A COMPARATIVE STUDY ON CENTRAL ANALGESIC ACTIVITIES OF ETHANOL AND AQUEOUS EXTRACTS OF *GLYCYRRHIZA GLABRA* ROOT IN ALBINO RATS

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Summary

Objective: To study the central analgesic activity of ethanol and aqueous extracts of *Glycyrrhiza glabra* in albino rats.
Materials and methods: The central analgesic effect of the ethanol and aqueous extracts of *Glycyrrhiza glabra* root at doses of 100, 200 and 400 mg/kg was evaluated against the standard drug pentazocine 10 mg/kg body weight. Albino rats of either sex of six numbers in each group was undertaken for study and evaluated by hot plate and tail immersion method.
Results: The results of the comparative study shows that ethanol extract of *Glucyrrhiza glabra* at 100, 200 and 400 mg/kg produced dose dependant analgesic effect in both the models of nociception. In hot plate method, the ethanol extract at 400mg/kg showed significant activity ($P<0.01$) after 60 minutes but the aqueous extract did not show any significant activity. In tail immersion method, both ethanol and aqueous extract at a dose of 200 and 400 mg/kg respectively showed significant ($P<0.05$ and 0.01) activity after 30 minute. The activity of ethanol extract was comparable with the standard drug pentazocine.
Discussion and conclusion: These findings demonstrate that *Glycyrrhiza glabra* showed significant analgesic activity against thermal stimuli in the tested animals. The activity probably may be mediated through central mechanism. So, it can be recommended for further studies.

Key words: Analgesic effect; hot plate test; tail immersion test; pentazocine; aqueous extract; ethanol extract; *Glycyrrhiza glabra* root.

Introduction

In the traditional system of medicine, the roots and rhizomes of *Glycyrrhiza glabra* L. (Family: Leguminosae) have been in clinical use for centuries. The most important bioactive components of *Glycyrrhiza glabra* root are triterpinoid saponin like glycyrrhizin, glycyrrhetinic acid (GA) and phenolics like liquiritin, liquiritigenin and glabridin (1). Most of the pharmacological activities shown by glycyrrhiza extract are attributed to its aglycone saponins, 18β-glycyrrhetinic acid. Glycyrrhetinic acid has a semisteroidal structure and its synthetic derivative, carbenoxolone has been used for peptic ulcer treatment (2). Glycyrrhetinic acid also shows various CNS activities like anticonvulsant activity in rat and mice (3), memory improvement activity in mice (4), antidepressant effect of glabridin, an isoflavans of *Glycyrrhiza glabra* (5), and cerebroprotective effect (6).

The saponins are naturally occurring surface-active glycosides with a distinct foaming characteristics mainly produced by plants (7). Phenolics are defined as a class of polyphenols which are important secondary metabolite of plant (8).On the basis C-skeleton, polyphenols are classified as flavonoids and pnenolic acid (8). It has been reviewed and reported that most of the plants containing flavonoids and saponins possess anti-inflammatory and analgesic activity (9,10). An extensive search of the literature reveals no reports on analgesic activity of the plant. Thus the present investigation was planned to perform a comparative study on analgesic activity of aqueous and ethanol extract of *Glycyrrhiza glabra* root.

Materials and Methods

Selection and collection of plant material

The *Glycyrrhiza glabra* root was selected for the present study was based on the presence of active constituents like saponin glycolsides and flavonoids. The powdered root of *Glycyrrhiza glabra* was procured from the Yucca enterprises, Mumbai in the year 2007.

Preparation of crude ethanol and aqueous extract of *Glycyrrhiza glabra*

The crude ethanol and aqueous extract of *G*lycyrrhiza *glabra* was prepared by macerating dried powdered root with respective solvent for 24 h. The macerated powdered roots were then extracted in a soxhlet extractor for 36 h, 1-2 cycles per hour. The crude extracts were evaporated to dryness using a rotary evaporator and a yield of 77 g (15.4%w/w) for aqueous and 80 g (16%w/w) for ethanol extract was obtained. The resultant extract was then stored in a refrigerator for further investigation (11). Fresh solutions of each extracts of *Glycyrrhiza glabra* were prepared in each day of the experiment by reconstituting the weighed quantity of the crude extract in a minimum amount of distilled water for oral administration.

Experimental animal

Albino rats of 150 to 200 g were procured from Gosh enterprise, Kolkata and were maintained in the college animal house with temperature (25 ± 1°C) on a 12 h light/dark cycle, with free access to standard pellet diet and water for seven days for acclimatization. Experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC), Roland institute of pharmaceutical sciences, Berhampur, Odisha

(India) (Regd no.926/ab/06/CPCSEA dated 22.02.2006). Two sets of animals were used in the study for the evaluation of analgesic activity in hot plate test and tail immersion test. Each set of animal was further divided into groups according to the treatment, consisting of six animals each.

