EVALUATION OF ANTIANXIETY EFFECTS OF DEGLYCYRRHINATED LICORICE ON ALBINO RATS

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ABSTRACT

Context: Glycyrhiza glabra has antidepressant, anti-stress and anti-anxiolytic potential. It can be hypothesized that Deglycyrrhinated licorice (DGL) to be effective in anxiety too. Aims: To evaluate the anti-anxiety effects of Deglycyrrhinated licorice (DGL) in albino rats and compare the antianxiety activity of DGL against standard drug diazepam (2-3 mg/kg), using animal models Elevated plus maze (EPM) Light and dark arena (LDA) apparatus Settings and Design: Animals were divided into four groups, each consisting six rats. DGL extract, Diazepam and Saline was given to the test groups and after 45 min, animals were exposed to EPM and light–dark test for normal duration (5 min), sufficient to assess the anxiety levels in rodents. Statistical analysis used: One-way ANOVA. Results: In EPM and Light & dark study DGL 1 group showed antianxiety effect compared to control group, but antianxiety effect is inferior to diazepam group and DGL 2 group possessed very mild insignificant antianxiety effect, which is inferior to both diazepam group and DGL 1 group. Conclusions: Deglycyrrhinated licorice possesses antianxiety effect but inferior to Diazepam and therefore can be used as add on therapy.

KEYWORDS: Anti-anxiety, Deglycyrrhinated licorice, Diazepam, Elevated plus maze, Light and dark arena, anxiety.
INTRODUCTION
Globally most prevalent psychological behavioural disorder is anxiety disorder.\textsuperscript{[1]} Anxiety disorders have chronic or relapsing course. It includes personal distress, impaired social and occupational functions, hampered quality-of-life, and overall substantial economic loss.\textsuperscript{[2]} The prevalence of anxiety remains same that could be attributed to the unclear neurobiological understanding of pathophysiology or the inconsistent efficacy of current pharmacological treatment, despite a steady increase in the development of anxiolytic drugs.\textsuperscript{[3]}

Chronic stress is caused due to, too much of mental stress or depression and exhaustion. In modern life, stress has become an integral part of human being.\textsuperscript{[4]} Diazepam (DZM) is most commonly used in the management of anxiety, but it has side effects such as occurrence of dependence, tolerance, varied alertness, and cognition. Withdrawal symptoms also occur after the cessation of DZM.

For thousands of years Glycyrrhiza glabra has been known in pharmacy. Glycyrrhiza glabra (Papilionaceae/ licorice, Fabaceae) is a plant with a rich ethnobotanical history. The roots are used as a folk medicine both in Europe and in Eastern countries.\textsuperscript{[4]} The main components are the saponins, triterpene, glycyrrhizin and glycyrrhetic acid.\textsuperscript{[5]} The roots of G. glabra are globally used in traditional systems of medicines. G. glabra is reported to have anticancer, anti-ulcer, antiviral, anti-diabetic, anti-oxidant, anti-inflammatory, anti-thrombic, anti-fungal, anti-malarial, anti-bacterial, immunostimulant, estrogenic, anti-allergenic, expectorant activities\textsuperscript{[6]} and anti-anxiolytic activities.\textsuperscript{[7]} 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.

One of the side effects is licorice-induced hypertension and edema which occurs due to glycyrrhizic acid a component of licorice. In deglycyrrhinated licorice the glycyrrhizin component is eliminated to an extent.\textsuperscript{[8]}

As Glycyrrhiza glabra has antidepressant, anti-stress and anti-anxiolytic potential. It can be hypothesized that Deglycyrrhinated licorice (DGL) to be effective in anxiety too. With this background the present study evaluates the effect of DGL on behavioural alterations during anxiety in rats.
MATERIAL AND METHODS

Animals
Albino rats *Wistar* strain of 4 – 6 weeks, weighing (200 – 250g) obtained from Central Animal House KSHEMA, Derlakatte, Mangalore. The animals grouped and housed in cages and maintained under standard laboratory conditions (Temperature 25 ± 2°C) with dark and light cycle (12h/12h). They allowed free access to standard dry pellet diet and water *ad libitum*. The experiment carried out according to the guidelines of the committee for the purpose of control and supervision of experiments on Animals (CPCSEA), New Delhi, India and approved by the Institutional Animal Ethics Committee (IAEC).

Chemicals
Diazepam: Diazepam - diazepam injection (Calmpose®, Ranbaxy, India).

Instrument
Elevated plus maze apparatus and Light Dark Arena apparatus for screening anxiolytic activity.

Plant material
Deglycyrrhinated licorice (DGL) a licorice root extract (GutGard®) obtained from Natural remedies, Bengaluru, India. DGL composition contains less than 0.2% of glycyrrhizin and safety studies by Natural remedies claims that acute oral toxicity study with a higher dose of 5000mg/kg did not manifest any toxicological signs in rats and was found to be non-mutagenic in Ames test.

