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Oriental Pharmacy and Experimental Medicine 2006 **6(3)**, 179-185

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Anxiolytic effect of chronic ginger treatment using elevated T- maze in mice

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SUMMARY

We investigated the effects of chronic administration of different extracts of ginger rhizome [pet ether extract (PE); toluene fraction (TF) of pet ether extract] on anxiety models: the elevated T- maze (ETM) (for inhibitory avoidance and escape measurements) and the open field test. Ondansetron (1 mg/kg), PE (10, 30 &100 mg/kg) and TF (10 & 30 mg/kg) were administered orally for 15 days. On the 14th day mice were previously exposed for 30 min to one of the open arms of the T-maze, 24 h before the test. On 15th day mice had two exposures to the enclosed and open arm of the ETM followed by exposure to the open field apparatus. The number of line crossings in the apparatus was used to assess locomotor changes. Cumulative Concentration Response Curve of 5-HT was plotted using rat fundus which were pre-treated in a similar way. Treatment with Ondansetron (1 mg/kg), PE (100 mg/kg), TF (10 mg/kg) and TF (30 mg/kg) significantly ($P < 0.05$) impaired inhibitory avoidance performance but did not impair escape latency. Concentration response curve of 5-HT was shifted towards the right with suppression of maxima in rats treated with PE and TF. The results suggest that PE and TF of Ginger rhizome exerts anxiolytic like behaviour in a specific subset of defensive behaviour, particularly those related to generalized anxiety disorder.

Key words: Elevated T- maze; Ginger; Anxiolytic; Ondansetron

INTRODUCTION

Anxiety affects one eighth of the total population in the world. It has been recognized that anxiety is not a unitary phenomenon. The etiology of most anxiety disorders although not fully understood, it has become an important area of research interest in the recent past. Numerous plants have been reported to possess anxiolytic activity. Saponins from *Albizzia lebbeck* (Mimosaceae) (Une *et al.*, 2001), saponins from *Bacopa monniera* (Scrophulariaceae) (Bhattacharya and Ghosal, 1998), essential oil of *Stachys lavanduifolia* (Labiatae) (Rabbani *et al.*, 2003), triterpenes from *Sesbania grandiflora* (Leguminosae) (Kasture *et al.*, 2002) are the active principles mediating anxiolytic effects. The rhizome part of *Zingiber officinale* (Zingiberaceae) commonly known as ginger possesses carminative, diaphoretic, laxative, expectorant, antispasmodic (Kirtikar and Basu, 1993), hypocholesterolaemic (Giri *et al.*, 1984), and hypoglycaemic activity (Sharma and Shukla, 1977). Traditionally it has been used for colic, flatulent dyspepsia, and specifically for flatulent intestinalcolic. Its main constituents include carbohydrates (upto 50%), lipids (6 - 8%), oleo-resin (about 33%), volatile oils (1 - 3%) and other constituents like amino acids,

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minerals and vitamins. It has been investigated for the prevention of motion sickness. Ginger contains gingerols and galanolactone, both having antagonistic activity at 5-HT_3 receptor (Huang *et al.,* 1991). The 5-HT_3 receptor antagonistic properties (Yamahara *et al.,* 1989) of ginger accounts for its anti-emetic effects (Bone *et al.,* 1990; Fischer *et al.,* 1991). In search for alternatives to benzodiazepines, the 5-HT_3 receptor antagonists are currently being considered for their potential use in fear and anxiety related disorders (Costall and Naylor, 1992). Hasenohrl *et al.* (1996) have demonstrated the anxiolytic and antiemetic activity of a combined extract of *Zingiber officinale* (Zingiberaceae) and *Ginkgo biloba* (Ginkgoaceae). Vishwakarma *et al.* (2002) have studied the anxiolytic and anti- emetic activity of the benzene fraction of acetone soluble part of pet ether extract of *Zingiber officinale*. Behavioral models with animals are necessary for finding new clinically effective drugs and can be helpful for clarifying the neurobiological basis of various psychiatric disorders. Different types of pathological anxiety exist, e.g. phobias, post-traumatic stress disorder, generalized anxiety disorder (GAD), panic disorder (PD), obsessive compulsive disorder (OCD). There is a clear need for animal models that may represent distinct human anxiety disorders. Since the rhizome part of ginger has not been studied for its chronic anxiolytic effects, the objective of the present article is to explore the potential of ginger rhizome through the use of elevated T-maze (ETM) and open field test (OFT). The ETM was derived from elevated plus maze to allow the measurement, in the same animal, two types of anxiety related responses, a conditioned (inhibitory avoidance) behaviour, which relates to GAD and unconditioned (escape latency) behavior, which relates to PD. The animals were also subjected to OFT in order to avoid confusing results due to treatment effects on loco motor activity.

