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Antiinflammatory and Antinociceptive Effects of *Galega purpurea* Root

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Abstract: The present study was designed to investigate anti-inflammatory and antinociceptive activities of *Galega purpurea* root. In this study both acute and chronic inflammation models were used to evaluate the anti-inflammatory activity of the extract and four different animal models were employed to investigate the antinociceptive activity of the extract. In acute model carrageenan, dextran, histamine and serotonin models were used to induce inflammation in rat hind paw and cotton pellet-induced granuloma method was used for chronic inflammation model. Acetic acid-induced writhing, method hot plate method, tail flick response and tail immersion methods were used to evaluate the antinociceptive effect of the extract. The methanol extract of *Galega purpurea* root exhibited significant, dose-dependent activity on the tested experimental animal models. Also the extract significantly reduced the acetic acid-induced abdominal contractions and the increased reaction time of mice in hot plate method, tail flick response and tail immersion method. This study has shown that the methanol extract from the roots of *Galega purpurea* does possess significant antiinflammatory and antinociceptive activity in laboratory animals at the doses tested and the results were comparable to those observed for the standard drugs indomethacin, acetyl salicylic acid and morphine.

Key words: *Galega purpurea*, antiinflammatory, antinociceptive

INTRODUCTION

Medicinal herbs have been used as a form of therapy for the relief of pain throughout history (Almeida *et al*., 2001). A case in point is *Galega purpurea* (Papilionaceae) a plant popularly known as kolinji in Tamil which thrives in Southern parts of India. It grows on hard stony ground too difficult to be rooted. The various parts of the plant are widely used in the folk medicine for the treatment of cough, asthma, bilious febrile attacks, arthritis and rheumatism. Decoction of the root useful in the management of enlargement and obstruction of the liver, spleen and kidney. Also the root is useful in the treatment of dyspepsia, chronic diarrhoea and ulcers (Nadkarni, 1976). In view of this and evidence from the existing information show that this plant may possess some important biological activities. There is no scientific information about the traditional use, phytochemical, pharmacological and toxicological activities of this plant. A part of a continuing research for a novel plant-derived antiinflammatory and antinociceptive agent, a methanol extract of roots of *Galega purpurea* was preliminary screened for antiinflammatory and antinociceptive activity and it showed the promising effects on the various animal models. The present study was performed in order to investigate its antiinflammatory and antinociceptive activities on both acute and chronic inflammation.

MATERIALS AND METHODS

Plant material: The roots of the plant *Galega purpurea* (Family: Papilionaceae) were collected from Erode district of Tamilnadu, India. The plant material was taxonomically identified by Botanical Survey of India, Kolkata. A voucher specimen (No. GMG 03/05) has been preserved in our laboratory for future reference. The collected plant material was dried under shade and then powdered with a mechanical grinder and stored in airtight container. The dried powder material of the root was defatted with petroleum ether and the marc thus obtained was then extracted with methanol in a soxhlet apparatus. The solvent was completely removed under reduced pressure and a semisolid mass was obtained (MEGP, yield 7.3%). The dried MEGP was suspended in normal saline and used for the present study.

Phytochemical screening: The extract was screened for the presence of various constituents employing standard screening test (Trease and Evans, 1983). Conventional protocol for detecting the presence of steroids, alkaloids, tannins, flavonoids, glycosides, etc., was used.

Animals: Studies were carried out in Pharmacology Laboratory, Division of Pharmacology and Pharmaceutical Chemistry, Department of Pharmaceutical Technology,

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Jadavpur University, Kolkata, India using male Wistar albino rats weighing 180-200 g and male Swiss albino mice weighing 20-22 g. They were obtained from the animal house of Jadavpur University, Kolkata. The mice were grouped and housed in poly acrylic cages (38×23×10 cm) with not more than six animals per cage and maintained under standard laboratory conditions (temperature 25±2°C) with dark/light cycle (14/10 h). They were allowed free access to standard dry pellet diet (Hindustan Lever, Kolkata, India) and water *ad libitum*. The animals were acclimatized to laboratory conditions for 10 days before commencement of the experiment. All procedures described were reviewed and approved by the University Animal Ethical Committee.

