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

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SUMMARY

The roots of the plant Korean ginseng have been extensively used in the traditional Chinese herbal medicine. The effects of chronic administration of Korean ginseng extract (KGE) were investigated on two different anxiety models: the elevated T-maze (for inhibitory avoidance and escape measurements) and the open field test (OFT). Diazepam (1 mg/kg), KGE (10, 30 and 100 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 elevated T-maze 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 Diazepam (1 mg/kg) and KGE (10, 30 and 100 mg/kg) significantly ($P < 0.05$) impaired inhibitory avoidance performance but did not impair escape latency. In OFT, diazepam facilitated locomotion as compared to vehicle and other treatment groups. KGE at any of the selected doses did not impair locomotion. Concentration response curve of 5-HT was shifted towards the right with suppression of maxima in rats treated with KGE. The results suggest that KGE exerts anxiolytic like behaviour in a specific subset of defensive behaviour, particularly those related to generalized anxiety disorder.

Key words: Elevated T-maze; Ginseng; Anxiolytic; Diazepam

INTRODUCTION

Anxiety is a common emotion in humans and may be understood as a pathological counterpart of normal fear. It has been recognized that anxiety is not a unitary phenomenon. The etiology of most anxiety disorders although not fully understood, it has come into sharper focus of research interest in the recent past. Numerous plants have been reported to possess anxiolytic activity. Saponins from *Albizzia Lebbeck* (Une *et al.*, 2001), saponins from *Bacopa Monniera* (Bhattacharya and Ghosal, 1998), gingerols from *Zingiber Officinale* (Vishwakarma *et al.*, 2002), triterpenes from *Sesbania Grandiflora* (Kasture *et al.*, 2002) are the active principles mediating anxiolytic effects. Korean (Panax) ginseng has a blood pressure lowering effect (Stavro *et al.*, 2004), antistress and anabolic activity (Grandhi *et al.*, 1994) and improves learning and memory (Jui *et al.*, 1999). It has a true adaptogenic action. It contains triterpene glycosides named ginsenosides which account for the majority of plants medicinal action. At least 13

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ginsenosides have been identified falling into 2 groups based upon the aglycone portion: protopanaxodiol and protopanaxotriol. They are classified according to an alphanumeric system i.e. Ra, Rb, Rb2, Rc, etc. The plant also contains sterols, acetylenic compounds and peptidoglycans named Panaxans. (Wren, 1988; Mills, 1991). Ginseng has shown behavioral changes which seem to be related to the regulation of gamma-amino-butyric acid (GABA) ergic neurotransmission. Ginseng saponin prolonged pentobarbitone sleeping time and delayed the onset of convulsion in high dose (Oh *et al.*, 1969; Jung and Jin, 1996). Ginsenoside Re is a potent inhibitor of neurotransmitter inhibitor, specially, GABA (Tsang *et al.*, 1983). Ginsenosides interact with ligand-bindings of GABAA and GABAB receptors (Kimura *et al.*, 1994). 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. There is a clear need for animal models that may represent distinct human anxiety disorders. The objective of the present article is to explore the potential of Korean ginseng as an anxiolytic 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) behaviour which relates to PD. The animals were also subjected to OFT in order to avoid confusing results due to treatment effects on locomotor activity. Cumulative concentration response curve (CCRC) of 5-HT were recorded using the fundic portion of the rats at the end of the treatment period to confirm the 5-HT hypothesis involved in anxiety.

MATERIALS AND METHODS

Extract

The Korean Ginseng Extract (KGE) was obtained as a gift sample from Glenmark Pharmaceuticals, Nashik. It was manufactured by Pangin Biotech Co. Ltd, Korea. The standardized and controlled ginseng slender tail roots were extracted 3 times under 70°C for about 8 h in the extraction apparatus with 70% of ethanol. The extract was concentrated in vacuo at a reduced pressure of 500 - 600 mmHg under 60 - 70°C till the ginseng extract was obtained. It contained 18%(w/w) of saponins.

Animals

Male albino mice (22 - 25 g) were obtained from Serum Institute Pune. Animals were housed into groups of five 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. The Institutional Animal Ethical Committee approved the protocol of this study.

Drugs and chemicals

Diazepam (Calmpose, Ranbaxy) was used as a standard anxiolytic drug. Serotonin hydrochloride was purchased from Sigma-Aldrich Chemicals, Mumbai. Diazepam and KGE were dissolved in saline and administered orally.

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 20% alcohol after each trial. Mice in groups of five were administered KGE (10, 30 and 100 mg/kg), diazepam (1 mg/kg) or saline orally. On day 14, individual mouse were pre-exposed for 30 min to one of the open arm 24 h before the test. 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 using an inter-trial interval of 30 s (avoidance 1 and 2). Thirty s 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 using an inter-trial interval of 30 s (escape 1 and 2). A cut-off time of 300 s was employed for each trial (avoidance and escape test).

OFT: The apparatus consisted of wooden box (96 × 96 × 5 cm). The floor of the box was divided into 16 squares. Immediately after being tested in the ETM, each animal was placed for 5 min in the open field apparatus for the evaluation of locomotor 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 KGE (30, 100 mg/kg) and vehicle. Rats were then sacrificed by stunning, fundus was removed and placed in Kreb's solution. A strip of fundus was mounted in a bath containing Kreb's 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).

