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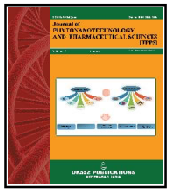

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Screening of *in vivo* anti-inflammatory and analgesic potential of methanolic extract of roots of *Glycyrrhiza glabra* L. in rats

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Abstract

The anti-inflammatory and analgesic activity of methanolic extract of roots of *Glycyrrhiza glabra* L. was evaluated by carrageenan induced paw oedema and acetic acid induced writhing reflex, respectively, in rats using different doses. Both the activities were compared with indomethacin (10 mg/kg). The methanolic extract of roots of *G. glabra* (250 and 500 mg/kg) produces the significant (p<0.05) reduction in the formation of oedema induced by carrageenan. In the acetic acid induced writhing model, the extract had a good analgesic effect characterized by a significantly reduction (p<0.05) in the number of writhes when compared to the control and standard. Hence, the methanolic extract of roots of *G. glabra* (250 and 500 mg/kg) shows positive anti-inflammatory and analgesic activity at dose dependent manner particularly by attenuating the peripheral pain mechanism.

1. Introduction

Traditional medicine, defined by the World Health Organization (WHO) as complementary, alternative, non-conventional, or indigenous medicine that was developed based on the theories, beliefs, and experiences inherent to various cultures, whether or not they can be interpreted, is used to maintain health as well as to prevent, attenuate, or treat physical and mental illnesses (WHO, 2000). On the 7.5 billion individuals that inhabit the earth, about 4.5 billion use traditional medicines as their main healthcare option. 0.93 billion Indians, who make up 17.84 % of the world's population, still rely on traditional remedies to maintain their essential health (Usha *et al*., 2018; Cordell, 2002). For their primary healthcare, both urban inhabitants in wealthy countries and rural communities in developing countries use these drugs, which are widely used today (Mehrotra, 2021; Das, 2021). The foundation of the ancient Indian medical system known as Ayurveda is made up of medicinal herbs (Bhatt, *et al*., 2021; Palai and Patra, 2021; Sumathi, *et al*., 2021).

Glycyrrhiza glabra L. (Leguminosae) also known as liquorice in English, mulethi in Hindi, and jethimadha in Telugu, is grown in Europe, Persia, Afghanistan, and India. *G. glabra* is a hard herb or understory shrub that grows to a height of up to 6 feet. It has multifoliate, imparipinnate leaves, axillary spikes of papilinaceous flowers that range in colour from lavender to violet, compressed pods, and reniform seeds. The substance is made up of the dried, peeled or unpeeled subterranean stems and roots and is referred to as liquorice in the trade. March brings flowers, and August brings fruit.

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The extract of roots of *G. glabra* was reported as antibacterial activity, antifungal activity (Gopal, 2009), antihepatotoxic activity (Alaaeldin, 2007), antihyperlipidemic activity (Asgary *et al.*, 2007), antiulcer activity, antioxidant activity (Mukherjee *et al*., 2010). Traditionally, the root has been used as a tonic, demulcent, expectorant, diuretic, and mild laxative. The root's demulcent properties make it useful for conditions like cough, hoarseness, sore throat, asthma, and dysuria as well as inflammatory affection or irritable conditions of the bronchial tubes, bowels, and genitourinary passages (Nadkarni, 1982). Triterpenoids saponin glycyrrhizin (2-9%), glycyrrhizinic acid (0.5-0.9%), and the aglycone of glycyrrhizin are the main bioactive components of root. It includes a significant amount of the flavonoid liquiritin, which gives the root its distinctive yellow colour (Anonymous, 2002). There are no reports on systematic and scientific studies of the analgesic and anti-inflammatory properties of *G. glabra* roots despite its historical use as such. In the current work, we examine the methanolic extracts of roots of *G. glabra* for anti-inflammatory and analgesic properties.