Chemicals: Carrageenan (SD Fine Chemicals Limited, Bombay), 5-Hydroxy tryptamine hydrochloride (serotonin), histamine (Sigma, USA) were used in this study and indomethacin (Recon, Bangalore), Aspirin (USV, Bombay) and morphine (MM Pharma, New Delhi) were used as the standard drugs.

Acute toxicity test: The animals were divided into six groups containing eight animals in each group. MEGP was suspended in normal saline and administered orally as a single dose to groups of mice at different concentrations (500, 750, 1000, 1250, 1500 and 2000 mg kg^{-1} b.w.). These animals were observed for a 72 h period. The number of deaths was expressed as a percentile and the LD_{50} was determined by probit a test using the death percentage versus the log dose (Thompson and Weil, 1952).

Antiinflammatory activity

Carrageenan-induced rat hind paw oedema model: The rats were divided into five groups (n = 6). Group 1 served as control and received normal saline (0.9% NaCl, 5 mL kg^{-1} b.w.) and the groups 2, 3 and 4 were treated orally with MEGP 100, 250 and 500 mg kg^{-1} b.w., respectively. Group 5 received the standard drug indomethacin (10 mg kg^{-1} b.w.). The administration of test drugs was 30 min prior to injection of 0.1 mL of 1% w/v freshly prepared suspension of carrageenan in normal saline in the right hind paw subplantar of each rat. The paw volume was measured initially and then at 1, 2, 3 and 4 h after the carrageenan injection by using plethysmometer (Winter and Porter, 1957). The antiinflammatory effect of MEGP was calculated by the following equation:

$$\text{Antiinflammatory activity (\%)} = \left(\frac{1\text{-}D}{C}\right) \times 100$$

Where, D represents the percentage difference in paw volume after the administration of drugs to the rats and C represents the percentage difference of volume in the control groups (Suleyman *et al*., 1991).

Dextran-induced rat hind paw oedema model: The animals were treated in a manner similar to that of carrageenan-induced paw oedema model; dextran (0.1, 1% w/v in normal saline) was used in place of carrageenan (Winter and Porter, 1957).

Histamine-induced rat hind paw oedema model: In this model hind paw oedema in the right foot of a rat was induced by subplantar injection of 0.1 mL of 1% w/v freshly prepared histamine in normal saline and the paw volume was measured as mentioned earlier (Suleyman *et al*., 1991).

Serotonin-induced rat hind paw oedema model: In another model oedema of the right hind paw of the rat was induced by subplantar injection of 0.1 mL 1% w/v freshly prepared serotonin in normal saline. Group division and the treatment of the animals were the same as the carrageenan-induced rat hind paw oedema model and the paw volume was measured as mentioned in Winter *et al*. (1962).

Cotton pellet-induced granuloma: The cotton pellets-induced granuloma in rats was studied according to the method D'Arcy *et al*. (1960). The animals were divided into five groups of six animals in each group. The rats were anaesthetized and sterile cotton pellets weighing 10±1 mg were implanted subcutaneously into both sides of the groin region of each rat. Group 1 served as control and received the vehicle (normal saline, 5 mL kg^{-1} b.w.). The extract MEGP at the concentration of 100, 250 and 500 mg kg^{-1} b.w. was administered orally to groups 2, 3 and 4, respectively for seven consecutive days from the day of cotton pellet implantation. Group 5 received the standard drug indomethacin (10 mg kg^{-1} b.w.) for the same period. On 8th day the animals were anaesthetized and the pellets together with granuloma tissues were carefully removed and made free from extraneous tissues. The wet pellets were weighed and then dried in an oven at 60°C for 24 h to constant weight, after that the dried pellets were weighed again. Increment in the dry weight of the pellets was taken as a measure of granuloma formation. The antiproliferative effect of MEGP was compared with control.

Antinociceptive activity: Evaluation of antinociceptive properties of the root extract of the plant was carried out by the chemical, mechanical and thermal noxious stimuli.