MATERIALS AND METHODS

Plant material

Dried rhizome of *Zingiber officinale* was purchased from local source in May 2004. They were authenticated by Dr. SC Pal of Department of Pharmacognosy, NDMVP Samaj's college of Pharmacy, Nasik.

Preparation of extract

One kg of dried rhizome of *Zingiber officinale* was finely powdered and defatted with petroleum ether by cold extraction process. The constituents of pet ether extract were separated by column chromatography using neutral alumina. The pet ether extract (PE) and its toluene fraction (TF) were used for the investigation. The pet ether extract gave a yield of 3.38% w/w, and TF gave a yield of 0.5% w/w.

Flow Chart

Dried powdered rhizome of *Zingiber officinale*
↓
Cold extraction using petroleum ether
↓
PE subjected to column seperation
↓
TF

Animals

Male albino mice (22 - 25 g) and wistar rats (200 - 250 g) were obtained from National Toxicology Centre, Pune. Mice were housed into 7 groups of five each and rats into 4 groups of five each at an ambient temp of 25 ± 1°C. Animals had free access to food (Hindustan Lever, India) and water. They were deprived of food but not water 4 h before the experiment. All experiments were conducted between 08:00 - 16:00 h. The Institutional Animal Ethical Committee approved the protocol of this study.

Drugs and chemicals

Ondansetron (Emset, Cipla, India) was used as a standard anxiolytic drug. The PE and TF of *Zingiber officinale* were suspended in PEG-400 (just sufficient to dissolve). Serotonin hydrochloride was purchased from Sigma- Aldrich Chemicals, Mumbai. Pet ether

and toluene were purchased from Modern Scientific, Nashik.

Anxiety studies

ETM

The apparatus is elevated 38.5 cm above the floor, has three arms of equal dimensions (30 × 5 cm). One arm was enclosed by walls (15 cm) and stood perpendicular to two open arms of the ETM (Carvalho-Netto *et al.*, 2004). The apparatus was cleaned with alcohol 70% after each trial. Mice in groups of five were administered orally PE (10, 30 &100 mg/kg), TF (10 & 30 mg/kg), ondansetron (1 mg/kg) or vehicle for 15 days. In the pre-test session, on day 14 individual mouse was pre-exposed for 30 min to one of the open arm. Pre-exposure shortens escape latency, improving it as an escape index. On day 15 animals were individually placed at the distal end of the enclosed arm and the time taken to withdraw from this arm with all four paws was recorded (baseline). The procedure was then repeated for one additional trial (avoidance 1 and 2) using an inter-trial interval of 30 s. Thirty seconds after completion of the avoidance task, mice were individually placed at the distal end of the open arm, and time taken to withdraw from this arm was recorded (escape latency). The procedure was then repeated for one additional trial (escape 1 and 2) using an inter-trial interval of 30 s. A cut-off time of 300 s was employed for each trial (avoidance and escape test).

Open field test

The apparatus consisted of wooden box (96 × 96 × 5 cm). The floor of the box was divided into 16 (6 × 6 cm) squares. Immediately after being tested in the ETM, each animal was placed for 5 min in the open field apparatus for the evaluation of loco motor activity. During this time the total number of lines crossed was recorded. (Turner, 1972).

