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ORIGINAL RESEARCH ARTICLE

Hydroalcoholic Extract of *Glycyrrhiza glabra* Linn. Attentuates Chronic Fatigue Stress Induced Behavioral Alterations in Mice.

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ABSTRACT

The present study evaluates the protective effect of hydroalcoholic extract of *Glycyrrhiza glabra* on chronic fatigue stress (CFS) induced behavioral alterations. The exposure of mice to chronic fatigue stress for 15 days demonstrated an increased immobility time, increased anxiety, impaired memory, reduction in muscle co-ordination, reduced activity and increased pain perception. The altered behavioral parameters were attenuated significantly by the treatment of *Glycyrrhiza glabra* (100 and 200 mg/kg *p.o*) comparable to fluoxetine (10 mg/kg, *i.p.*). The study concludes that *Glycyrrhiza glabra* could be used as an alternative to conventional medicines for the treatment of chronic fatigue stress.

Keywords: *Glycyrrhiza glabra*; chronic fatigue stress; behavioral effects.

INTRODUCTION

Chronic fatigue syndrome (CFS) is an illness characterized by persistent and relapsing fatigue accompanied by numerous neuro and psychosomatic complaints [1]. Too much of exhaustation, mental stress or depression can lead to the chronic stress.
In this modern era, stress has become an integral p art of human life [2]. Depression is the most significant factor in differential diagnosis of chronic fatigue. Forced swimming is an experimental protocol used for inducing stress and depression in laboratory rodents [3].
Glycyrrhiza glabra (licorice, Fabaceae/Papilionaceae) is a plant with a rich ethnobotanical history. The roots are used as a folk medicine both in Europe and in Eastern countries. The main components are the triterpene, saponins, glycyrrhizin and glycyrrhetic acid[4]. The roots of *G. glabra* are very widely used in traditional systems of medicines all over the world. *G. glabra* is reported to have antiviral, anticancer, anti-ulcer, anti-diabetic, antiinflammatory, anti-oxidant, anti-thrombic, anti-malarial, anti-fungal, anti-bacterial, estrogenic, immunostimulant, anti-allergenic and expectorant activities [5]. Its roots were also demonstrated to have antidepressant activity in mice. Aqueous extract of *Glycyrrhiza glabra* was also reported to increase animal resistance to vibration stress [6].
As *Glycyrrhiza glabra* has antidepressant and antistress potential. It can be hypothesized to be effective in chronic fatigue stress too. With this background the present study evaluates the effect of aqueous extract of *G. glabra* on behavioral alterations induced by chronic fatigue stress.

MATERIAL AND METHODS

Collection and extraction of plant

Glycyrrhiza glabra roots were purchased from local market of Udaipur and were authenticated by Professor K.G. Ramawat, Laboratory of Ethnobotanical Research, Department of Botany, Faculty of Science, MLS University, Udaipur, Rajasthan, India through comparison with a voucher specimen present in the herbarium. The pulverized root powder was extracted using 50% hydroalcoholic solvent by double maceration method.

Phytochemical screening

The aqueous extract of *Glycyrrhiza glabra* were screened for the presence of triterpenoids, saponins, flavonoids lignins and extract gave positive results of all.

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Animals

Male Albino mice (Laka strain) weighing between 22 and 30 g bred in Government Veterinary College, Mhow (M.P.) were used. The animals were housed under standard laboratory conditions maintained under a natural light and dark cycle, and had free access to food and water. Animals were acclimatized to laboratory conditions before the experiment. The experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC) with CPCSEA no. 870/ac/05/CPCSEA.

Drugs

Fluoxetine was procured as gift sample from Lupin Ltd. Bhopal was used as a standard drug in this experiment. All the solutions of drugs were prepared just before administration.

Induction of Chronic Fatigue Stress:

In the present study, animals were individually forced to swim inside a rectangular glass jar (25×12×25 cm^3) containing 15 cm of water maintained at 23-25^oC; the total duration of immobility was recorded during a 6 min test. The animal was judged to be immobile when it ceased struggling and remained floating motionless in water, making only those motions that are necessary to keep its head over water. The animals were forced to swim 6 min every day for total of 15 days, the recording of immobility period was done on alternate days [7].