Acetic acid-induced writhing method: Mice were divided into five groups of six animals in each. Writhing test was used according to the method of Turner with slight modification (Turner, 1965). The MEGP at the different doses (100, 250 and 500 mg kg^{-1} b.w.) and standard drug acetyl salicylic acid (100 mg kg^{-1} b.w.) were administered orally, 1 h prior to the injection of acetic acid, vehicle control group received normal saline (0.9% NaCl, 5 mL kg^{-1} b.w.). Writhing was induced by administering 10 mL kg^{-1} b.w. of acetic acid solution (0.6%) intraperitoneally. The minutes after acetic acid injection, the mice were placed in a transparent box and the number of writhes was counted for a period of 10 min. Writhing movement was accepted as contraction of the abdominal muscles accompanied by stretching of hind limbs. There was a significant reduction in the number of writhes by drug treatments as compared to vehicle-treated animals. This was considered a positive analgesic response and the percentage inhibition of writhing was calculated and evaluated statistically.

Hot plate method: Five groups of six mice each were selected for the present study. Group 1 served as control and received the vehicle (normal saline, 5 mL kg^{-1} b.w.). The extract MEGP at the concentration of 100, 250 and 500 mg kg^{-1} b.w. was administered orally to groups 2, 3 and 4, respectively and group 5 received the standard drug morphine (5 mg kg^{-1} b.w., s.c). The mice were placed on an aluminium hot plate kept at a temperature of 55±0.5°C for a maximum time of 30 sec (Franzotti *et al*., 2000). Reaction time was recorded when the animals licked their fore-and hind paws and jumped; at before (0) and 15, 30, 45 and 60 min after administration of test drugs. The mice which reacted within 15 s and which did not show large variation when tested on four separated occasions were selected for the studies.

Tail flick response: Mice were randomly assigned to five groups of six animals each. A control group received normal saline (0.9% NaCl, 5 mL kg^{-1} b.w.). The methanol extract of *Galega purpurea* was given at the doses of 100, 250 and 500 mg kg^{-1} b.w. to the second, third and fourth group, respectively. Standard drug morphine (5 mg kg^{-1} b.w., s.c) was given to the fifth group which served as standard. Analgesic activity was measured 30 min after the administration of test and standard drugs (D'Amour and Smith, 1941). The tail of each mouse was placed on the nichrome wire of an analgesiometer (Techno, Lucknow, India), which was fixed at 5.5±0.5 amp. The time taken by the animal to withdraw (flick) its tail from the hot wire was taken as the reaction time. The reading was taken after 30 min of administration of the test drugs. The mice which reacted within 15 sec and which did not show large variation selected for studies.

Tail immersion method: Mice were divided into five groups of six animals each. Group 1 received normal saline (0.9% NaCl, 5 mL kg^{-1} b.w.) (control) and groups 2, 3 and 4 received 100, 250 and 500 mg kg^{-1} of MEGP, respectively. Group 5 received the standard drug morphine (5 mg kg^{-1} b.w., s.c). They were held in position in a suitable restrainer with tail extending out. The tail (up to 5 cm) was then dipped into a pot of water maintained at 55±0.5°C. The time in seconds to withdraw the tail out of water was taken as the reaction time. The reading was taken after 30 h of administration of the test drugs (Palanichamy and Nagarajan, 1990). The mice which reacted within 15 sec and which did not show large variation selected for the studies.

Statistical analysis: Values are mean±SEM. Statistical significance was determined by ANOVA, followed by student t-test. Values with $p<0.001$ were considered as statistically significant.

RESULTS

Phytochemical screening: Preliminary phytochemical screening of the methanol extract of *Galega purpurea* revealed the presence of steroids, alkaloids, flavonoids, tannins, glycosides. Further separation of the specific phytochemical is in process.

Acute toxicity study: In the acute toxicity assay no deaths were observed during the 72 h period at the doses tested. At these doses, the animals showed no stereotypical symptoms associated with toxicity, such as convulsion, ataxy, diarrhoea or increased diuresis. The median lethal dose (LD_{50}) was determined to be higher than the highest dose tested i.e., 2.0 g kg^{-1} b.w.

