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Anxiolytic activity of *Glycyrrhiza glabra* Linn.

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Abstract

Objective: To study anxiolytic activity of hydroalcoholic extract of roots and rhizomes of *Glycyrrhiza glabra*. Materials and methods: Mice received varying doses (10-300mg/kg i.p.) of hydroalcoholic extract of *Glycyrrhiza glabra* and anxiolytic activity was assessed using different paradigms like elevated plus maze, foot shock-induced aggression, and amphetamine-induced stereotypy. Diazepam or ondansetron served as standard anxiolytic agents. Results: In all the animal models of anxiety, lower doses of hydroalcoholic extract were more effective in alleviating anxiety. The extract and standard anxiolytic agents increased duration of occupancy of mice in open arm, increased latency to foot shock-induced aggression and reduced number of fighting bouts and delayed the onset of amphetamine-induced grooming, biting, sniffing and repetitive head movements. Conclusion: The hydroalcoholic extract of roots and rhizomes of *Glycyrrhiza glabra* possesses anxiolytic activity.

Key Words: *Glycyrrhiza glabra*, Anxiolytic activity, elevated plus maze, amphetamine, foot shock.

1. Introduction

Glycyrrhiza glabra Linn (Leguminosae), commonly known as liquorice contains a triterpenoid saponin glycyrrhizin (2-9%) [1], a mixture of potassium and calcium salts of glycyrrhizinic acid [2]. It also contains glabranin A and B, glycyrrhetol, glabrolide, isoglabrolide [3], isoflavone *viz.*, formononetin, glabrone, neoliquiritin, hispaglabridin A and B; coumarins *viz.*, herniarin, umbelliferone; triterpene sterols *viz.*, onocerin, β-amyrin, and stigmasterol [2,4]. Liquorice is used in the traditional system of medicine as demulcent, antacid, anti-ulcer [5,6] emolient, anti-inflammatory [3], tonic, expectorant, diuretic, laxative, sedative. It also possesses antipyretic [7], antimicrobial, antiherpes [8] and antimutagenic [9] activity. Hirabayashi, [10] has reported antiviral activity of glycyrrhizin, a triterpenoid saponin which is 50 times sweeter than sucrose [11], and its derivatives against human immuno-deficiency (type I) virus and the herpes simplex type I virus. Glycyrrhizin also possesses antimicrobial activity [12]. Since the roots are

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reported to possess sedative activity, we have investigated its anxiolytic potential using elevated plus maze, foot shock- induced aggression in mice.

2. Materials and methods

2.1 Preparation of extract

The shade dried roots and rhizomes (1.0kg) were crushed and extracted with 70% v/v ethyl alcohol using Soxhlet's apparatus. The extract was concentrated under reduced pressure and evaporated in air to dryness (yield 170g). The extract was stored in refrigerator and reconstituted in water for injection.

2.2 Animals

Albino Swiss mice weighing 22-25g of either sex were housed into groups of five under standard laboratory condition of temperature and humidity. They had free access to water. Food (Lipton India Ltd. pellets) was withdrawn 8hrs before the experiment.

2.3 Drug treatments

The extract was injected intraperitoneally (i.p.) in varying doses (10, 30, 100 or 300mg/kg) 30 min before amphetamine (Sigma, USA) or the tests. The 5-HT_3 receptor antagonist, ondansetron (Cheminor, India) was used as a standard anxiolytic in a dose of 0.5mg/kg i.p. in the elevated plus maze experiment whereas, diazepam (Sigma, India, 1.0mg/kg i.p.) was used in amphetamine antagonism and foot shock aggression test. A person observed the behaviour of animals not aware of the drug treatments.

2.4 Assessment of anxiolytic activity

2.4.1 Elevated plus maze

The anxiolytic activity was assessed using elevated plus maze (EPM) as described by Pellow [13]. In brief, the EPM consisted of two open arms (25x5cm) crossed with two enclosed arms (25x5x20cm) The arms were connected with a central square of 5x5cm. The apparatus was elevated to the height of 25cm in a dimly illuminated room. The mice treated with the extract (10-100mg/kg) or vehicle were placed individually in the centre of the EPM facing an enclosed arm and the time spent in open and closed arm was recorded for 5min.

The number of entry in the enclosed arm was also noted during this time. An entry was defined as all four paws in the arm. The ratio of time spent in open versus closed arm was calculated. The EPM was cleaned with hydrogen peroxide after each trial.

2.4.2 Foot shock-induced aggression in mice [14]

Pairs of male mice were placed in a box with a grid floor consisting of steel rods with a distance of 6mm. A constant shocker delivered a 60Hz current for 5 sec followed by 5 sec intermission for 3 min. The test compound (10-100mg/kg) or the standard drug, diazepam (1mg/kg) was administered 30min before the test.

Each pair of mice was dosed and tested without previous exposure. The latency to fighting and total number of fights was recorded during the 3min period. The fighting behaviour consisted of vocalization, leaping, running, rearing and facing each other with some attempts to attack. Five pairs of mice were used for each treatment.

2.5 Statistical Analysis

All observations are presented as mean ± SEM. The data was analyzed by Student's *t* -test (unpaired). Differences were considered significant at the 5% level.