Experimental Groups

Group I: Normal control (unstressed), received vehicle *p.o*.

Group II: Experimental control (stressed), received vehicle *p.o*.

Group III and IV: Received *Glycyrrhiza glabra* (100 and 200mg/kg *p.o*.), 60 min before exposure to chronic fatigue stress.

Group V: Received standard drug fluoxetine in dose of 10 mg/kg *i.p*. 30 min before exposure to chronic fatigue stress.

Behavioural Assessment:

Various behavioral parameters were assessed in mice 24 h after the last chronic forced swim test. All the animals were tested for behavioral alterations like immobility time, anxiety, memory, muscle relaxation, locomotion and hyperalgesia.

Measurement of Locomotor Activity:

Locomotor activity was recorded using an actophotometer for a period of 5 minutes. An array of 16 infrared emitters, detector pairs measured animal activity along a single axis of motion, the digital data being displayed on the front panel meters as ambulatory movements. Mice were acclimatize to the observation chamber for a period of 2 minute. Ambulatory and rearing movements were noted. Locomotion was expressed in terms of total photobeam count for 5 minutes per animals[8].

Measurement of anxiety in mirror chamber test:

The mirror chamber consisted of a wooden chamber having a mirror chamber enclosed within it. During the 5 min test session the following parameters was noted (i) latency to enter the mirror chamber (ii) the total time spent in mirror-chamber, (iii) the number of entries animal made into the mirror chamber. Animals were placed individually at the distal corner of the mirror chamber at the beginning of the test. An anxiogenic response was defined as decreased number of entries and time spent in the mirror chamber [9].

Measurement of memory- elevated plus maze test:

Cognitive behavior was noted by using elevated plus-maze learning task[8]. Transfer latency (TL), the time in which animal moves from the open arm to enclosed arm, was utilized as an index of learning and memory process. The elevated plus maze consisted of two open arms (16×5 cm^2) and two closed arms 16×12×5 cm^3) with an open roof. The maze was elevated to a height of 25 cm from the floor. The animal was placed individually at the end of either of the open arms and the transfer latency was noted on the first day. If the animal did not enter an enclosed arm within 90s, it was gently pushed in to the enclosed arm and the transfer latency was assigned as 90s. To become acquainted with the maze, the animals were allowed to explore the plus maze for 20s after reaching the closed arm and then returned to its home cage. Retention was examined 24h after the 1st day trial. Percent retention was calculated from the difference in the transfer latencies of day 1 and day 2[8].

Measurement of muscle coordination- rota rod test

Evaluation of muscle coordination (muscle strength and grip) was carried out using horizontal rotating rod (rota rod) test, which determines an animal's ability to support its own body weight by holding on to the rotating rod. The loss of muscle grip is an indication muscle relaxation which is recorded by fall-off time. Mice were placed on a horizontally rotating (25 rpm) rota-rod apparatus

(Techno, India).The fall-off time was recorded for each mouse and the longest period any animal was kept on the rod was 300s[7].

Measurement of hyperalgesia:

Tail-immersion assay was used to assess hyperalgesia in mice after PTZ kindling. Each mouse was placed individually in restraining cages leaving the tail hanging out freely. The terminal 1cm part of the tail was immersed in a water bath maintained at 52°C. The withdrawal latency was defined as the time for the animal to withdraw its tail from water. A cutoff time of 15s was used to prevent damage to the tail [10].

Statistical Analysis

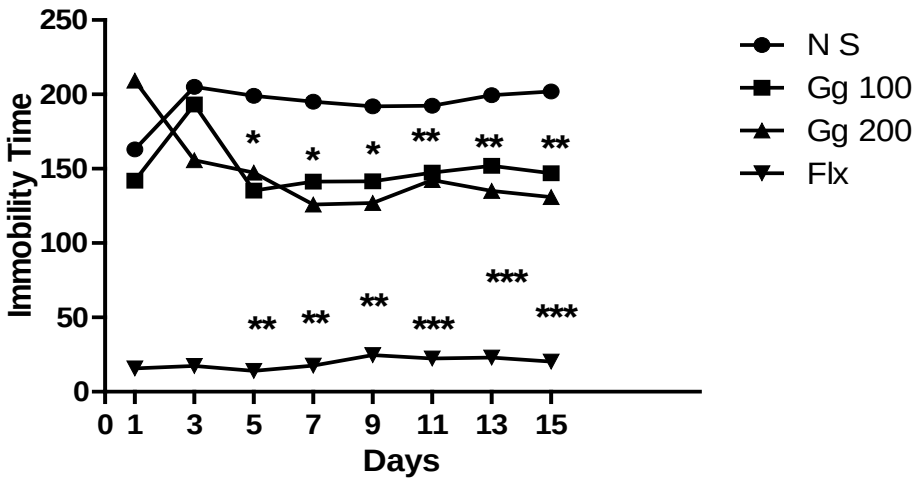
All the values are expressed as the mean± SEM. The data were analyzed by using one-way ANOVA followed by Turkey's test, P<0.05 was considered statistically significant.