Statistics

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

RESULTS

ETM: Results showed that with two exposures to the ETM, KGE impaired inhibitory avoidance and did not facilitate escape latency. Treatment with diazepam (1 mg/kg) impaired inhibitory avoidance performance in the ETM. One -way ANOVA showed a significant effect of treatment [F (4,20)=26.65; $P < 0.05$] and [F (4,20)=14.17; $P < 0.05$] for Avoidance-1 and Avoidance-2 respectively. The Dunnett's test showed that KGE (10, 30 and 100 mg/kg) decreased the latency to leave the enclosed arm ($P < 0.05$) as compared to vehicle. Diazepam (1 mg/kg) did not impair the escape latency for all the trials as compared to control. One-way ANOVA showed a significant effect of treatment [F (4,20) = 0.55; $P < 0.05$] and [F (4,20) = 3.92] for Escape-1 and Escape-2 respectively (Table 1).

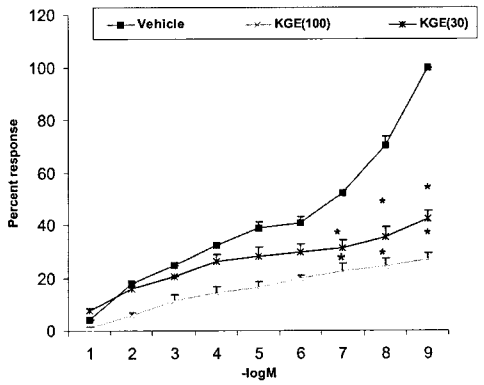
OFT: Diazepam facilitated locomotion as compared to vehicle and other treatment groups. KGE at any of the selected dose did not impair locomotion [F

Table 1. Effect of KGE on inhibitory avoidance and escape latency in ETM. n = 5, values are mean ± S.E.M. *$P < 0.05$ when compared to vehicle (ANOVA followed by Dunnett's test)

Sr no	Treatment (mg/kg)	Inhibitory Avoidance (sec)		Escape latency (sec)	
		Avoidance-1	Avoidance-2	Escape-1	Escape-2
1	Vehicle	16 ± 1.34	65.2 ± 11.99	24 ± 7.02	71.0 ± 12.03
2	Diazepam (1)	4.33 ± 0.80*	8.83 ± 1.51*	29.33 ± 13.91	28.61 ± 13.67
3	KGE (10)	9.2 ± 0.66*	24.2 ± 3.23*	26.6 ± 6.11	26.8 ± 5.18
4	KGE (30)	6.8 ± 1.2*	15.4 ± 6.08*	16.4 ± 4.35	28.4 ± 4.46
5	KGE (100)	3.4 ± 0.67*	9.2 ± 1.5*	16.4 ± 4.61	28.6 ± 9.6
	F (4,20)	26.65	14.17	0.55	3.92

Table 2. Effect of KGE on locomotion in OFT in mice. n = 5, values are mean ± S.E.M. *$p < 0.05$ when compared to vehicle (ANOVA followed by Dunnett's test)

Sr. no	Treatment (mg/kg)	Squares traversed
1	Vehicle	75.8 ± 14.54
2	Diazepam (1)	121 ± 8.59*
3	KGE (10)	65.4 ± 12.69
4	KGE (30)	70.4 ± 8.68
5	KGE (100)	61.6 ± 5.16
	F (4,20)	14.99



(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 KGE (30 and 100 mg/kg) on CCRC of 5-HT on isolated rat fundus strip in Vehicle, KGE (30), KGE (100) 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.

(4,20) = 14.99; $P < 0.05$] (Table 2).

In vitro studies: The CCRC of 5-HT was significantly ($P < 0.05$) shifted to the right in rat fundus treated with KGE (30 and 100 mg/kg) for 15 days (Fig. 1).

DISCUSSION

The purpose of the present work was to explore the potential of KGE in anxiety through the use of ETM and OFT. 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 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 OFT in order to avoid confusing results due to treatment effects on locomotor activity.

The results showed an anxiolytic effect in one of the tasks-inhibitory avoidance-in the ETM. Chronic administration of KGE (10, 30 and 100 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 (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 agonist diazepam and the 5HT1A partial agonist buspirone (Graeff *et al.*, 1993, 1998; Viana *et al.*, 1994). In agreement with these results, anxiolytic effects of KGE have recently been described by us using three other models of anxiety; the elevated plus maze, the light dark model and the hole board apparatus.

The classic anxiolytic diazepam impaired IA, but failed to change the escape latency. This finding corroborates with the previous finding in rats (Graeff *et al.*, 1993; Viana *et al.*, 1994), which demonstrated that diazepam selectively impaired IA, without influencing escape latency. A shift of CCRC of 5-HT towards the right with suppression of maxima using rat fundus supports the 5-HT antagonistic properties of KGE.

In conclusion, the present results show that anxiolytic effect of chronic treatment of KGE is possibly due to the 5-HT antagonistic and a GABA potentiating property of the extract. It exerts anxiolytic

like effects in a specific subset of defensive behaviour, particularly those that have been related to GAD.

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