**ABSTRACT**

Present work was done to investigate the antiinflammatory and antinociceptive activity of aqueous and ethanolic extracts of *Glycyrrhiza glabra* by using different pain models in Swiss albino mice of either sex. Antinociceptive activity was evaluated at 50-200 mg/kg i.p. in mice using various experimentally induced pain models like Acetic acid induced abdominal constriction, Formalin induced analgesia and Tail flick method. Aqueous and ethanolic extracts of *Glycyrrhiza glabra* inhibited Acetic acid induced abdominal constriction, with maximal effect 57.33 and 57.53% inhibition respectively at 200 mg/kg. In Formalin induced paw licking model, both extract exhibited more pronounced antinociceptive effect in inflammatory phase than in neurogenic phase (maximal effect were 43.24% and 40.93% for aqueous and ethanolic extract, respectively, at 200 mg/kg i.p.). A dose dependant response was observed in Tail flick method but statistically significant maximal response was seen in mice at the dose of 200 mg/kg i.p. From the results it was concluded that both extracts exhibited anti-nociceptive activity by central and peripheral mechanism.

**Keywords:** Antiinflammatory, Antinociceptive, *Glycyrrhiza glabra*, Fabaceae, pain models.

**INTRODUCTION**

Exploitation of plants has lead to the search of many medicinal useful compounds and their precursors. Medicinal plants and the active principles isolated from them are of emmence importance to humanity in their fight against diseases. Almost all developed countries are switching towards herbal medicines. In the light of this facts the researchers of phytochemistry should come with renewed zeal to exploit the unexploited flora of mother earth. Scientific interest in medicinal plants has burgeoned in recent times due to increased efficiency of new plant derived drugs and rising concerns about the side effects of conventional medicine. Inflammation is seen in conditions such as Alzheimer’s disease, cancer, irritable bowel syndrome and hepatic diseases. It is believed that controlling inflammation may help to alleviate these conditions or even prevent them. Thus the present investigation was carried out to evaluate the anti-nociceptive potential of roots of *Glycyrrhiza glabra* Linn.

Genus *Glycyrrhiza* belongs to family *Fabaceae*. *Fabaceae* (*papilionaceae*) is a family of order *Fabale*, which contains other species of liquorice such as *G. uralensis*, *G. inflata*, *G. aspera*, *G. korshinskyi*, *G. eurycarpa*, *G. echinata*, *G. lepitopa*, *G. hirsuta* and 30 more species. Glycyrrhiza, literally means sweet root, has been used by human beings for at least 4000 years. Hippocrates used liquorice to prescribe to treat cough, asthma and other respiratory diseases. Traditionally Dioscoride used decoction of roots to relieve fever, mouth aphpae, respiratory and digestive disorder. Nowadays root decoction is often used as an antitussive, respiratory balm.

*Glycyrrhiza glabra*, which is called as Liquorice in India, has been reported to have several pharmacological effects such as Antioxidant1, Anticonvulsant2, Antiviral, Antiallergic3, Antithrombotic, Antitumorogenic, Antimicrobial4, Antiinflammatory, Antiartritic5 and Memory strengthening capacity6. The phytochemical studies on Glycyrrhiza glabra have shown the presence of constituents such as Triterpenes, saponins (glycyrrhizin, glycyrrhizinic acid and glycyrrehetic acid), flavonoids (liquiritin, isoiriquitin), coumarins (liquoric acid), triterpenoid, essential oil and other active principles (polysaccharides)7-10. This plant is furthermore, believed to have demulcent, antacid, anti-ulcer, tonic, laxative, sedative, expectorant, anti hypertensive, spasmylytic, diuretic11 and antipyretic effects12.