RESULTS

Effect of hydroalcoholic extract of G*lycyrrhiza glabra* on chronic fatigue stress induced immobility period.

In the present study forced swimming test was employed to induce a state of chronic fatigue in animals. Animals were made to swim daily for 6 minutes over a total period of 15 days. The immobility period of the treated groups was less as compared to experimental control (stressed) group on day 15. *Glycyrrhiza glabra* 100 and 200 mg/kg, showed very significant reversal of immobility period as compared to experimental control (stressed) animals, comparable to fluoxetine (10 mg/kg *i.p.*) (**Fig 1**).

Fig 1. Effect of chronic administration of *Glycyrrhiza glabra* on mean immobility period on alternate days.



Effect of different doses of *Glycyrrhiza glabra* (100 and 200mg/kg *p.o.*) and fluoxetine (10mg/kg, *i.p.*) on the immobility time on alternate day. *Significant P < 0.05, ** Very Significant P < 0.005, *** Highly Significant P < 0.0001 as compared to that of normal (stressed) mice. NS: Normal Stressed, Gg 100 & Gg 200: Glycyrrhiza glabra 100 mg/ kg and 200 mg/kg *p.o.*, Flx: fluoxetine 10 mg/kg *i.p.*

Effect of hydroalcoholic extract of *Glycyrrhiza glabra* on Anxiety

In animals the chronic stress exposure resulted in increased latency to enter, decrease in number of entries and time spent in mirror chamber as compared to normal (unstressed) mice. Daily treatment with *Glycyrrhiza glabra* (100 & 200 mg/kg*)* & fluoxetine (10 mg/kg *i.p.*), of stressed mice significantly, decrease latency to enter, increased the number of entries and time spent in mirror chamber as compared to normal control (stressed) mice. (**Table.1**)

Table 1: Effect of hydroalcoholic ectract of *Glycyrrhiza glabra* on Anxiety.

S.No.	Groups	Latency to Enter (Sec.) (Mean ±SEM)	No. Entries (Mean ±SEM)	Time Spent (Sec.) (Mean ±SEM)
1	Normal (unstressed)	5.667±0.8	10.667±0.333	5.667±0.881
2	Experimental control (stressed)	8.000±1.5	3.000±0.5774	2.000±1.528
3	*Glycyrrhiza glabra* (100mg/kg)	5.000±0.5**	6.333±0.3**	3.000±0.5*
4	*Glycyrrhiza glabra* (200mg/kg)	2.333±0.8***	8.000±0.5***	4.333±0.8**
5	Fluoxetine (10 mg/kg)	1.333±0.8***	8.53±0.7***	5.88±0.8***

Effect of different doses of *Glycyrrhiza glabra* (100 and 200mg/kg *p.o.*) and fluoxetine (10mg/kg, *i.p.*) on the Latency to enter, No. of Entry & time spent on 13th day. *Significant P < 0.05, ** Very Significant P < 0.005, *** Highly Significant P < 0.0001 as compared to that of experimental control (stressed)

Effect of hydroalcoholic extract of *Glycyrrhiza glabra* on Memory

Patients with chronic fatigue often have difficulties with concentration and memory. Transfer latency in the elevated plus maze task has been taken as an index of memory impairment. In this study chronic stress for 15 days impair the memory of animals and daily

treatment with *Glycyrrhiza glabra* (100 & 200 mg/kg) and fluoxetine (10 mg/kg *i.p.*) Prevented the memory dysfunction in stressed mice significantly respectively as compared to normal control (stressed) mice. (**Table.2**)

Table 2: Effect of hydroalcoholic extract of *Glycyrrhiza glabra* on Memory.