3. Results

3.1 Elevated plus maze

The mice treated with vehicle spent 224.0 ± 11.03 sec in enclosed arm and 66.4 ± 8.7sec in open arm and mice entered 8.8 ± 0.58 times in

an enclosed arm. The mice treated with the hydroalcoholic extract spent significantly less time in the enclosed arm and more time in the open arm. The occupancy of mice (receiving 10mg/kg of AE) in enclosed arm reduced to 115 ± 10.0sec ($P<0.05$) whereas, occupancy in open arm increased to 131.0 ± 7.3sec ($P<0.01$). In higher doses the time spent in open arm increased dose dependently. The ratio of time spent in open/enclosed arm increased significantly ($P<0.05$) by the extract as well as ondansetron.

However, the number of entries in the enclosed arm decreased significantly only in animals that received 100mg/kg of the extract. Ondansetron exhibited more potent anxiolytic activity than the extract. The observations are given in Table 1.

3.2 Foot shock-induced aggression

After application of foot shock, vehicle treated mice started fighting after 2.0 ± 0.44sec and fought 17.2 ± 2.08 times in 3min. The foot shock induced aggression was delayed by the extract in a dose of 10mg/kg and the number of fighting bouts reduced to 9.6 ± 0.74 ($P<0.05$). The extract exhibited an inverted 'U' shape dose response curve and the higher dose (100mg/kg) exhibited diminished anti-aggression effect. Diazepam (1mg/kg) was more potent than the extract. The observations are given in Table 2.

4. Discussion

Current evidence suggests that anxiety has a neurobiologic basis. It is thought to be caused by dysfunction of one or more neurotransmitters and their receptors. Drugs affecting noradrenergic β-receptors, serotonin receptors, GABA receptors, adenosine and cholecystokinin can modulate anxiety [15].

Recently, it has been reported by Sibille *et al.*, [16] that genetic inactivation of the serotonin 5-HT_{1A} receptor in mice results in down-regulation of major $GABA_A$ receptor alpha subunits, reduction of $GABA_A$ receptor binding, and benzodiazepine-resistant anxiety. Further it has been suggested that there are multiple types of anxiety states and serotonin nervous system plays a major role in anxiety [17].

In the present study we therefore used ondansetron, a 5-HT_3 receptor antagonist, as well as diazepam, a $GABA_A$ receptor agonist as reference anxiolytics. The present study indicates that the hydroalcoholic extract of *Glycyrrhiza glabra* exhibited anxiolytic activity

Table 1.
Effect of hydroalcoholic extract of *Glycyrrhiza glabra* on occupancy of mice in open/closed arm of the elevated plus maze

Treatment (mg/kg)	Time spent in sec (mean ± SEM) in closed arm	open arm	Entries in open arm	Ratio of open closed arm
Vehicle	224.0 ± 11.03	66.4 ± 8.70	8.8 ± 0.58	0.3 ± 0.05
AE (10)	115.6 ± 10.08*	131.0 ± 7.37**	8.8 ± 0.96	0.86 ± 0.16*
AE (30)	148.4 ± 11.28**	132.6 ± 9.87**	8.0 ± 0.77	0.92 ± 0.12*
AE (100)	130.8 ± 21.65*	153.4 ± 20.21**	3.75 ± 0.47*	1.1 ± 0.43*
Ondan (0.5)	45.4 ± 10.46**	149.0 ± 14.06**	7.2 ± 0.73	4.0 ± 1.06*

n = 5, Ondan = Ondansetron. AE - hydroalcoholic extract of *G. glabra.*
* $P<0.05$, ** $P<0.01$ compared to vehicle treated group (Student's *t* test).

Table 2.
Effect of hydroalcoholic extract of *Glycyrrhiza glabra* on foot shock-induced aggression in paired mice.

Treatment (mg/kg)	Latency to fighting in sec (mean ± SEM)	Number of fighting bouts (mean ± SEM)
Vehicle	2.0 ± 0.44	17.2 ± 2.08
AE (10)	4.8 ± 0.8*	9.60 ± 0.74*
AE (30)	8.8 ± 0.96**	7.4 ± 0.92*
AE (100)	4.0 ± 0.83	8.6 ± 0.97*
Diazepam (1)	11.6 ± 3.2**	4.6 ± 0.67**

n = 10, AE - Hydroalcoholic extract of *G. glabra.*
*P<0.05, **P<0.01 compared to vehicle treated group (Student's *t* - test).

in a variety of animal models of anxiety. Increase in occupancy of animals in the open arm or decrease in the time spent in the enclosed arm of the elevated plus maze indicates anxiolytic activity of a drug [13].

Both ondansetron and the hydroalcoholic extract exhibited anxiolytic activity. The extract exhibited maximum anxiolytic activity after a dose of 10mg/kg and higher doses i.e. 30 and 100mg/kg showed less prominent response compared to the dose of 10mg/kg. An inverted U shaped dose effect curve has been reported earlier by many workers and such effect is observed during the late stage of anxiety [18-21]. Such inverted U shape dose effect curve may also be because of other ingredients present in the extract. The occupancy of mice in the enclosed arm was increased significantly. However, the number of entries in the enclosed arm reduced significantly only at the dose of 100mg/kg. The ratio of time spent in open to closed arm after the extract, increased in a dose dependent manner.

Anxiolytic agents delay onset of fighting and reduce the number of fighting [22]. The extract as well as diazepam, an established anxiolytic agent, increased latency to fighting and reduced the number of fighting episodes. An inverted U shape dose response curve was noted in this experiment also.

Thus it is concluded that the hydroalcoholic extract of *Glycyrrhiza glabra* roots and rhizomes possesses potential for further research to isolate an anxiolytic agent and to study its mode of action.

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