Septilin, a herbal preparation, containing powder of *Glycyrrhiza glabra* has proven to have anti-inflammatory and analgesic effect13. The literature survey revealed that there are no scientific studies carried out regarding anti-inflammatory and anti-nociceptive activities on the roots of *Glycyrrhiza glabra* to substantiate their therapeutic claim. Hence in the present study the aqueous and ethanolic extract of roots of *Glycyrrhiza glabra* were examined for its antiinflammatory and antinociceptive activities on the roots of *Glycyrrhiza glabra* to substantiate their therapeutic claim. Hence in the present study the aqueous and ethanolic extract of roots of *Glycyrrhiza glabra* were examined for its antiinflammatory and antinociceptive activities.

**MATERIAL AND METHODS**

Plant roots were purchased from commercial source and authenticated by Aagarkar Research Institute, Pune. Roots were pulverized using grinder and made into coarse powder which further used for extract preparation.
Aqueous extract:-

Pulverized powders of *Glycyrrhiza glabra* was soaked in distilled water for 24 hours and filtered. The filtrate was evaporated in air to get dry extract.

Ethanolic extract:-

The pulverized powder was filled in column of soxhlet’s apparatus and kept it for extraction till clear ethanol appears to be falling from side tube. Ethanol was evaporated in air.

Chemicals

Ethanol, codeine phosphate and ibuprofen were purchased from Research Laboratory (Fursungi, Pune) and Diclofenac sodium and carageenan were obtained as from local market. Extracts of Glycyrrhiza were diluted with saline water.

Preliminary phytochemical screening

*G. glabra* was studied for its preliminary phytochemical screening for the detection of various plant constituents.

Preparation of test drugs

Both the extracts were dissolved in saline water and used for administration to the animals in all these pain models.

Experimental animals

Albino rats of Wistar strain (150-200g) and Adult Swiss albino mice (20-26g) of either strain were obtained from Serum Institute of India Ltd., Hadapsar, Pune, India. The animals were randomly allocated to treatment groups (six animals per group, per treatment) in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24±2°C with relative humidity of 30 to 70%. A 12:12 light dark cycle was followed. All animals had free access to water and standard pelleted laboratory animal diet. The animals fasted over 8 hours before, during and after the experiment. All the experimental protocol was approved by Institutional Animal Ethics Committee.

Formalin induced paw licking in mice

This test was performed in accordance with the method of Dubuisson and Dennis. The extracts of *Glycyrrhiza glabra* administered intraperitoneally (50-200mg/kg) to the different group of mice and after 15 minutes later 20µl of 1% formalin was injected subcutaneously under the dorsal surface of the hind paw and the animals were observed in the chambers. The time spend for licking the paw was counted for 30 minutes post formalin injection and considered as indicative of pain stimuli. The formalin test had two distinctive phases possibly reflecting different types of responses. Out of this the first phase of nociceptive response is neurogenic phase that normally peaks at 5 minutes and continues for 10 minutes while last 10 minutes of the counting period are indicative of inflammatory phase. Results are compared with control and standards (codeine phosphate)14-15.

Tail flicking test in mice

This method was carried out by using the hot wire of analgesiometer. Initially the ethanolic extract of *Glycyrrhiza glabra* (50-200 mg/kg i.p.) and Codeine phosphate were administered i.p. Then the tail of mice was placed on the hot wire of analgesiometer and the time taken by mice to flick the tail was measured. A cut off period of 15 sec is pondered to avoid tissue damage. The difference between times required for flicking the tail of mice of saline treated control and mice treated with ethanolic extract of *Glycyrrhiza glabra* and standard was expressed as Antinociception14-15.

Statistical analysis

The results are expressed as mean ± SEM. The statistical significance of difference between the means was analyzed by one way non parametric ANOVA and Dunnett’s test for antinflammatory and Turkey’s test for antinociceptive activity. P<0.05 was considered statistically significant.

RESULTS

Preliminary phytochemical screening

Preliminary phytochemical screening of *Glycyrrhiza glabra* showed the presence of triterpene saponins, flavonoid glycoside, protein and starch16.