Antiinflammatory activity

Carrageenan-induced rat hind paw oedema model: In the carrageenan-induced rat paw oedema model of antiinflammatory activity, the methanolic extract of roots of *Galega purpurea* showed a significant inhibitory effect on the oedema formation from the first hour to fourth hour. The highest inhibitory effect was found during the third hour where the inhibition was found to be 20.34, 32.20 and 37.29% ($p<0.001$) at the doses of 100, 250 and 500 mg kg^{-1} b.w. of MEGP, respectively. These findings were comparable to the standard drug indomethacin (10 mg kg^{-1} b.w.) where the inhibition was 40.68% (Table 1).

Table 1: Effect of *Galega purpurea* root extract on Carrageenan-induced rat hind paw oedema

		Paw volume differences (mL)			
Treatments	Dose (mg kg^{-1})	1 h	2 h	3 h	4 h
Control (0.9% NaCl)	5 mL	0.58±1.1	0.61±1.3	0.59±1.2	0.58±1.1
MEGP	100	0.54±0.10*	0.51±0.09	0.47±1.1**	0.42±1.2*
		(6.90)	(16.39)	(20.34)	(27.59)
MEGP	250	0.50±0.10*	0.45±0.13**	0.40±1.2*	0.38±0.9
		(13.79)	(26.23)	(32.20)	(34.48)
MEGP	500	0.47±0.12*	0.44±0.08	0.37±0.9*	0.36±0.2*
		(18.96)	(27.87)	(37.29)	(37.93)
Indomethacin	10	0.46±3.4*	0.42±1.3*	0.35±1.5*	0.37±1.6
		(20.69)	(31.15)	(40.68)	(36.21)

Values are mean±SEM (n = 6) for control and mean±SEM (% of inhibition) (n = 6) for each treatment, *: p<0.001; **: p<0.01 experimental groups compared with control group

Table 2: Effect of *Galega purpurea* root extract on Dextran-induced rat hind paw oedema

		Paw volume differences (mL)			
Treatments	Dose (mg kg^{-1})	1 h	2 h	3 h	4 h
Control (0.9% NaCl)	5 mL	0.48±0.01	0.47±0.02	0.51±0.01	0.48±0.01
MEGP	100	0.43±0.04*	0.37±0.03	0.32±0.03**	0.30±0.04
		(10.42)	(21.28)	(37.25)	(37.50)
MEGP	250	0.40±0.03*	0.31±0.02*	0.29±0.02	0.27±0.02*
		(16.67)	(34.04)	(43.14)	(43.75)
MEGP	500	0.36±0.04*	0.28±0.03	0.26±0.01*	0.25±0.04**
		(25.00)	(40.43)	(49.02)	(47.92)
Indomethacin	10	0.33±0.01*	0.26±0.01*	0.25±0.01*	0.23±0.03
		(31.25)	(44.68)	(50.98)	(52.08)

Values are mean±SEM (n = 6) for control and mean±SEM (% of inhibition) (n = 6) for each treatment, *: p<0.001; **: p<0.01 experimental groups compared with control group

Table 3: Effect of *Galega purpurea* root extract on Histamine-induced rat hind paw oedema

		Paw volume differences (mL)			
Treatments	Dose (mg kg^{-1})	1 h	2 h	3 h	4 h
Control (0.9% NaCl)	5 mL	0.53±1.3	0.55±1.0	0.58±1.5	0.54±1.0
MEGP	100	0.48±1.1*	0.47±0.9	0.41±1.3**	0.37±1.2
		(9.40)	(14.55)	(29.31)	(31.48)
MEGP	250	0.46±0.9*	0.44±1.2**	0.38±1.1*	0.34±0.78*
		(13.21)	(.0020)	(34.48)	(37.04)
MEGP	500	0.43±1.2*	0.39±0.08	0.32±0.9*	0.30±0.82*
		(18.87)	(29.09)	(44.83)	(44.44)
Indomethacin	10	0.41±1.1*	0.37±1.1*	0.31±1.3*	0.29±1.4
		(22.64)	(32.73)	(46.55)	(46.29)

Values are mean±SEM (n = 6) for control and mean±SEM (% of inhibition) (n = 6) for each treatment, *: p<0.001; **: p<0.01 Experimental groups compared with control group

Dextran-induced rat hind paw oedema model: Both of MEGP (100, 250 and 500 mg kg^{-1} b.w.) and indomethacin (10 mg kg^{-1} b.w.) significantly decreased the dextran-induced paw oedema (p<0.001). MEGP at the doses of 100, 250 and 500 mg kg^{-1} showed an inhibition of 37.25, 43.14 and 49.02%, respectively and the results were comparable to that of the standard drug indomethacin (50.98%) (Table 2).