Determination of maximum tolerated dose

Maximum tolerated dose was determined as per OECD-423 guidelines (acute toxic class method) (12). Female albino rats (n = 3 per step) selected by random sampling technique. The rats were kept fasting for overnight provided with water, then the extracts (ethanol and aqueous) were administered orally at the dose level of 5 mg/kg body weight (volume should not exceed 2 ml/100 g body weight) by intra gastric tube, food was withheld for further 3-4 h. Immediately after dosing the rats were observed once in every 30 minutes during the first 24 h and daily thereafter, for a total of 14 days for any mortality. If mortality was observed for 2-3 animals, then the dose administered was assigned as toxic dose. If mortality was not observed the procedure was repeated for further higher dose such as 50, 300, 1000 and 2000mg/kg body weight.

Analgesic activity

Evaluation of central analgesic properties of aqueous and ethanol extract of *Glycyrrhiza glabra* root was carried out by using two thermal model of noxious stimuli i.e. hot plate reaction time method and tail immersion test. The rats were randomly divided into eight groups of six animal each (n=6). Group-I served as normal control and received distilled water (5 ml/kg, p.o.), group-II served as reference group and received pentazocine (10 mg/kg, s.c.), group III-VIII served as test group and received the extracts at the doses of 100, 200 and 400 mg/kg, orally.

Hot plate test

The test was performed using the procedure as described by hot plate methods of Eddy et al., 1950 (13). The basal reaction time of all animals towards thermal heat was recorded. The animals which showed fore paw licking or jumping response within 6-8 sec were selected for the study. Thirty minutes after the administration of test and reference compounds, the animals in all the five groups were individually exposed to the hot plate (Inco) maintained at 55 ± 1°C. A cut off period of 60 sec was observed to avoid damage to the rat paw (14). The reaction time, which is the time taken for the animal to start licking the paw or jump from the hot plate or lifting one of its hind paw was taken as the hot plate latency (15). The reaction time was observed after each 30 min interval up to 90 min. The results were presented in table no.1.

Tail immersion test

The test was performed using the procedure as described by tail immersion methods of Ghosh, 2005 (16). Prior to analgesic experiments, all the animals were screened for the sensitivity test by immersing gently the tail of the rat up to 5cm in the hot water maintained at 55 ± 1°C. The animals immersing the tail from hot water with in 5 sec were selected for the study. The basal reaction time of all animals towards thermal heat was recorded. Thirty minutes after the administration of test and reference compounds, the animals in all the five groups were individually exposed to hot water and the reaction time was measured after each 30 min interval up to 90 min. The reaction time (in seconds) was taken as the time when the animals withdrew their tails completely

from the hot water bath with the cut off time being 60 sec (17). The mean reaction time in each group was determined. The results were presented in table no.2.

Statistical Analysis

All the results are expressed as mean ± standard error of mean (SEM). Data were analyzed by two-way analysis of variance (ANOVA) followed by Bonfori multiple comparison test as post hoc test using the software prism, version 5.00 for windows. The level of statistical significance considered was $p< 0.05$, when compared with the control group.

Results

Maximum Tolerated Dose

The result of toxicity study showed that both the extracts of *Glycyrrhiza glabra* exibited mortality at a dose of 2000mg/kg body weight. This indicates that both aqueous and ethanol extract is safe up to a single dose of 1000 mg/kg body weight. So, the dose selected for analgesic activity was 100, 200 and 400 mg/kg body weight.

Analgesic activity

This study establishes the central analgesic activity of *Glycyrrhiza glabra* root extract. In hot plate test there was no significant difference in basal reaction time observed between all the treatment group signifies that all the rats have equal sensitivity level to heat. The standard drug pentazocin (10 mg/kg, i.p) shows significant ($p<0.05$ and <0.001) increase in reaction time after 60 and 90 min respectively, compared to distilled water treated rats. The aqueous extract of *Glycyrrhiza glabra* was not showing any significant activity, where as ethanol extract at a dose level 100 and 200 mg/kg, body weight showed significant ($p<0.001$) increase in reaction time after 90 min but at 400 mg/kg body weight, it showed significant ($p<0.001$) increase in reaction time after 60 and 90 min, compared to distilled water treatment rats. The analgesic activity of the ethanol extract was comparable with the standard drug pentazocin, the data are shown in table no.1.

In tail immersion test there was no significant difference in basal reaction time observed between all the treatment group signifies that all the rats have equal sensitivity level to heat. The standard drug pentazocin (10 mg/kg, i.p) shows significant ($p <0.001$) increase in reaction after 30 min, compared to distilled water treated rats. The aqueous extract of *Glycyrrhiza glabra* only at higher doses i.e. 400mg/kg showed a significant ($p<0.001$) analgesic activity after 30 as well as 90 min, when compared with distilled water treated rats. The ethanol extract showed dose dependant increase in reaction time at all dose level in all time intervals. The ethanol extract at doses 200 and 400 mg/kg body weight showed significant ($p<0.01$ and $p<0.001$ respectively) activity after 30 min, when compared with distilled water treated group. The analgesic activity of the aqueous and ethanol extract was comparable with the standard drug pentazocin, the data are shown in table no.2.

The analgesic studies revealed that the ethanol extract of *Glycyrrhiza glabra* roots exhibited potent analgesic (central analgesic activity) effect against thermal noxious stimuli, because the aqueous extract showed activity only in higher dose. The ethanol extract also produced dose dependant analgesic effect.

