ORIGINAL ARTICLE

Analgesic and anti-inflammatory activities of the hydroalcoholic extract from *Gloriosa superba* Linn

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Gloriosa supberba (family: Liliaceae) is widely used as a medicinal plant, and the alkaloids from the plant (Colchicines and Gloriosine) are used in the treatment of gout and rheumatism. We evaluated the analgesic and anti-inflammatory activities of hydroalcoholic extract (50% v/v) of dried aerial parts of *G. superba*. The analgesic activity of the extract was evaluated by using Eddy's hot plate method and acetic acid-induced writhing method. The anti-inflammatory activity was evaluated by using the cotton wool granuloma model and the carrageenan-induced paw edema model. The percentage inhibitions of writhes or percentage protection were found to be 64.09%, 78.56% and 81.45% for extract at a dose 100, 200 and 400 mg/kg body weight, respectively, in the acetic acid-induced writhing method (P <0.01) when compared with control. The percentage increase in reaction time at 90 minutes were 21.02%, 79.96% and 158.05% for extract at a dose of 100, 200 and 400 mg/kg body weight, respectively, in Eddy's hot plate method (P <0.01) when compared with control. The percentage inhibition of paw edema was increased with time and gave maximum effect at 2 hours, then declined in case of standard extract 400 mg/kg body weight. Only the 200 and 400 mg/kg body weight extracts exhibited significant result (P <0.05) when compared with control. The rats exhibited 9.59%, 28.72% and 45.8% inhibition of granuloma mass formation after the 7 days treatment with 100, 200 and 400 mg/kg body weight of extract when compared with control (P <0.05) in cotton pellet granuloma.

Key words: *Gloriosa supberba*, analgesic activity, anti-inflammatory activity

INTRODUCTION

During thousands of years of early human existence many natural materials were identified for combating human ailments. The earliest mention of the medicinal use of plants has been found in Rigveda.

Gloriosa superba is highly valued in both traditional and modern therapies. Its seeds and tubers (active content Colchicine) are used mainly for treating gout and rheumatism. *G. superba* is a good abortifacient[1] causing expulsion of fetus from the womb. Roots are purgative, cholagogue, anthelmintic, bitter, acrid, astringent and germicidal. It cures leprosy, swelling, piles, chronic ulcers and colic pain in bladder. Paste is an antidote in snakebite. The powder of root is given for treatment of rheumatic fever. Various plant parts are used in spleen complaints, sores, tumours and syphilis. The extract of plant is CNS depressant.[2]

G. superba (family: Liliaceae) is widely used as a medicinal plant, and the alkaloids from the plant (Colchicines and Gloriosine) are used in the treatment of gout and rheumatism.[3] Hence, the objective of present study was to evaluate the analgesic and anti-inflammatory activities of *G. superba* to scientifically justify the traditional claims.

MATERIALS AND METHODS

Preparation of Hydroalcoholic Extract

G. superba was collected from Thrissur, Kerala, India, during October–November 2005 and was identified and authentified by Dr C D Vargheese M.Sc, Ph.D; Department of Botany, St: Thomas College; Thrissur. A voucher specimen (No: 4247) is deposited in the Department of Pharmacognosy, NGSM Institute of Pharmaceutical Sciences, Nanthoor, Mangalore – 575005, India. The dried parts were powdered (2 kg) and soaked in ethanol (50%) and kept aside for 7 days. After 7 days, the solvent from the total extract was distilled off and the concentrate was evaporated on a water bath to a syrupy consistency and then evaporated to dryness (150 g).

Selection of Animals

Wistar albino rats of either sex weighing between 150 and 200 g and albino mice of either sex weighing between 20 and 30 g were obtained from KSHEMA, Deralakatte Mangalore. These animals were used for the acute toxicity, anti-inflammatory and analgesic activities. The study was

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approved by Institutional Ethics Committee for animal experimentation KSHEMA/IAEC, Mangalore, (approval no: 038/2006).

The animals were stabilized for 1 week; they were maintained under standard condition at room temperature; 60 ± 5% relative humidity and 12-hour light dark cycle. They had been given standard pellet diet supplied by Hindustan Lever Co., Bombay, and water ad libitum throughout the course of the study. The animals were handled gently to avoid giving them too much stress, which could result in an increased adrenal out put.

Acute Toxicity Studies

The preliminary pharmacological studies were conducted to assess the acute pharmacological effects and LD_{50} of the crude extract. The acute toxicity study was carried out in adult female albino rats by the 'up and down' method.[4] The animals were fasted overnight and next day extracts of the herb *G. superba* dissolved in normal saline was administered orally at different dose level. Then the animals were observed continuously for 3 hours for general behavioural, neurological and autonomic profiles and then every 30 minutes for next 3 hour and finally death after 24 hours.[5]

Selection of Doses

For the assessment of analgesic and anti-inflammatory activities, three dose levels were chosen in such a way that, middle dose was one-tenth of the maximum dose during acute toxicity studies, and a low dose, which was 50% of the one-tenth dose, and a high dose, which was twice that of one-tenth dose (200 mg/kg, 400 mg/kg, 100 mg/kg).