Histamine-induced rat hind paw oedema model: The results shows that the methanol extract at the doses of 100, 250 and 500 mg kg^{-1} b.w. significantly (p<0.001) reduced the oedema formation of rat paw at 1, 2 and 3 h after histamine injection. The effects were dose-dependent at the doses tested (100, 250 and 500 mg kg^{-1} b.w.). Peak inhibitory effects 34.48 and 44.83% were observed for 250 and 500 mg kg^{-1} b.w., respectively (Table 3).

Serotonin-induced rat hind paw oedema model: There was a dose-dependent significant (p<0.01) reduction in serotonin-induced rat paw oedema at 100, 250 and 500 mg kg^{-1} b.w. of MEGP and at 10 mg kg^{-1} indomethacin over a period of 4 h as shown in Table 4. MEGP at the dose of 500 mg kg^{-1} b.w. exhibited maximum activity of 40.00% while the standard drug indomethacin (10 mg kg^{-1} b.w.) shows 42.50% inhibition.

Cotton pellet-induced granuloma: It was seen that MEGP was responsible fore anti-inflammatory effect which would

Table 4: Effect of *Galega purpurea* root extract on Serotonin-induced rat hind paw oedema

		Paw volume differences (mL)			
Treatments	Dose (mg kg^{-1})	1 h	2 h	3 h	4 h
Control (0.9% NaCl)	5 mL	0.39±1.3	0.41±1.5	0.40±1.4	0.42±1.2
MEGP	100	0.36±1.1*	0.34±1.4**	0.30±1.1**	0.32±1.2**
		(7.69)	(17.07)	(25.00)	(23.81)
MEGP	250	0.35±0.9*	0.32±1.2**	0.29±1.6**	0.30±1.1**
		(10.26)	(21.95)	(27.50)	(28.57)
MEGP	500	0.33±1.2*	0.30±0.88**	0.24±1.4**	0.25±0.8*
		(15.38)	(26.83)	(40.00)	(40.47)
Indomethacin	10	0.31±1.3*	0.28±1.3*	0.23±1.1*	0.27±1.2*
		(20.51)	(31.71)	(42.50)	(35.71)

Values are mean±SEM (n = 6) for control and mean±SEM (% of inhibition) (n = 6) for each treatment, *: p<0.001; **: p<0.01 experimental groups compared with control group

Table 5: Effect of *Galega purpurea* root extract on cotton pellet-induced granuloma in rats

Treatments	Dose (mg kg^{-1})	Weight of cotton pellets (mg) (wet)	Percentage Inhibition	Weight of cotton pellets (mg) (dry)	Percentage inhibition
Control (0.9% NaCl)	5 mL	183.17±14.3	-	48.62±3.60	-
MEGP	100	136.14±0.14*	25.68	37.37±0.14*	23.14
MEGP	250	94.46±0.10*	48.43	30.22±0.13*	37.84
MEGP	500	83.47±0.02	54.43	24.78±0.05*	49.03
Indomethacin	10	78.25±6.30*	57.28	23.54±2.40*	51.57

Values are mean±SEM (n = 6) for control and mean±SEM (% of Inhibition) (n = 6) for each treatment, *: p<0.01 Experimental groups compared with control group

Table 6: Effect of *Galega purpurea* root extract on acetic acid-induced writhing test in mice

Treatments	Dose (mg kg^{-1})	No. of writhes	Percentage inhibition
Control (0.9% NaCl)	5 mL	45.14±0.02	-
MEGP	100	33.53±0.03**	25.72
MEGP	250	28.71±0.02	36.40
MEGP	500	16.34±0.04**	63.80
ASA	100	10.63±0.01***	76.45