Table 1

Analgesic activity of various extracts of *glycyrrhiza glabra* on hot plate test in rat

Treatment	Dose (Kg^{-1}) (p.o.) (# i.p.)	Reaction time in seconds			
		Basal	30 min	60 min	90 min
Distilled water	10 ml	6.24 ± 0.94	6.84 ± 1.26	6.44 ± 0.54	7.68 ± 1.24
Pentazocin	10 mg #.	6.55 ± 1.18	9.43 ± 1.21	15.24 ± 1.27*	23.99 ± 1.7***
AEGG	100 mg	4.82 ± 0.93	9.16 ± 1.33	10.56 ± 1.44	10.30 ± 2.1
AEGG	200 mg	5.97 ± 1.06	8.85 ± 1.04	10.95 ± 1.38	14.56 ± 1.78
AEGG	400 mg	6.15 ± 0.91	7.29 ± 0.97	8.51 ± 1.06	9.39 ± 0.92
EEGG	100 mg	7.43 ± 0.95	9.92 ± 1.41	10. 71 ± 2.23	17.41 ± 2.7***
EEGG	200 mg	4.94 ± 0.63	11. 37 ±1.08	14.55 ± 1.76*	19.63 ±1.44***
EEGG	400 mg	4.79 ± 0.53	11. 16 ±1.83	17.13 ±1.6***	24.06 ± 2.3***

Values are expressed as mean ± SEM (n=6); Statistical analysis of data was carried out by two way ANOVA followed by Bonfori multiple comparison test,* $p<0.05$, ** $p < 0.01$ and ***$p<0.001$ compared to distilled water treated group; AEGG = Aqueous extract of *Glycyrrhiza glabra*; EEGG = Ethanol extract of *Glycyrrhiza glabra*.

Table 2

Analgesic activity of various extracts of *glycyrrhiza glabra* on tail immersion test in rat

Treatm-ent	Dose (Kg^{-1}) (p.o.) (# i.p.)	Reaction time in seconds			
		Basal	30 min	60 min	90 min
Distilled Water	10 ml	1.34 ± 0.31	1.80 ± 0.21	1.34 ± 0.20	1.41 ± 0.18
Pentaz-ocin	10 mg #.	1.29 ± 0.23	4.57±0.4***	4.02 ±0.4 ***	3.16 ± 0.2*
AEGG	100 mg	1.64 ± 0.26	3.03 ± 0.15	2.29 ± 0.11	2.04 ± 0.17
AEGG	200 mg	1.90 ± 0.33	2.65 ± 0.32	1.99 ± 0.24	1.82 ± 0.09
AEGG	400 mg	2 ± 0.34	3.54 ± 0.64*	4.60 ± 0.3***	3.93 ± 0.2***
EEGG	100 mg	1.80 ± 0.23	2.85 ± 0.20	3.35 ± 0.24**	3.14 ± 0.22*
EEGG	200 mg	1.22 ± 0.2	4.2 ± 0.31**	3.36 ± 0.22**	3.01 ± 0.18*
EEGG	400 mg	1.52 ± 0.23	4.51 ±0.3***	3.94 ± 0.4***	3.73 ± 0.37*

Values are expressed as mean ± SEM (n=6); Statistical analysis of data was carried out by two way ANOVA followed by Bonfori multiple comparison test,* $p<0.05$, ** $p < 0.01$ and ***$p<0.001$ compared to distilled water treated group; AEGG = Aqueous extract of *Glycyrrhiza glabra*; EEGG = Ethanol extract of *Glycyrrhiza glabra*.

Discussion

Hot plate test and tail immersion tests are the most common tests of nociception that are based on phasic stimulus of high intensity (18). Analgesic effect mediated through central mechanism indicates the involvement of endogenous opioid peptides and biogenic amines like 5-HT (19,20).The ability of the extract to prolong the reaction latency to pain induced thermally in rats suggests central analgesic activity (21). The result from hot plate and tail immersion test gave evidence for the analgesic activity of the root extract. The activity may be attributed due to presence of flavonoids and other bioactive compounds like saponins. It was reported that inhibition of pain could arise not

only from the presence of opioids and/or opiodiomimetics but could also arise from the presence of phenolic constituents (22). The literature review reveals that *glycyrrhiza glabra* contains several phenolic compounds like flavonoids, isoflavonoids and triterpine saponins like glycyrrhizin, which may exhibit the analgesic activity. The increase of brain serotonin level may also be responsible for analgesic activity (23,24). It was also reported in our earlier study that *Glycyrrhiza glabra* produces antidepressant activity due to increase in serotonin level in rat brain (25). Therefore the analgesic activities produced by *Glycyrrhiza glabra* may be related to increase brain serotonin level.

Conclusion

From the above investigation, it is quite apparent that ethanol extracts of *Glycyrrhiza glabra* root possesses potent analgesic effect against different stimuli and may be mediated through increase in brain serotonin. This is evidenced by significant increase in the reaction time by the extract in different experimental model.

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