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EVALUATION OF ANTIDEPRESSANT ACTIVITY OF AQUEOUS AND ETHANOLIC EXTRACTS OF *GLYCYRRHIZA GLABRA*

Arzoo Qureshi*, Dr. Prateek K. Jain, Arpit Shrivastava, Sameeksha Jain, Prakhar Nema, Dr. Harshita Jain
Adina Institute of Pharmaceutical Sciences, Saugor (M.P.), India.

*Corresponding Author's E mail: arzoonoshi@gmail.com

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ABSTRACT
The purpose of this study was to see how an aqueous extract (400 mg/kg) and an ethanolic extract (200 mg/kg) of G. glabra affected TST and FST in mice, with efficacy comparable to fluoxetine. G. glabra extracts demonstrated an antidepressant-like effect, however the exact mechanisms are unknown. According to the current findings, when tested in TST, the medicines sulpiride (a selective dopamine D2-receptor antagonist) and p-CPA (a serotonin synthesis inhibitor) greatly reduced the antidepressant-like action of G. glabra extracts. This suggests that G. glabra extracts raise norepinephrine, dopamine, and serotonin levels in mice's brains, which may have an antidepressant effect. In TST, the antidepressant effect of fluoxetine (a specific serotonin reuptake inhibitor) was drastically decreased by p-CPA, suggesting that fluoxetine has an antidepressant effect via the serotonergic system. Liquorice extract has antidepressant-like effects due to an increase in norepinephrine and dopamine levels in the brain. The antidepressant-like properties of liquorice may be owing to its inhibitory action on monoamine oxidase.

Keywords: Antidepressant, Forced swim test, Tail suspension test, *Glycyrrhiza glabra*.

INTRODUCTION

Glabra Glycyrrhia Yasti, Glycyrrhiza radix, Mulethi, and Liquorce are all names for the dried, unpeeled roots and stolons of Glycyrrhiza glabra Linn, which belongs to the Leguminosae family[1]. Yasti has a glycyrrhinic acid content of at least 3.0%. Glycyrrhizin or glycyrrhizic acid, a triterpenoid saponin, is the main ingredient of licorice. Glucose, sucrose, bitter principle glycyramarin resins, asparagin, and fat are among the other ingredients. The presence of flavonoids, which have an antigastric action and are effective in the treatment of peptic ulcers, is another essential chemical component of liquorice. Liquiritin and isoliquiritin are yellow-colored falvonoids. The existence of two methylisoflavones and a coumarin, liquo-coumarin[2], in Indian liquorice roots has been discovered. Carbenoxolone is a glycyrrhiza-derived

oleandane derivative. Preclinical investigations have indicated that co-administration of G. glabra polysaccharides to mice on a high-fat diet improves immunological response and raises the activity of several antioxidant enzymes. Experiments have also revealed that beta-glycyrrhetinic acid has immunomodulatory properties[3-4]. According to research5, it contains antioxidant, anti-inflammatory, and immunosuppressive properties. In a number of clinical trials, this plant was used to cure HCV infection. Glycyrrhizin, powdered Glycyrrhiza roots, licorice extracts, glycyrrhetinic acid, stearylglycyrrhetinate, pyridoxine glycyrrhetinate, and glycyrrhetinic acid 3--O-hemisuccinate (carbenoxolone) are used for their anti-inflammatory qualities. Furthermore, glabridin-containing glycyrrhiza flavonoids isolated from G. glabra are used in cosmetic compositions for their skin-whitening, anti-sensitizing, and anti-inflammatory properties.

In light of the foregoing information, the current study was conducted to investigate the effect of aqueous and ethanolic extracts of G. glabra in mice using the forced swim test and tail suspension test, as well as to investigate the possible underlying mechanisms of the extracts' antidepressant-like activity. To standardise the animal models of depression and assess the antidepressant activity of the extracts, established antidepressant medications like fluoxetine were used.

Materials and Methods

For the experiment, Swiss male albino mice (3 months old and weighing roughly 20-30 g) were obtained. The Institutional Animal Ethics Committee (IAEC) authorised the experimental protocols, and laboratory animals were cared for according to the recommendations of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

Drugs and chemicals — *Glycyrrhiza glabra* L. dried roots were acquired from a commercial source. They were pulverised into a coarse powder. In this research, fluoxetine hydrochloride, glacial acetic acid, sodium hydroxide pellets, Tween 80, and carboxy methyl cellulose were used.

Preparation of extracts

Glycyrrhiza glabra was soaked in 95% ethanol and stored for 24 hours. Then, using a Soxhlet device, this wet medication was extracted with ethanol (95 percent) at 70 degrees Celsius for 48 hours6. The extract was collected, and the ethanol was removed using distillation. In a vacuum, the extract was evaporated to dryness and kept in the refrigerator. The extract yield was 26.05 percent.