Values are mean±SEM (n = 6) for control and mean±SEM (% of inhibition) (n = 6) for each treatment. **: p<0.01; ***: p<0.05 experimental groups compared with control group

be calculated depending on the wet and dry weight of cotton pellets. According to these results the antiproliferative effects of MEGP (500 mg kg^{-1} b.w.) and indomethacin (10 mg kg^{-1} b.w.) were calculated as 54.43 and 57.28%, respectively. After they were dried, the antiproliferative effects were calculated on the basis of dry weight pellets; the inhibition of inflammation by MEGP (500 mg kg^{-1} b.w.) and indomethacin (10 mg kg^{-1} b.w.) were established as 49.03 and 51.57% (p<0.01), respectively (Table 5).

Antinociceptive activity: The extract at the doses of 100, 250 and 500 mg kg^{-1} b.w. showed significant antinociceptive effect in all the four different models for nociception used to investigate the antinociceptive effects of the methanolic extract of *Galega purpurea* root and the results were dose-dependent.

Acetic acid-induced writhing method: The methanol extract from the roots of *Galega purpurea* strongly reduced the abdominal constrictions induced by the intraperitoneal administration of acetic acid solution. The effects produced by MEGP were dose-dependent and the values were found to be significant (p<0.01) at the doses tested, when compared to control. MEGP at the doses of 100, 250 and 500 mg kg^{-1} b.w. exhibited 25.72, 36.40 and 63.80 inhibition, respectively, where the inhibition for the standard drug acetyl salicylic acid was found to be 76.45% (Table 6).

Hot plate method: The MEGP produced significant (p<0.01) analgesic activity at all the doses tested. In this method MEGP considerably increased the animals reaction time to the heat stimulus. Values were found to be significant and dose-dependent. The highest reaction time of 11.94±0.6 was observed at a dose of 500 mg kg^{-1} b.w. compared with the control group value of 5.52±0.3. The results were comparable to that of the standard drug morphine (13.34±0.18) (Table 7).

Tail flick response: The results shows that the administration of MEGP at different doses (100, 250 and 500 mg kg^{-1} b.w.) and morphine (5 mg kg^{-1} b.w., s.c.) prolonged significantly the mouse tail reaction time when the animal's tail was subjected to heat generated by the tail flick apparatus. Meanwhile, the response to heat stimuli of the control group was not altered during the period of the experiment. Administration of MEGP at the doses of 100, 250 and 500 mg kg^{-1} b.w. exhibited 31.11, 55.56 and 75.56% inhibition, respectively, while the standard drug morphine produced 95.55% inhibition (Table 8).

Table 7: Effect of *Galega purpurea* root extract on hot plate test in mice

		Latency (s)				
Treatments	Dose (mg kg^{-1})	0 min	15 min	30 min	45 min	60 min
Control (0.9% NaCl)	5 mL	6.38±0.2	6.44±0.6	6.01±0.4	5.96±0.1	5.52±0.3
MEGP	100	6.40±0.3*	7.72±0.2*	7.94±0.1*	8.31±0.2*	8.49±0.5*
MEGP	250	6.39±0.17	7.90±0.3*	8.12±0.2*	8.50±0.3*	9.11±0.2*
MEGP	500	6.41±0.5*	9.24±0.4*	9.39±0.4*	10.61±0.2	11.94±0.6*
Morphine	5	6.40±0.2	11.30±0.4*	13.11±0.2	13.25±0.3	13.38±0.18

Values are mean±SEM (n = 6); *: p<0.01 experimental groups compared with control group

Table 8: Effect of *Galega purpurea* root extract on tail flick response in mice

Treatments	Dose (mg kg^{-1})	Reaction time (s)	Percentage inhibition
Control (0.9% NaCl)	5 mL	4.5±0.22	-
MEGP	100	5.9±0.25**	31.11
MEGP	250	7.0±0.19**	55.56
MEGP	500	7.9±0.30**	75.56
Morphine	5	8.8±0.31**	95.55

Values are mean±SEM (n = 6); **: p<0.01 experimental groups compared with control group