***In-vitro* studies**

Three groups of five rats each were treated with PE (50 mg/kg), TF (10 mg/kg) and Ondansetron (1 mg/kg) for 15 days. Rats were then sacrificed by stunning, fundus was removed and placed in Krebs solution. A strip of fundus was mounted in a bath containing Krebs solution. The physiologic salt solution had the following composition (mM): NaCl, 118; KCl, 4.7; $CaCl_2$, 2.5; $MgSO_4$, 1.2; $NaHCO_3$, 25; KH_2PO_4, 1.2 and Glucose, 11. The physiologic salt solution had a pH of 7.4. It was warmed to 37°C and aerated with 95% O_2 and 5% CO_2 (Carbogen). Each strip was placed under optimum resting tension (1.5 g) and allowed to equilibrate for 30 min with frequent changes of Kreb's solution at 10 min interval. Contractile response to each dose was recorded for 90 s (Goyal, 1999).

Gas chromatography mass spectroscopy

TF was dissolved in chloroform and subjected to Gas chromatography mass spectroscopy (Perkin Elmer, U.S.A.) and the mass spectrum was obtained.

Statistics

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

RESULTS

ETM

Results showed that with two exposures to the ETM, PE and TF impaired inhibitory avoidance and did not facilitate the escape latency much. Treatment with Ondansetron (1 mg/kg) impaired inhibitory avoidance performance in the ETM. One-way ANOVA showed a significant effect of treatment [$F(6,28) = 34.64$; $P < 0.05$] and [$F(6,28) = 11.63$; $P < 0.05$] for Avoidance-1 and Avoidance-2 respectively. The Dunnett's test showed that PE (100 mg/kg), TF(10 mg/kg) and TF (30 mg/kg) increased the latency to leave the enclosed arm ($P < 0.05$) as compared to vehicle and other treatment groups. Ondansetron (1 mg/kg) did not impair the escape latency for all

Table 1. Effect of PE and TF on inhibitory avoidance and escape latency in ETM

Sr no	Treatment (mg/kg)	Inhibitory Avoidance (s)		Escape latency (s)	
		Avoidance-1	Avoidance-2	Escape-1	Escape-2
1	Vehicle	16±1.34	5.2±11.99	24±7.02	71.0±12.03
2	Ondansetron (1)	5.6±0.4*	17.4±4.75*	61±10.33*	89.8±15.79
3	PE (10)	17.6±0.81	51.8±10.79	25.4±5.06	46.4±13.54
4	PE (30)	9±0.54*	108±20.83	38.4±7.27	84.0±16.55
5	PE (100)	6.6±0.50*	20±4.23*	72±13.78*	100±12.14
6	TF (10)	9.8±1.24*	17.6±1.50*	59.6±9.59	164.6±36.77
7	TF (30)	5.8±0.37*	15.2±2.15*	23.8±2.81	85.8±111.49
	F (6,28)	34.64	11.63	5.56	3.74

n = 5, values are mean ± S.E.M. *P < 0.05 when compared to vehicle (ANOVA followed by Dunnett's test).

the trials as compared to control. One–way ANOVA showed a significant effect of treatment [F (6, 28) = 5.56; P < 0.05] and [F (6, 28) = 3.74] for Escape-1 and Escape-2 respectively. The Dunnett's test showed that PE (100 mg/kg) significantly increased the latency to leave the open arm (P < 0.05) as compared to vehicle treated group (Table 1).

Open field test

Ondansetron facilitated locomotion as compared to vehicle and other treatment groups. [F(6, 28) = 2,77; P < 0.05] (Table 2).

***In-vitro* studies**

The Cumulative Concentration Response Curve (CCRC) of 5-HT was significantly (P < 0.05) shifted to the right in rat fundus treated with PE (50 mg/kg), TF (10 mg/kg) and Ondansetron (1 mg/kg) for 15 days (Fig. 1).