Drug treatment
Deglycyrrhinated licorice, which is a licorice root extract preparation (GutGard®), was obtained by Natural remedies, India. The DGL dose 10mg/kg and 13.5mg/kg was suspended in distilled water and administered orally to rats.

Injection of diazepam (Calmpose®) was diluted in normal saline. Administration i.p. injection was 1 ml/100 g of rat.

Laboratory models for testing anxiety
Models based upon spontaneous (unconditioned) responses
Exploratory behaviours: Elevated plus maze Light-dark Arena
Research Design

a) Elevated plus maze (EMP)

The elevated plus-maze is currently one of the most widely used models of animal anxiety,[9,10] and has been validated for use with both rats and mice.[11,12] Therefore, we chose this test to investigate the anxiolytic potential.

This test has been widely validated to measure anxiety in rodents. The plus – maze combines three potential anxiogenic factors- novelty, height and open space. Briefly, the cross shaped maze consists of four arms that are interconnected by a central platform. Two opposing arms are surrounded by side and end walls (closed arms), whereas remaining two arms are unprotected (open arms). The setup consists of a maze of two open arm (25cm×5cm), crossed with walls (35cm high) and central platform (5cm×5cm). The maze is suspended 50cm above the room floor. The animal placed on the central platform, facing one of the enclosed arms and observed for 5 min. during the 5-min test period, the time spent in open and closed arm, was recorded.

Animals weighed, numbered, and divided into five groups, each consisting six rats. One group was used as control (saline), second for standard drug (diazepam) treatment, third and fourth group for extract treatment (dose minimum and maximum). Animals were placed individually in the center of the maze, head facing toward open arm and stopwatch was started. The following parameters were noted for 5 min. (1) First preference of mouse to open or closed arm. (2) Number of entries in open arm (an arm entry defined as the entry of four paws into the arm). (3) Average time each animal spends in open arm (Average time = total duration in the arm/number of entries) was calculated. Saline and diazepam i.p (2-3mg/kg) were injected to the control and standard groups respectively. Extract was given per oral to the test groups. After 30 min, animals were placed individually in the center of the maze. Finally, we compared the preference of the animals to open or enclosed arm, average time spent in open arm and the number of entries in open arm in each group.

b) Light and dark Arena (LDA)

Light dark exploration test is one of the few tests specifically designed for use of rats. The original maze is divided into two parts, 1/3 with opaque walls and a cover (dark compartment) whereas the remaining 2/3 is open illuminated (light compartment).
The door between the two compartments permits rat to move from one side to another. The rat was released in the light compartment and observed for 5 min. During that time the time spent in light and dark compartment, was recorded.

The transitions between the light and the dark box and time spent in the light box were recorded for 5 min immediately after the mouse stepped into the dark box. The apparatus was cleaned thoroughly between trials.

**Experimental protocol**

Animals were weighed, numbered, and divided into four groups, each consisting six rats. One group was used as control (saline), second for standard drug (diazepam) treatment, third, and fourth, group received deglycyrrhinated licorice (DGL) of 10mg/kg and 13.5mg/kg respectively through oral feed). Saline and diazepam were injected intra peritoneal to the control and standard groups respectively and DGL extract was given to the test groups and after 45 min, animals were exposed to EPM and light–dark test for normal duration (5 min), sufficient to assess the anxiety levels in rodents.

**Groups are as follows**

Group 1: - Control group, treated with normal saline.
Group 2: - Diazepam group, 0.1mg/100mg, given i.p.
Group 3: - DGL 1, extract dose of 10 mg/kg, orally administered.
Group 4: - DGL 2, extract dose of 13.5 mg/kg, orally administered.

**Statistical Analysis**

All the results were expressed as Mean ± SEM. Data were analyzed by analysis of variance (ANOVA). $P < 0.05$ was considered as significant.

**RESULTS**

**Elevated Plus Maze**

Administration of diazepam (0.1 mg/100 gm) increased the amount of time spent in the open arms and the frequency of open arm entries compared to saline-treated (control) group [Table 1]. DGL extract of 10 mg/kg and 13.5 mg/kg increased the time spent in the open arms. Entries in the open arms increased with extract 10 mg/kg (group 3) compared to extract 13.5 mg/kg (group 4). [Table 1].
Table 1: EPM - Effect of different treatments on frequency and time spent in open arm.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean ± standard of frequency of open arm</th>
<th>Mean ± standard of time spent in open arm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.1 ± 2.5</td>
<td>60.5 ± 78.95</td>
</tr>
<tr>
<td>Diazepam</td>
<td>4.5 ± 2.9</td>
<td>95 ± 67.30</td>
</tr>
<tr>
<td>Dgl1</td>
<td>2.8 ± 1.9</td>
<td>71.6 ± 35.16</td>
</tr>
<tr>
<td>Dgl2</td>
<td>2.3 ± 2.1</td>
<td>26.6 ± 25.8</td>
</tr>
</tbody>
</table>

Light and Dark Box Test

Diazepam (0.1 mg/100 gm) increased the time spent in light compartment compared to saline-treated group [Table 2]. Increase in the time spent in the light compartment was seen with administration of 10 and 13.5 mg/kg of DGL extract compared to saline-treated (control) group [Table 2]. DGL extract dose of 10 mg/kg spent more time in light compared to DGL extract of 13.5mg/Kg dose. [Table2].