Table 9: Effect of *Galega purpurea* root extract on tail immersion test in mice

Treatments	Dose (mg kg^{-1})	No. of writhes	Percentage inhibition
Control (0.9% NaCl)	5 mL	4.8±0.61	-
MEGP	100	6.7±0.55*	39.58
MEGP	250	7.7±0.43**	60.42
MEGP	500	8.3±0.69**	72.92
Morphine	5	9.2±0.51*	91.66

Values are mean±SEM (n = 6); *: p<0.001; **: p<0.01 experimental groups compared with control group

Tail immersion method: In the tail immersion method the extract considerably increased the animals reaction time to the heat stimulus. Values were found to be significant (p<0.01) and the effects were dose-dependent at the doses tested. Pre-treatment with MEGP at the doses of 100, 250 and 500 mg kg^{-1} b.w. showed 39.58, 60.42 and 72.92% inhibition, respectively. And the percentage inhibition produced by the standard drug morphine was found to be 91.66% (Table 9).

DISCUSSION

In living animal tissues, inflammatory processes involve the release of several mediators, including prostaglandins, histamine, thermo-attractants, cytokines, proteinase and so on; as well as substances that regulate adhesion of molecules and the processes of cell migration, activation and degranulation (Hollander *et al*., 2003). Various forms and models of inflammatory reactions have been detected. For example, inflammatory responses from the airways of asthmatic patients, bone and joints inflammation, microbial infection, anaphylaxis and allergic conditions and so forth. Thus the adoption of different antiinflammatory and analgesic experimental models for the assessment of phytomedicines used in the traditional health care system for the management of pain, asthma, arthritis, rheumatism and so on, are considered desirable and justifiable (Omisore *et al*., 2004).

Carrageenan-induced rat paw oedema model is one of the most widely used primary test for the screening of new antiinflammatory agents (Winter *et al*., 1962). The oedema formation is a biphasic event. The initial phase, hyperemia observed during the first hour, is attributed to the release of histamine and serotonin (Vinegar *et al*., 1969) and the delayed oedema is due to the release of bradykinin and prostaglandins (Di Rosa *et al*., 1971; Flower *et al*., 1985). Dextran-induced paw oedema is known to be mediated both by histamine and serotonin (Ghosh *et al*., 1963). Dextran-induces fluid accumulation because of mast cell generation with little protein and few neutrophils. Carrageenan induces a protein rich exudates containing large number of neutrophils (Lo *et al*., 1982).

The present study established the antiinflammatory activity of methanol extract of *Galega purpurea* The extract produced marked inhibition of carrageenan-induced rat paw inflammation, a test which has a significant predictive value for antiinflammatory agents acting by inhibiting the mediators of acute inflammation (Olajide *et al*., 1999). Also carrageenan-induced paw inflammation is a test largely used to study both steroidal and non-steroidal antiinflammatory drugs. Carrageenan induces an inflammatory reaction in two different phases. The initial phase which occurs between 0 and 2 h after injection of carrageenan, has been attributed to the release of histamine, serotonin and bradykinin on vascular permeability (Vinegar *et al*., 1987). The inflammation volume reaches its maximum approximately 3 h post-treatment after which it begins to decline (Garcia *et al*., 2004). The late phase which is also a complement-dependent reaction has been shown to be due to over production of prostaglandin in tissues (Di Rosa, 1974). The results obtained from the present study suggest that the inhibitory activity of the extracts observed in the first phase of carrageenan-induced inflammation may be due to inhibition of early mediators such as histamine and serotonin. The action on the second phase due to the inhibition of bradykinin and prostaglandins. Thus the extract inhibited the inflammation from the first hour acting on both the early as well the late phases. The extract also effectively inhibited the inflammation

produced by dextran, histamine and serotonin which suggest that the antiinflammatory activity of methanol extract of *Galega purpurea* is possibly mediated by inhibiting the action of these mediators.

Therefore, it is suggested that the mechanism of action of MEGP may be related to prostaglandin synthesis inhibition as described for the antiinflammatory mechanism of indomethacin in the inhibition of the inflammatory process induced by carrageenan (Di Rosa *et al*., 1971).