2. Materials and Methods

2.1 Plant material

The roots of *G. glabra* were purchased locally from Amreli, Gujarat, India and authenticated by taxonomist, Bioscience Department, Sardar Patel University. Vallabh Vidyanagar, Gujarat, India.

2.2 Preparation of extracts of roots of *G. glabra*

G. glabra roots were sun-dried and ground into a coarse powder. The 42 mesh sieve was then used to filter the material. The powder was extensively treated to continuous hot extraction in a Soxhlet device using a weighted quantity (200 gm) of the powder. To get an extract sample, the extract was pressure evaporated in a Rotary evaporator until all solvent had been eliminated. The methanol extract of roots of *G. glabra* had a yield of 15.2 % weight to weight.

2.3 Animals

The male albino wistar rats of body weight 250 to 300 g were selected for all the experiments. Animals were kept in our animal house at an ambient temperature of 25°C and 45-55% relative humidity with 12 h/12 h dark/light cycle. Animal, were fed pellet diet (Pranav Agro Industries, Vadodara, Gujarat) and water *ad libitum*. The experiments on animal were conducted in accordance with the international accepted principles for laboratory animal use and the experimental protocols duly approved by the Institutional Ethical Committee (Reg. No. 949/PO/c/2006/CPCSEA).

2.4 Anti-inflammatory activity

2.4.1 Carrageenan induced paw oedema

Rats were divided into six groups; six animals were selected in each group. Prior to the experimentation day, they were water-starved over night. The control group was given 2 ml of 0.9% saline orally, the standard group was given an oral dosage of indomethacin 10 mg/kg, and the test groups were given oral doses of 250 and 500 mg/kg, respectively, of a methanolic extract of roots of *G. glabra*. One of the hind paws received an injection of 0.1 ml of 1% carrageenan in normal saline 60 min after the medication was given. To enable consistent dipping at successive readings, a mark was made on the leg at the malleolus. At zero hour, the mercury displacement technique was used to measure the paw oedema volume with the use of a plethysmograph (immediately after injecting carrageenan). The same process was carried out again at 1, 2, 3, 4, and 5 h, and the difference between the readings at those times was used to determine the actual edoema volume (Jha *et al*., 2010; Baneerjee *et al*., 2000). The algorithm was then used to determine the percentage inhibition of oedema in the various treatment groups.

$$\text{Percentage inhibition} = (1 - V_t/V_c) \times 100$$

where

V_t is edema volume in the drug treated group.

V_c is edema volume in the control group.

2.5 Analgesic activity

2.5.1 Acetic acid induced writhing

Six groups of six rats each had six chosen rats were used in the study. The control group was given 2 ml of 0.9% saline orally, the standard group was given 10 mg/kg of indomethacin, and the test groups were given 250 and 500 mg/kg of the methanolic extract of roots of *G. glabra*, respectively. 10 ml/kg of acetic acid (0.6%) intraperitoneally injected to cause abdominal constriction. The animals were pretreated 30 min before the acetic acid injection with either the reference medication or the test drug at varied dosages; the same process was done for each animal individually. Twenty minutes after the acetic acid injection, the number of writhing episodes was counted. Rats pretreated with the test medication showed reduced abdominal constriction rates, which was a measure of the analgesic effect (Bhutia *et al*., 2010).

2.6 Statistical analysis

All data were represented as mean ± Standard error of mean (SEM) or as percentages. The statistical analysis involving two groups were done by means of student's *t* test, whereas analysis of variance (ANOVA) and Dunnett's multiple comparison tests were used to compare several groups with a control to estimate the significance of difference between individual groups; *p* values of 0.05, 0.01 or less were considered significant.

3. Results

3.1 Anti-inflammatory activity of methanolic extract of roots of *G. glabra*.

The methanolic extract of roots of *G. glabra* was evaluated for anti-inflammatory and analgesic activity in albino wistar rats, respectively. The methanolic extract of roots of *G. glabra* showed significant anti-inflammatory activities when administered orally. The low dose of roots of *G. glabra* (250 mg/kg) inhibits 34.32 % while high dose (500 mg/kg) inhibits 40.29 % of carrageenan induced paw oedema as compared to control group at 5th h as shown in Table 1.