Aqueous extract- Coarse powder of roots was extracted with distilled water by double maceration for 48 hr. The extract was filtered through muslin cloth. The filterate was evaporated to dryness in vacuum and kept in a refrigerator. The yield of extract was 26.63%.

G. glabra aqueous extract was diluted in 0.25 percent carboxy methyl cellulose (w/v). Tween 80 (10% v/v) was used to emulsify the ethanolic extract. Fluoxetine was dissolved in a standard saline solution (0.9 percent sodium chloride).

Tests for evaluating antidepressant activity

Suspension of the tail TST is a mouse behavioural test that may be used to screen antidepressant medicines and compare the effects of different treatments on depression-related behaviors7. Mice's tails are taped together to prevent them from escaping or clinging to nearby surfaces. They are 50 cm above the ground, suspended on the edge of a table. Throughout the test, which normally lasts six minutes, the subsequent escape behaviours are measured. In drug research, the tail suspension test is a valuable approach for high-throughput screening of prospective antidepressant drugs.

Porsolt (1981) proposed the "behavioural despair" model for testing antidepressants in mice[8-9]. Forced swim test (Porsolt test)—Porsolt (1981) proposed the "behavioural despair" model for assessing antidepressants in mice[8-9]. In this experiment, a mouse was immersed in water in a glass jar (25 x 12 x 25 cm3) with fresh water at a height of 15 cm and kept at 25°C for 2 minutes, following which each animal acquired a characteristic stationary posture. Antidepressants (along with a few other medications) lessen the time spent immobilised. Unsolvable problems were imposed on an unresponsive, anxious, and immovable animal. These can be divided into two categories: searching, which is marked by high motor activity and energy consumption, and waiting, which is marked by immobility and energy conservation. The sequences of options between various types of activities are referred to as the searching and waiting technique. Antidepressants shift the balance between these two forms of behaviour to favour searching, according to the data. The Porsolt forced swim test is based on the observation that when a rat is forced to swim without the ability to escape, after a brief period of intense activity, it stops swimming completely and only makes the movements necessary to keep its head above water. Antidepressants reduce immobility time, which is a significant measure of the efficiency of antidepressants.

Measurement of locomotor activity—To rule out the influence of celastrus oil on immobility time, horizontal locomotor activities of control and test animals are measured for a period of 10 minutes using a photoactometer[10-11].

Drug protocol— Animals were divided into 24 groups and each group comprised a minimum of 4 animals each (Table 1)

All data is provided as a mean standard deviation in statistical analysis. The data was analysed using one-way ANOVA, followed by Dunnett's test. The data for locomotor activity scores was analysed using the Student's paired t-test. In all tests, the statistical significance threshold was set at P 0.05.

Group no.	Treatment
	Forced Swim Test
I	Control group (vehicle for aqueous extract): 0.25% w/v carboxy methyl cellulose (CMC) was administered s.c. for 10 successive days.
II	Fluoxetine (20 mg/kg.) was administered s.c. for 10 successive days.
III, IV, V	Aq. extract of G. glabra (100, 200 & 400 mg respectively) was administered s.c. for 10 successive days, immobility period was recorded.
VI	Control group (vehicle for ethanolic extract): 10% v/v Tween 80 was administrated s.c. for 10 successive days. At 90 minute after administration on tenth day, immobility period was recorded.
VII	Fluoxetine (20 mg/kg.) was administered s.c. for 10 successive days.
VIII, IX, X	Ethanolic extract of *G. glabra* (100, 200 & 400 mg respectively) was administered s.c. for 10 successive days. At 90 minute after administration on tenth day, immobility period was recorded.
	Tail Suspension Test
XI to XX	Each group of FST except that immobility period was recorded using TST.
	Locomotor Activity
XXI	Control group
XXII	Effect of aqueous extract (200 mg/kg, po) of *G. glabra* on locomotor function of mice were recorded using photoactometer to rule out the increase in locomotor performance of mice due to the extract.
XXIII	Control group
XXIV	Effect of ethanolic extract (100 mg/kg,po) of *G. glabra* on locomotor function of mice were recorded using photoactometer to rule out the increase in locomotor performance of mice due to the extract.