Likewise, the granulomatous tissue formation is related to the chronic inflammatory process, which is characterized by several phases (Swingle *et al*., 2000). Thus the results of the cotton pellet implantation model for antiinflammatory activity further support the antiinflammatory activity of the crude methanol extract of *Galega purpurea* root.

Inhibition of prostaglandin synthesis could give rise to analgesic activity. So, the extract was further investigated for its possible antinociceptive activity. Four different animal models were employed to investigate the potential antinociceptive activity of methanol extract of *Galega purpurea* in this study. The methods for investigating antinociception were selected such that both centrally and peripherally mediated effects were investigated. Acetic acid-induced abdominal constriction method is widely used for the evaluation of peripheral antinociceptive activity (Gene *et al*., 1998). Also called the abdominal constriction response, it is very sensitive and able to detect antinociceptive effects of compounds and dose levels that may appear inactive in other models like the tail flick test (Collier *et al*., 1968; Bentley *et al*., 1981). Acetic acid, which is used to induce writhing, causes algesia by liberation of endogenous substances which in turn excite the pain nerve endings (Taesotikul *et al*., 2003). Local peritoneal receptors are postulated to be partly involved in the abdominal constriction response (Bentley *et al*., 1983). This method has been associated with prostanoids in general, e.g., increased levels of PGE2 and PGF2α in peritoneal fluids (Derardt *et al*., 1980) as well as lipoxygenase products by some researchers (Levini *et al*., 1984; Dhara *et al*., 2000). According to the percentage of inhibition on the number of writhes obtained with different doses of MEGP, we found that the intensity of the analgesic effect was similar to that of standard drug acetyl salicylic acid. Aspirin and related drugs can inhibit cyclo-oxygenase in peritoneal tissues, thus interfering with mechanism transduction in primary afferent nociceptors (Fields, 1987). Therefore, the results of the acetic acid-induced writhing strongly suggest that the mechanism of action of this extract may be linked partly to the blockade or release of endogenous substances like lipoxygenase and/ or cyclo-oxygenase.

Hot plate test was also assayed to characterize the analgesic activity of the extract. Morphine, used as a reference drug also produced a significant antinociceptive effect during all the observation times when compared with control values. The hot plate method is considered to be selective for opioid like compounds in several animal species, but other centrally acting drugs including sedatives and muscle relaxants have also shown activity in this test (Hiruma-Lima *et al*., 2000). The validity of this test has been shown even in the presence of substantial impairment of motor performance (Plummer *et al*., 1991). Thus the results obtained from the present study indicates that the methanol extract of *Galega purpurea* root relieved the pain through both central and peripheral mechanisms.

The extract also had a significant and dose-dependent effect on the various acute pain models namely tail flick and tail immersion tests. Centrally acting analgesic drugs elevate pain threshold of animals towards heat and pressure. The effect of the extract on these pain models indicates that it might be centrally acting.

Several flavonoids isolated from medicinal plants have been discovered to possess significant antiinflammatory and antinociceptive effects (Duke, 1992). It is therefore possible that both the antiinflammatory and antinociceptive effects observed with this extract may be attributable to its flavonoid components, shown to be present during phytochemical analysis. The oral LD_{50} obtained with this study also suggest it may have a reasonable safety margin with regards to acute toxicity further justifying its wide application in various communities and lack of any reported side effects with the traditional use of this plant.

CONCLUSIONS

In conclusion, this study has shown that the methanol extract from the roots of *Galega purpurea* does possess significant antiinflammatory and antinociceptive activity in laboratory animals at the doses tested and the results were comparable to those observed for the standard drugs indomethacin, acetyl salicylic acid and morphine. It is also suggested that the mechanism of antiinflammatory action of MEGP might be associated with he inhibition of prostaglandin synthesis as observed for most non-steroidal drugs. Also the present study indicates that the extract possess analgesic properties which are mediated via peripheral and central inhibitory mechanisms.

Thus the results obtained from the present study support the traditional use of this plant in some painful

and inflammatory conditions. It is important to point out that work is in progress to isolate and to characterize the active compounds present in the methanol extract of *Galega purpurea* root.

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