Table 2. Effect of PE and TF on locomotion in open field test in mice

Sr. no	Treatment (mg/kg)	Squares traversed
1	Vehicle	75.8±14.54
2	Ondansetron (1)	121.4±8.21*
3	PE (10)	108.2±16.71
4	PE (30)	88±6.05
5	PE(100)	88±10.91
6	TF (10)	107.6±3.35
7	TF (30)	73±10.38
	F (6,28)	2.77

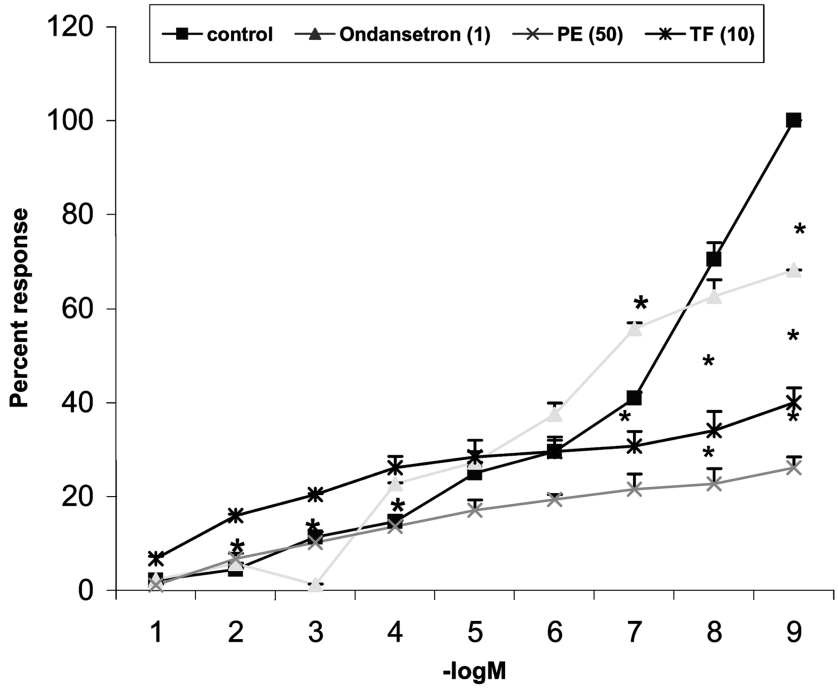
n = 5, values are mean ±S.E.M. *P < 0.05 when compared to vehicle (ANOVA followed by Dunnett's test).

Gas chromatography mass spectroscopy

The GC-MS showed presence of [6]-gingerol, [8]-gingerol, [10]-gingerol, [6]-shagoal, [8]- shagoal [10]-shagoal and Zingiberone having molecular weights 294, 332, 360, 276, 306, 332 and 194 respectively. They contributed to 13.31%, 0.73%, 0.87%, 26.39%, 15.87%, 0.73%, and 1.51% respectively (Maryadele *et al.,* 2001).

DISCUSSION

The purpose of the present work was to explore the potential of ginger rhizome in anxiety through the use of ETM and open field test. The ETM was derived from elevated plus maze to investigate conditioned anxiety (Inhibitory Avoidance-IA) and unconditioned fear (Escape latency-EL) in the same animal; these responses have been related to GAD and PD, respectively. The selective sensitivity of inhibitory avoidance and escape latencies to anxiolytic and panicolytic drugs, respectively, has encouraged the use of the ETM model for the study of the GAD and PD (Graeff *et al.,* 1993, 1998; Viana *et al.,* 1994). Previous studies (Vianna *et al.,* 1994; Graeff *et al.,* 1996) revealed that the initial latency to leave the open arm was not significantly



(1 = 8.35, 2 = 8.14, 3 = 7.85, 4 = 7.54, 5 = 7.24, 6 = 6.84, 7 = 6.63, 8 = 6.33, 9 = 6.03)

Fig. 1. Effect of PE-50 and TF-10 on CCRC of 5-HT on isolated rat fundus strip in Control, Ondansetron (1), PE (50), TF (10) treated groups. *P < 0.05 when compared to control group (ANOVA followed by Dunnett's test). n = 5, Vertical lines represent S.E.M. Figures in parenthesis i.e ( ) indicate in mg/kg dose of body weight of animal.

different from the first latency to withdraw from the closed arm. It is likely that exploration interferes with open arm escape. Therefore, animals were also subjected to open field test inorder to avoid confusing results due to treatment effects on loco motor activity.