Acetic acid induced abdominal constrictions in mice

The i.p. injection of acetic acid (0.6%) results in constrictions of abdominal muscle together with a stretching of hind limbs. The ethanolic extract of *Glycyrrhiza glabra* (50-200mg/kg) and Diclofenac sodium (10mg/kg) were administered i.p. 15 min prior to acetic acid injection to different groups of mice. The number of abdominal constrictions was counted for 20 minutes from the time immediately after acetic acid injection. Antinociception was expressed as the number of abdominal constrictions between saline treated control and animals pretreated with aqueous and ethanolic extract of *Glycyrrhiza glabra*14-15.

Acetic acid induced abdominal constrictions in mice

Aqueous and ethanolic extract of *Glycyrrhiza glabra* elicited a dose dependent inhibition of abdominal constrictions compared with control group. Aqueous extract of *Glycyrrhiza glabra* produced 42.04% inhibition at 100mg/kg dose and maximal inhibition 57.33% (P<0.01) at 200mg/kg. The standard Diclofenac Sodium had shown 73.25% inhibition at10mg/kg,i.p. The ethanolic extract produced 41.84% inhibition at 100mg/kg dose and maximal inhibition 57.53% (P<0.01) at 200mg/kg and compared with standard Diclofenac Sodium with inhibition of 76.47%.

Table 1: Effect of aqueous extract of Glycyrrhiza glabra on Acetic Acid induced abdominal contractions.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose (mg/kg)</th>
<th>No. of abdominal contractions</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>—</td>
<td>26.2±1.2</td>
<td>——</td>
</tr>
<tr>
<td>AEGG</td>
<td>50</td>
<td>19.7±1.3</td>
<td>24.48%</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>15.2±1.2</td>
<td>42.04%</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>11.2±1.2</td>
<td>57.33%</td>
</tr>
<tr>
<td>Standard</td>
<td>10</td>
<td>7.0±1.0</td>
<td>73.25%</td>
</tr>
</tbody>
</table>

n = 6; * = P<0.01 (P<0.05 is considered significant), (One way ANOVA, Dunnett’s test).

Table 2: Effect of ethanolic extract of Glycyrrhiza glabra on Acetic Acid induced abdominal contractions.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose (mg/kg)</th>
<th>No. of abdominal contractions</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>—</td>
<td>25.5±1.5</td>
<td>——</td>
</tr>
<tr>
<td>EEGG</td>
<td>50</td>
<td>19.2±1.2</td>
<td>24.86%</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>14.8±1.2</td>
<td>41.84%</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>10.8±1.2</td>
<td>57.53%</td>
</tr>
<tr>
<td>Standard</td>
<td>10</td>
<td>6.0±1.0</td>
<td>76.47%</td>
</tr>
</tbody>
</table>

n = 6; * = P<0.01 (P<0.05 is considered significant), (One way ANOVA, Dunnett’s test).

Formalin induced paw licking in mice

Aqueous and ethanolic extract of Glycyrrhiza glabra exhibited no effect during the neurogenic phase (0-5 min) of formalin induced licking in mice at 50mg. It was observed that aqueous and ethanolic extract show 14.37% and 9.67% inhibition at 50mg/kg, i.p., and 38.95% (P<0.01) and 35.08% (P<0.001) inhibition at 200mg/kg respectively which was compared with vehicle treated animals. The standard Codeine phosphate (12mg/kg) caused 66.02% inhibition. An inhibitory effect (29.70% and 25.10%) of aqueous and ethanolic extract of Glycyrrhiza glabra was observed at 100mg/kg and the statistically significant maximal inhibition 43.24% (P<0.01) and 40.93%(P<0.001) was observed at 200mg/kg. The standard Codeine phosphate (12mg/kg) also exhibited a statistical inhibition (66.98%) of the inflammatory phase.

