P, File SE. (1985) Validation of open-closed arm entries in elevated plus maze as measure of anxiety in the rat. *J. Neurosci. Methods* **14**, 149-167.

Pellow S, Johnston Al, File SE. (1987) Selective agonists and antagonists for 5-HT receptor subtypes and interactions with yohimbine and FG7142 using elevated plus maze in the rat. *J. Pharm. Pharmacol*. **39**, 917-928.

Rabbani M, Sajjadi HR. (2003) Anxiolytic effects of *Stachys lavandulifolia* vajl on the elevated plus maze model of anxiety in mice. *J. Ethnopharmacol.* **89,** 271-276.

Rabbani M, Sajjadi SE and Mohammadi A. (2007) Evaluation of the anxiolytic effect of *Nepeta persica* Boiss. in mice. *eCAM* **17,** 1093.

Rabbani M, Sajjadi SE, Vaseghi G, Jafarian A. (2004) Anxiolytic effects of *Echium amoenum* on the elevated plus-maze model of anxiety in mice. *Fitoterapia* **75**, 457-464.

Rabbani M, Sajjadi SE, Jafarian A, Vaseghi G. (2005) Anxiolytic effects of *Salvia reuterana* Boiss. on the elevated plus-maze model of anxiety in mice. *J. Ethnopharmacol.* **101**, 100-103.

Sanberg PR. (1980) Haloperidol induced catalepsy is mediated by post-synaptic dopamine receptors. *Nature* **284**, 472-473.

Shyamala CS, Suchalatha S. (2004) Effect of Arogh- a polyherbal formulation on the marker enzymes in isoproternol induced myocardial injury. *Indian J. Clin. Biochem.* **19,** 184-189.

Suchalatha S, Thirugnanasambantham P, Maheswaran E, Shyamala CS. (2004) Role of Arogh- a polyherbal formulation to mitigate oxidative in experimental myocardial infarction. *Indian J. Exp. Biol*. **42**, 224-226.

Trease EG, Evans WC. (1983) *Textbook of pharmacognosy*, 12 ed. Singapore: Alden Press.

Turner RA. (1972*) Screening procedures in pharmacology*, pp.99, New York: Academic press.

Une HD, Sarveiya VP, Pal SC, Kasture VS, Kasture SB. (2002) Nootropic and anxiolytic activity of saponins of *Albizzia lebbeck* leaves. *Pharmacol. Biochem. Behav*. **69**, 439-444.

Vishwakarma SL, Pal SC, Kasture VS, Kasture SB. (2002) Anxiolytic and antiemetic activity of *Zingiber officinale. Phytother. Res.* **16**, 621-626.

Wielosz M, Kleinork Z. (1979) Lithium induced head twitches in rats. *J. Pharm. Pharmacol*. **31**, 410-414.