S.No	Groups	Latency to Enter(sec.) Mean ±SEM
1	Normal (unstressed)	5.525±1.404
2	Normal control (stressed)	10.72±0.65
3	*Glycyrrhiza glabra* (100mg/kg)	4.543±0.12***
4	*Glycyrrhiza glabra* (200mg/kg)	3.827±0.9**
5	Fluoxetine (10mg/kg)	2.627±0.3***

Effect of different doses of *Glycyrrhiza glabra* (100 and 200mg/kg *p.o.*) and fluoxetine (10mg/kg, *i.p.*) on the duration of memory (Plus maze test) on 13th day. *Significant P < 0.05, ** Very Significant P < 0.005, *** Highly Significant P < 0.0001 as compared to that of experimental control (stressed).

Effect of hydroalcoholic extract of *Glycyrrhiza glabra* on Muscle Co-ordination.

The untreated chronically fatigued animals showed an early fall from rota rod as compared to normal (unstressed) animals. The treatment with *Glycyrrhiza glabra* (100 and 200 mg/kg) and fluoxetine (10 mg/kg *i.p.*) for 15 days increased the fall off time, significantly as compared to control (stressed) Animals(**Table.3**).

Table 3: Effect of hydroalcoholic extract of *Glycyrrhiza glabra* on Muscle Co-ordination.

S. No	Groups	Fall off Time(sec.) Mean ±SEM
1	Normal (unstressed)	210.91±2.324
2	Normal control (stressed)	115.20±2.450
3	*Glycyrrhiza glabra* (100mg/kg)	135.900±4.687**
4	*Glycyrrhiza glabra* (200mg/kg)	168.607±0.233***
5	Fluoxetine (10mg/kg)	189.23±0.33***

Effect of different doses of *Glycyrrhiza glabra* (100 and 200mg/kg *p.o.*) and fluoxetine (10mg/kg, *i.p.*) on the duration of muscle co-ordination (fall off time) on 13th day. *Significant P < 0.05, ** Very Significant P < 0.005, *** Highly Significant P < 0.0001 as compared to that of experimental control (stressed).

Effect of hydroalcoholic extract of *Glycyrrhiza glabra* on Locomotion

A decrease in locomotor activity was observed in control (stressed) mice as compared to normal (unstressed) mice. The treatment with *Glycyrrhiza glabra* (100 & 200 mg/kg) and fluoxetine (10 mg/kg *i.p.*) Increases the locomotor activity of animals significantly as compare to control mice. (**Table 4**).

Table 4: Effect of hydroalcoholic extract of *Glycyrrhiza glabra* on Locomotion.

S. No	Groups	Locomotor Activity Score (counts) Mean ±SEM
1	Normal (unstressed)	72.0±4.15
2	Normal control (stressed)	28.0±3.07
3	*Glycyrrhiza glabra* (100mg/kg)	38.0±6.5**
4	*Glycyrrhiza glabra* (200mg/kg)	60.0±2.4***
5	Fluoxetine (10mg/kg)	69.4±0.8***

Effect of different doses of *Glycyrrhiza glabra* (100 and 200mg/kg *p.o.*) and fluoxetine (10mg/kg, *i.p.*) on the locomotor activity on 13th day. *Significant P < 0.05, ** Very Significant P < 0.005, *** Highly Significant P < 0.0001 as compared to that of experimental control (stressed).

Effect of hydroalcoholic extract of *Glycyrrhiza glabra* on Hyperalgesia

The control (stressed) mice show hyperalgesic response as compared to normal (unstressed) mice. The daily treatment with *Glycyrrhiza glabra* (100 & 200 mg/kg) and fluoxetine (10 mg/kg *i.p.*) Increases the tail flicking time of stressed animals as compare to control mice. (**Table 5**)

Table 5: Effect of hydroalcoholic extract of *Glycyrrhiza glabra* on Hyperalgesia.