Tail flicking in mice

Aqueous and ethanolic extract of Glycyrrhiza glabra exhibited dose dependent inhibition of time required for flicking of the tail of mice compared with control group. It was observed that animal shows mild response towards time required for the flicking of tail at 100 mg/kg of aqueous and ethanolic extract of Glycyrrhiza glabra and the statistically significant maximal response was observed at 200mg/kg (P>0.05) for aqueous and ethanolic extracts respectively. The standard codeine phosphate (12mg/kg) was observed to show maximal response against time required for flicking of tail of mice.
DISCUSSION

In the present investigation aqueous and ethanolic extract of roots of Glycyrrhiza glabra was studied for its antinociceptive activity as this species of Genus Glycyrrhiza has not been studied for its antinociceptive activity yet. Various species from Genus Glycyrrhiza such as G. glabra, G. inflata showed the anti-inflammatory activity. Also the phytochemical investigation of aqueous as well as ethanolic extract of Glycyrrhiza glabra had shown the presence of saponin triterpenes, flavonoid glycosides, starch, proteins and tannins. So an attempt was done to elucidate Antinociceptive activity of roots of Glycyrrhiza glabra.

Thermal painful stimuli are known to be selective to centrally but not peripherally acting analgesic drugs. In the present study aqueous and ethanolic extract of roots of Glycyrrhiza glabra exhibited dose dependant antinociceptive response in tail flick test. A mild response was observed with the dose of 100mg/kg i.p. while as significant response was observed with the dose of 200mg/kg i.p. Still this response was less as compared to standard codeine phosphate (12mg/kg i.p.) It may lead to conclusion that Aqueous and ethanolic extract of Glycyrrhiza glabra is mild analgesic.

The intensity of analgesic effect of 200mg/kg i.p. dose of aqueous and ethanolic extract of rhizomes of Glycyrrhiza glabra was comparable to that with Diclofenac (10mg/kg i.p.) in acetic acid induced abdominal constrictions in mice. Acetic acid causes inflammatory pain by inducing capillary permeability and liberating endogenous substances that excite pain nerve endings. The mechanism of analgesic effect of ethanolic extract of roots of Glycyrrhiza glabra could be probably due to blockade of effect or the release of endogenous substance that excite pain nerve endings similar to that of codeine phosphate.

Aqueous and ethanolic extract of roots of Glycyrrhiza glabra also exhibited a significant inhibitory effect on the nociceptive response of the late phase of the chemical pain model, “Formalin test”. The formalin test is used to evaluate the mechanism by which an animal responds to moderate continuous pain generated in injured tissue. This test is characterized by two phases among which the early phase (immediately after injection) seems to be caused by C-fiber activation due to peripheral stimulus and the late phase (starting approximately 20min. after formalin injection) appears to depend on inflammatory reaction in the peripheral tissues.

The major triterpene saponin, glycyrrhizin, possesses expectorant, antiviral, anti-inflammatory and anti-allergic activity. The antinociceptive activity of G. glabra is due to presence of glycyrrhetinic acid and its derivatives and this is partially due to inhibition of PGE2 production similar to cortisol by inhibiting phospholipase A2 and acting to reduce pain and inflammation. By inhibition of phospholipase A2 conversion of phospholipids to arachidonic acid is inhibited which in turn inhibit synthesis of PGE2 which are stimulator of pain. A glycyrrhizinic acid derivative, glyderinine, showed stronger antipyretic, analgesic and anti-inflammatory activities than hydrocortisone and amidopyrine.

Aqueous and ethanolic extract of Glycyrrhiza glabra administered intraperitoneally exhibits significant antinociceptive activity when assessed in pain model (acetic acid induced abdominal constrictions) of nociception in mice, while moderate antinociceptive action was observed in thermal model (tail flick) and chemical test (formalin test) at the dose of 200mg/kg i.p. While benzene, acetone and chloroform extracts of Glycyrrhiza glabra had shown insignificant nociception. Present data supports the analgesic potential of aque-
ous and ethanolic extract of *Glycyrrhiza glabra*. Further pharmacodynamic investigations are required to understand the precise mechanism of Antinociception exhibited by extracts of *Glycyrrhiza glabra*.

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**REFERENCES**


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