Table 2: Light & Dark Arena - Effect of different treatments on frequency and time spent in light field.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean ± standard of frequency of light compartment</th>
<th>Mean ± standard of frequency of light compartment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.0 ± 1.4</td>
<td>62.5 ±10.32</td>
</tr>
<tr>
<td>Diazepam</td>
<td>4.6 ± 1.0</td>
<td>76.3± 33.60</td>
</tr>
<tr>
<td>Dgl1</td>
<td>3.3 ± 1.5</td>
<td>86.6 ±38.42</td>
</tr>
<tr>
<td>Dgl2</td>
<td>5.1 ± 1.9</td>
<td>66 ± 25.81</td>
</tr>
</tbody>
</table>

DISCUSSION

Anxiety, like all emotions, has cognitive, neurobiological and behavioural components. It is a negative emotion that occurs in response to perceived threats that can come from internal or external sources and can be real or imagined.\cite{[13]} The incidence of anxiety in the community is very high and associated with lot of morbidity.\cite{[14]}

For the last 40 years Benzodiazepines have been used for treatment of several forms of anxiety, but it has unwanted side effects, and therefore alternative treatment with favourable side-effect profiles, acceptable benefits & moderate costs are of interest, especially in primary care settings. The fact that benzodiazepines have ataxic and sedative side effects is well known.\cite{[15],[16]} Medicinal plants are a good source to find new remedies for these disorders. Research has been conducted to investigate natural anxiolytic drugs as well as new antidepressant principles, in search for more specific, an alternative, and perhaps cost effective therapy.\cite{[17]}
Current evidence suggests that anxiety has a neurobiological basis. It could be due to dysfunction of one or more neurotransmitters and their receptors. Drugs affecting serotonin receptors, noradrenergic β-receptors, GABA receptors, cholecystokinin and adenosine can modulate anxiety.\[^{18}\] Recently, it has been reported by Sibille et al.,\[^{19}\] that genetic inactivation of the serotonin 5-HT\(_{1A}\) receptor in mice results in downregulation of major GABA\(_{A}\) receptor alpha subunits, reduction of GABA\(_{A}\) receptor binding, and benzodiazepine-resistant anxiety. It is also further suggested that there are multiple types of anxiety states and serotonin nervous system plays a major role in anxiety.\[^{20}\]

The result in the study indicates that the DGL extract have anti-anxiety effect on rats. In EPM study diazepam group showed anti-anxiety effect when compared with control group with increased frequency of entry in open arm and more time spend in open arm. Which is similar to earlier study which showed that Diazepam and other benzodiazepines exert anxiolytic effects.\[^{21,22}\] DGL 1 group (10 mg/kg) showed increased anti-anxiety effect in open arm, compared to control group and DGL 2 group (13.5 mg/kg) but anti-anxiety effect was less than Diazepam group. Whereas DGL 2 group showed insignificant anti-anxiety effect compared to control group but it was less than diazepam group and DGL 1 group. The anxiolytic-like activity was also observed in the light/dark box. In this test Diazepam group showed anti-anxiety effect when compared to control group with increased frequency of entry in light compartment and more time spent in light compartment. DGL 1 group showed increased duration of time spent in light compartment compared to all other three groups i.e. control group, diazepam group and DGL 2 group, but the frequency of entry in light compartment was the least when compared to other three groups. Whereas DGL 2 group showed more duration of time spent in light compartment compared to control group but less than in diazepam and DGL 1 group.

Earlier study on anti-anxiety effect of Glycyrrhiza glabra was done by S. Ambawade et al and Rahul Trivedi et al and found to possess anxiolytic activity.\[^{7,23}\] But there has been no study done earlier on anti-anxiety effect of Deglycyrrhinated licorice, and hence in our study we tried to evaluate if Deglycyrrhinated licorice also possess anxiolytic activity.

**CONCLUSION**

The present study demonstrated that the aqueous extract of Deglycyrrhinated licorice possess dose-dependent anxiolytic activity. But result shows that DGL is inferior to Diazepam and therefore can be used as add on therapy. Further, there is need to isolate, characterize, and
screen the active principles that are responsible for its anxiolytic activity. Furthermore, there is a need to find out the exact mechanism by which the plant extract exerts above effects. Further studies are needed to separate and confirm the active components and its effect on anxiety.

ACKNOWLEDGEMENT
Authors are thankful to ‘Natural remedies’, Bangalore, India for providing us the DGL extract preparation.

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