RESULTS

Effect of G. glabra aqueous and ethanolic extracts on TST and FST-induced immobility periods Aqueous extract (100, 200, and 400 mg/kg, s.c.) given to mice for 10 days in both TST and FST significantly reduced immobility time in a dose-dependent manner, suggesting a significant antidepressant-like effect. Among three doses administered for ten days, a dose of 400 mg/kg, s.c. of aqueous extract had the most potent antidepressant-like effects, as evidenced by the greatest decrease in immobility length. A mild dose (100 mg/kg) of ethanolic extract given to mice for 10 days, on the other hand, had no effect on their behaviour. In both TST and FST, the maximal dose (400 mg/kg) of the ethanolic extract significantly increased the immobility duration when compared to the control group. When compared to the control in TST and FST, the middle dose of the ethanolic extract (200 mg/kg) significantly shortened the immobility duration, suggesting a significant antidepressant-like effect. When fluoxetine (20 mg/kg, s.c.) was given for 10 days instead of the control, the immobility duration was significantly reduced. The efficacy of aqueous extract (400mg/kg) and ethanolic extract (200mg/kg) was found to be comparable to that of fluoxetine in both FST and TST. (Tables 2, 3, 4, and 5)

Effect on locomotor activity- When mice were given aqueous extract (400 mg/kg, s.c.) for 10 days, there was no significant difference in locomotor function (3819.1) compared to the control (386.38.1). Ethanolic extract (200 mg/kg, s.c.) provided for 10 days had no effect on mice's locomotor function (251.78.9) when compared to the control (254.38.0). (Tables 6,7).

Table 2 – Effect of Gly. Glabra aqueous extract on immobility period of mice using forced swim test (FST) [Values are mean ± SE]

Group no.	Treatment	No. of animals	Dose	Immobility period (sec)
I	Vehicle for aqueous extract	4	10 ml	175.5 ± 3.5
II	Fluoxetine	4	20 mg	149.8 ± 2.7
III	GG extract (aqueous)	4	100 mg	154.0 ± 2.3
IV	GG extract (aqueous)	4	200 mg	151.8 ± 4.9
V	GG extract (aqueous)	4	400 mg	119.2 ± 3.3

Statistical analysis of data was carried out by one-way ANOVA followed by Dunnett's test. $F(9, 47) = 46.61$; $P<0.0001$ $P < 0.05$ when compared with vehicle for aqueous extract group

Table 3 – Effect of Gly. Glabra ethanolic extract on immobility period of mice using forced swim test (FST) [Values are mean ± SE]

Group no.	Treatment	No. of animals	Dose	Immobility period (sec)
VI	Vehicle for ethanolic extract	4	10 ml	176.7±3.9
VII	Fluoxetine	4	20 mg	149.8 ± 2.7
VIII	GG extract (ethanolic)	4	100 mg	176.8 ± 2.1
IX	GG extract (ethanolic)	4	200 mg	155.0 ± 3.1
X	GG extract (ethanolic)	4	400 mg	196.7 ± 1.9

Statistical analysis of data was carried out by one-way ANOVA followed by Dunnett's test. F (9, 47) =46.61 ; P<0.0001 P < 0.05 when compared with vehicle for ethanolic extract group

Table 4 – Effect of Gly. Glabra aqueous extract on immobility period of mice using tail suspension test (TST) [Values are mean ± SE]

Group no.	Treatment	No. of animals	Dose	Immobility period (sec)
XI	Vehicle for aqueous extract	4	10 ml	193.3 ± 2.6
XII	Fluoxetine	4	20 mg	149.2 ± 6.7
XIII	GG extract (aqueous)	4	100 mg	182.2 ± 2.0
XIV	GG extract (aqueous)	4	200 mg	159.2 ± 3.2
XV	GG extract (aqueous)	4	400 mg	96.4 ± 3.9

Statistical analysis of data was carried out by one-way ANOVA followed by Dunnett's test. F (9, 47) =46.61 ; P<0.0001 P < 0.05 when compared with vehicle for aqueous extract group

Table 5 – Effect of Gly. Glabra ethanolic extract on immobility period of mice using tail suspension test (TST) [Values are mean ± SE]

Group no.	Treatment	No. of animals	Dose	Immobility period (sec)
XVI	Vehicle for ethanolic extract	4	10 ml	195.7 ± 4.3
XVII	Fluoxetine	4	20 mg	149.2 ± 6.7
XVIII	GG extract (ethanolic)	4	100 mg	194.7 ± 2.2
XIX	GG extract (ethanolic)	4	200 mg	152.6 ± 2.6
XX	GG extract (ethanolic)	4	400 mg	221. ± 5.4

Statistical analysis of data was carried out by one-way ANOVA followed by Dunnett's test. F (9, 47) =46.61 ; P<0.0001 P < 0.05 when compared with vehicle for ethanolic extract group

Table 6 - Effect on locomotor activity in aqueous extract

Group no.	Treatment	Dose	Activity score
XXI	Control	100 mg	386.3±8.1
XXII	Aqueous extract	200 mg	381±9.1

Table 7 - Effect on locomotor activity in aqueous extract

Group no.	Treatment	Dose	Activity score
XXIII	Control	100 mg	254.3±8.0
XXIV	Ethanolic extract	200 mg	251.7±8.9

Discussion:

In both TST and FST, the aqueous (400 mg/kg) and ethanolic (200 mg/kg) extracts of G. glabra showed a robust antidepressant-like effect in mice, with efficacy comparable to fluoxetine. All major antidepressant medication classes are detected by these assays, which are very sensitive and specific.