Deakin and Graeff (1991) suggested that different 5-HT pathways and receptor subtypes modulate the neural substrates of depression, panic, and generalized anxiety. According to this assumption, the ascending 5-HT pathway that originates in the dorsal raphe nucleus (DRN), runs along the medial forebrain bundle, and innervates the amygdala and frontal cortex facilitates active escape or avoidance behaviors that occur in response to potential or distal threat (Blanchard *et al.*, 1989). These behavioral strategies rely on learning and, thus, relate to conditioned or anticipatory anxiety and, possibly, GAD. Postsynaptic 5-$HT_{2A/2C}$ and 5-HT_3, receptors are likely to be activated by this pathway. In turn, the DRN-periventricular pathway innervates the periventricular and periaqueductal gray matter. In these regions 5-HT inhibits inborn fight or flight reactions triggered by proximal danger (Blanchard *et al.*, 1989), acute pain, or asphyxia that may relate to panic disorder. This function of 5-HT is likely to be mediated by both 5HT-$_{2A/2C}$ and 5-HT_{1A} postsynaptic receptors.

Compounds representatives of three kinds of anxiolytics- namely the agonist of benzodiazepene receptor, diazepam; the 5-HT_{1A} partial agonist, buspirone; and the non selective 5-HT_2 antagonist, ritanserin have been shown to selectively impair inhibitory avoidance while leaving one-way escape

unchanged (Graeff *et al.,* 1998). These results are compatible with the view that inhibitory avoidance relates to GAD. On the other hand impairment of open arm escape has been described with the chronic administration of the anti-panic compound, imipramine (Custodio Teixeira *et al.,* 2000). In our study, the results showed an anxiolytic effect in one of the tasks i.e inhibitory avoidance- in the ETM. Chronic administration of PE (30 and 100 mg/kg) and TF (10 and 30 mg/kg) impaired inhibitory avoidance without affecting escape latency in animals pre-exposed to one of the open arms. The results confirm with the previous data (Custodio Teixeira *et al.,* 2000) and suggest that pre-exposure provides a better index for escape. The extract acts in a way similar to compounds used in clinical practice to treat GAD, i.e., the benzodiazepine receptor agonist diazepam and the $5HT_{1A}$ partial agonist buspirone (Graeff *et al.,*1993, 1998; Viana *et al.,*1994) and 5-HT_3 antagonists. In agreement with these results, anxiolytic effects of ginger rhizome have recently been described by us (paper communicated) using three other models of anxiety; the elevated plus maze, the light–dark model and the hole board apparatus. A shift of CCRC of 5-HT towards the right with suppression of maxima using rat fundus has further confirmed the 5-HT antagonistic properties of PE and TF.

In conclusion, it appears that the mechanism of anxiolytic action of ginger rhizome after chronic administration observed with the ETM is of 5-HT_3 receptor antagonistic nature. Thus the present results show that ginger rhizome exerts anxiolytic like effects in a specific subset of defensive behaviour, particularly those that have been related to GAD.

ACKNOWLEDGEMENTS

The authors are grateful to Prin.V. M Aurangabadkar of M.G.V's Pharmacy College for providing necessary laboratory facilities and to Dr. VS Kasture for her kind support and encouragement. Dr. KJ Shrivastav, Dy. Director of National Horticultural Research and Development Foundation, Nashik is also hereby equally acknowledged.

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