S. No	Groups	Tail Flicking Time(sec.) Mean ±SEM
1	Normal (unstressed)	10,15±1.9
2	Normal control (stressed)	2.403±1.0
3	*Glycyrrhiza glabra* (100mg/kg)	5.560±0.5**
4	*Glycyrrhiza glabra* (200mg/kg)	7.190±0.8***
5	Fluoxetine (10mg/kg)	9.06±0.4***

Effect of different doses of *Glycyrrhiza glabra* (100 and 200mg/kg *p.o.*) and fluoxetine (10mg/kg, *i.p.*) on the pain perception (Tail flicking time) on 13th day. *Significant P < 0.05, ** Very Significant P < 0.005, *** Highly Significant P < 0.0001 as compared to that of experimental control (stressed).

DISCUSSION

Chronic fatigue is relatively a common disorder. In addition to the characteristic persistent fatigue, the patients often complain of a number of symptoms including headache, joint pain, gastrointestinal disturbances, cognitive dysfunction, visual disturbances, and paraesthesia. It is well established that there is a high lifetime prevalence of affective symptoms, such as depression, dysthymia and anxiety in the chronic fatigue population and there are many overlapping symptoms between chronic fatigue and major depression. Various antidepressant drugs have found clinical utility in this syndrome. However, patients suffering from chronic fatigue demonstrated poor response to conventional antidepressant therapy [11]. Fluoxetine, a selective serotonin reuptake inhibitor is reported to be useful for the treatment of psychiatric disorders [12]. *Glycyrrhiza glabra* also demonstrated antidepressant effect [13].

The core finding of the present study is that hydroalcoholic extract of *Glycyrrhiza glabra &* fluoxetine attenuates various behavioral alterations due to chronic fatigue induced by daily exposures of mice to forced swim.

In the present study, the forced swimming has been used to induce a state of chronic fatigue in animals [14].Animals were made to swim daily for 6 min over a total period of 15 days. It was found that the immobility period increased to maximum

on day 4 and persisted up to day 15. These chronically fatigued animals showed an increased anxiety response in mirror chamber, increased pain sensation (hyperalgesic response), memory dysfunctions and increased immobility period and reduced muscle co-ordination.

Repeated experiences to forced swim are known to increase immobility as compared with unstressed rats[15]. In the present study, a similar result was observed in stressed mice as compared to unstressed control group and the effect was attenuated by the daily administration with *Glycyrrhiza glabra* and flouxetine, thereby confirming the anti-stress as well as antidepressant effect.

Cognitive problems are another most disruptive and disabling symptoms of chronic fatigue. Patients with chronic fatigue often have difficulties with concentration and memory[7]. In the present study, chronically fatigued mice demonstrated memory impairment and the daily treatment with *Glycyrrhiza glabra* prevented the memory dysfunction in mice.

Previous studies have demonstrated that various stress conditions induce hyperalgesia to thermal, chemical and mechanical stimuli[16]. The dysfunction of the hypothalamo-pituitary-adrenocortical axis (HPA axis) and multiple neurotransmitter systems in the central nervous system (CNS), including endogenous opioid, serotonergic and noradrenergic systems, has been reported[17]. Antidepressants have been known to induce a dose-dependent antinociceptive effect in mice[7]. Since chronically stressed animals present a hyperalgesic response[18,19], this raises the possibility of alterations induced by chronic stress in a variety of components associated with the regulation of nociception. In the present study, forced swimming stress produced hyperalgesia in mice that was attenuated by daily treatment with *Glycyrrhiza glabra*, thus showing antihyperalgesic effect.

Hyperactivity of central nervous system has also been strongly implicated in the pathophysiology of anxiety. Forced swimming stress has also been reported to induce 2-3-fold rise of plasma cortisol level[20]. Increased cortisol level has been linked with anxiety-like behavior and painful response in humans[21,22]. *Glycyrrhiza glabra* and flouxetine in the present study produced antianxiety effect possibly by decreasing plasma cortisol levels. Further, an increase in fall off time from rota rod also indicates an improvement in muscle coordination in the treated animals.

In summary, the present study revealed that *Glycyrrhiza glabra* is effective in reversing chronic fatigue induced memory dysfunction, immobility, hyperalgesia, anxiety and muscle inco-ordination in mice. It could be used as one of the alternative to the conventional medicines available for the treatment of the disease.

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