TST immobility depicts a state of despair that can be eased by a variety of medicines that have been demonstrated to help people with depression. Mice in the FST are similarly forced to swim in a restricted space from which they cannot escape. Animals experience behavioural sadness as a result of this, which is claimed to be similar to human depression. The TST has been demonstrated to be less stressful than the FST and to have greater pharmacological sensitivity. When compared to controls, both aqueous and ethanolic extracts had no effect on mice's locomotor activity, showing that they had no motor effects. It backs up the idea that the extracts have a one-of-a-kind antidepressant effect. G. glabra extracts demonstrated an antidepressant-like effect, however the exact mechanisms are unknown. According to the current findings, the medications sulpiride (a selective dopamine D2-receptor antagonist) and p-CPA (a serotonin synthesis inhibitor) were found to be efficacious in TST when the rats were given prazosin (a 1-adrenoceptor antagonist). This suggests that G. glabra extracts raise norepinephrine, dopamine, and serotonin levels in mice's brains, which may have antidepressant effects. In TST, the antidepressant effect of fluoxetine (a specific serotonin reuptake inhibitor) was drastically decreased by p-CPA, suggesting that fluoxetine has an antidepressant effect via the serotonergic system. Liquorice extract has antidepressant-like effects due to an increase in norepinephrine and dopamine levels in the brain. The antidepressant-like properties of liquorice may be owing to its inhibitory action on monoamine oxidase.

The current data suggest that extracts of Glycyrrhiza glabra, both aqueous and ethanolic, exhibited an antidepressant-like effect in mice in both FST and TST, and that it was comparable to fluoxetine. Interactions with the adrenergic, dopaminergic, and serotonergic systems appear to mediate the extracts' antidepressant-like effects. As a result, Glycyrrhiza glabra extracts may be effective in the treatment of a wide range of disorders.

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References

1. Sharma V and Agrawal RC. *Glycyrrhiza glabra*: a plant for the future. Mintage J Pharm Med Sci. 2013;2(3):15–20.
2. Zadeh JB, Kor ZM and Goftar MK. Licorice (Glycyrrhiza glabra Linn) as a valuable medicinal plant, Inter. J. Advance. Biol. Biomed Res. 2013; 1: 1281–1288.
3. Asl MN and Hosseinzadeh H. Review of pharmacological effects of Glycyrrhiza sp. and its bioactive compounds, Phytother Res. 2008; 22: 709-724.

4. Fiore C, Eisenhut M, Ragazzi E, Zanchin G and Armanini D. A history of the therapeutic use of liquorice in Europe, Journal of Ethnopharmacology. 2005; 99(3): 317–324.
5. Adel M, Alousi LA and Salem HA. Licorice: a possible anti-inflammatory and anti-ulcer drug. AAPS Pharm Sci Tech. 2005; 6:74–82.
6. Harborne JB. Phytochemical methods: A guide to modern techniques of plant analysis. Third edition, Chapman and Hall, UK. 1998; 292 – 293.
7. Steru L, Chermat R, Thierry B and Simon P. The tail suspension test: A new method for screening antidepressants in mice. Psychopharmacology (Berl). 1985; 85(3):367-70
8. Dhingra D, Sharma A. Evaluation of antidepressant-like activity of glycyrrhizin in mice. Int J Pharmacol. 2005; 37(6):390-4.
9. Sanmukhani J, Anovadiya A and Tripathi CB. Evaluation of antidepressant like activity of Curcumin and its combination with fluoxetine and imipramine: An acute and chronic study. Acta Pol Pharm. 2011;68(5):769-75.
10. Rajkowska G, Miguel-Hidalgo JJ, Wei J, Dilley G, Pittman SD, Meltzer HY, et al. Morphometric evidence for neuronal and glial prefrontal cell pathology in major depression. Biol Psychiatry. 1999;45(9):1085-98.
11. Guragac Dereli FT, Ilhan M and Kupeli Akkol E. Discovery of new antidepressant agents: In vivo study on Anthemis wiedemanniana Fisch. & Mey. J. Ethnopharmacol. 2018; 226: 11–16.