Table 1: Anti-inflammatory effect of methanolic extract of roots of *G. glabra*

Groups	Paw oedema (Mean ± SEM)					Oedema inhibition (%)				
	1h	2 h	3 h	4 h	5 h	1 h	2 h	3 h	4 h	5 h
Control (2 ml of 0.9 % saline)	0.46 ± 0.04	0.66 ± 0.05	0.80 ± 0.04	0.73 ± 0.09	0.67 ± 0.03	-	-	-	-	-
Test 1 (250 mg/kg)	0.38 ± 0.03*	0.60 ± 0.04*	0.55 ± 0.05*	0.45 ± 0.03*	0.44 ± 0.04*	17.39	9.09	31.35	38.35	34.32
Test 2 (500 mg/kg)	0.32 ± 0.05*	0.47 ± 0.04*	0.47 ± 0.04*	0.37 ± 0.05*	0.40 ± 0.05*	30.43	28.78	41.25	49.31	40.29
Indomethacin (10 mg/kg)	0.18 ± 0.09*	0.19 ± 0.03*	0.18 ± 0.09*	0.15 ± 0.04*	0.16 ± 0.04*	60.86	71.21	77.50	79.45	76.11

SEM = standard error of mean *p<0.05 compared to control.

Table 2: Analgesic effect of methanolic extract of roots of *G. glabra*

Groups	Writhing (Mean ± SEM)	Writhing inhibition (%)
Control (2 ml of 0.9 % saline)	48 ± 0.4	-
Test 1 (250 mg/kg)	41 ± 0.2*	14.58
Test 2 (500 mg/kg)	37 ± 0.6*	22.91
Indomethacin (10 mg/kg)	25 ± 0.4*	47.91

SEM = standard error mean * p<0.05 as compared to control.

3.2 Analgesic activity of methanolic extract of roots of *G. glabra*

The methanolic extract of roots of *G. glabra* administered orally was shown analgesic activity against acetic acid induced analgesic response in rats. It significantly inhibited 14.58 % and 22.91 % writhing at low dose (250 mg/kg) and high dose (500 mg/kg), respectively, as described in Table 2.

4. Discussion

The paw edoema caused by carrageenan is regarded as a model for the exudative phase of inflammation, where the development of edoema is characterized as biphasic. The initial phase begins shortly after carrageenan injection because many cytokines, including histamine, serotonin, and kinins, are released. Due to the production of a chemical like prostaglandins, the second phase lasts longer (Vogel, 2002). According to the results of the current investigation, the methanolic extract of roots of *G. glabra* at doses of 250 mg/kg and 500 mg/kg significantly reduced inflammation and provided analgesia in a dose-dependent way. The preliminary phytochemical analysis of *G. glabra* found that it included tannins, flavonoids, and the saponin glycoside glycyrrhizin (Roshan *et al*., 2012). By blocking the cyclooxygenase that produces inflammatory prostaglandins, the flavonoids are known to have anti-inflammatory and analgesic properties (Serafini *et al*., 2010). Since many plants' anti-inflammatory and analgesic properties have been linked to their chemical components, such as flavonoids, it is hypothesized that the impact may be caused by these components, which would confirm the findings of the current study (Reddy *et al*., 2007; Zakaria *et al*., 2001). Therefore, the *G. glabra* root methanolic extract (250 and 500 mg/kg) exhibits positive anti-inflammatory and analgesic action in a dose-dependent manner, mainly through attenuating the peripheral pain mechanism.

5. Conclusion

The methanol extract of roots of *G. glabra* significantly and dose dependently suppressed the inflammation as well as has analgesic activity in rats. Further isolation, characterization and biological evaluation of active constituents are required to confirm this preliminary finding, which may provide the rationale for the ethnopharmacological uses of roots of *G. glabra*.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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