Hydroalcoholic extract at a dose of 100, 200, 400-mg/kg-body weight were given to rats and mice in normal saline and administered orally.

Screening Methods for Analgesic Activity

Eddy's hot plate method

The mice of either sex were weighed and divided into five different groups (n = 6 in each group). Group I served as control. Group II (Pentazocine 46.8 mg/kg body weight) served as standard and groups III, IV and V were treated with extracts at a dose of 100, 200 and 400 mg/kg body weight, respectively. The reaction time of animals was noted down on hot plate at 30, 60, 90, 120 and 150 minutes after the treatment. The basal reaction was time taken by observing hind paw licking or jump response (whichever appear first) in animals while placed on hot plate, which was maintained at constant temperature 55°C. A cut off period of 10 seconds was observed to avoid damage to the paws. The percentage increase or decrease in reaction time (as index of analgesia) at each time interval was calculated.[6]

$$\text{Percentage increase in reaction time} = \left[\frac{R_t}{R_c} - 1\right] \times 100$$

Where R_t is reaction time in treated group and R_c is reaction time in control group

Acetic acid induced writhing method

Male albino mice weighing between 20-25 g were selected for the study. The animals were divided into 5 groups (n=6 in each group). All animals received 0.1 ml acetic acid 0.6 % v/v. i.p. and first group served as control. Group II served as positive control and received Diclofenac. The groups III, IV, V received 100, 200 and 400 mg/kg body weight of hydro alcoholic extract of G.superba respectively 30 minutes prior to the administration of acetic acid i.p. The writhing effect was indicated by the stretching of abdomen with simultaneous stretching of at least one hind limb. This was observed for 30 minutes and change in number of writhings in test group compared with standard treated and control treated groups.[6] The percentage inhibition was calculated by using the formula,

$$\text{Percentage inhibition} = 1 - (N_T/N_C) \times 100$$

Where N_T is average number of writhings in treated group and N_C is average number of writhings in control group

Screening Methods for Anti-Inflammatory Activity

Carrrageenan induced rat paw edema (acute inflammatory model)

The method of Winter *et al*. was used to study anti-inflammatory activity using plethysmograph to measure the paw volume. Male albino rats (weighing between 150 – 200 g) were divided into 5 groups (n=6 animals in each group). Group I served as control (vehicle treated), group II served as positive control (Diclofenac treated), groups III, IV, V received 100, 200 and 400 mg/kg body weight of hydro alcoholic extract of *G.superba*. A mark was made on both the hind paws just below the tibio-torsal junction.[7] 30 minutes after treatment, an inflammatory edema was induced in the left hind paw by injection of 0.1 ml of 1% of carrageenan solution in the plantar tissue of the paw of all the animals. The right paw served as a reference. The initial paw volume was measured plethysmographically within 30 seconds after injection. The relative increase in paw volume was measured in all groups at 30 minutes, 1, 2 and 3 hrs after carrageenan injection. The percentage increase in the paw volume over the initial reading was calculated. This increase in the paw volume in-group II, III, IV and V were compared with group I. The percentage inhibition of edema volume was calculated by using the formula,

$$\text{Percentage inhibition} = \left[1 - \frac{V_t}{V_c}\right] \times 100$$

Where V_t is increase in paw volume in treated groups and V_c is increase in paw volume in control groups

Cotton pellet granuloma method (Sub-acute inflammatory model)

The method of Goldstein *et al* was used with few modifications. Sterilized cotton pellet of 20 mg were implanted beneath the abdominal skin in axilla or groin region of the rat through a single incision along the midline under anesthesia using pentobarbitone (40 mg/kg i.p.). The male albino rats (150-200 g) were selected and divided into 5 groups (n=6 animals in each group). Group I served as control (vehicle treated), group II served as positive control (Diclofenac treated), groups III, IV, V received hydro alcoholic extract of *G.superba* at a dose of 100, 200 and 400 mg/kg body weight. The drugs were applied for 7 days. On the eighth day the animals were anesthetized with ether and the implanted pell *et al.* ong with granulation tissue were removed, freed from extraneous tissues and dried in an oven at 60°C for 24 hours.[8] The dried pellets were weighed and the gain in weight in each group was calculated. The difference in granulation tissue weights was noted. The increase in the granulation tissue weight of groups II, III, IV and V were compared with group I. The percentage inhibition was calculated by using the formula,

$$\text{Percentage inhibition} = \left[1 - \frac{W_t}{W_c}\right] \times 100$$

Where W_t is granuloma weight in treated groups and W_c is granuloma weight in control groups.

Statistical analysis

All the values were expressed as mean ± SEM and compared with the corresponding control values. *P*-values are calculated by using one-way ANOVA followed by Dunnett's *t*-test.

RESULTS

Acute Toxicity Studies of the Hydroalcoholic Extract of *G. supberba*

No death was observed even at the maximum administered dose of 2000 mg/kg body weight.

Effect of the Hydroalcoholic Extract of *G. supberba* in Eddy's Hot Plate Method

The treatment of mice with extract of *G. superba* at doses of 100, 200 and 400 mg/kg body weight exhibited a significant (P <0.01) increase in reaction time while compared to control. All the three doses of extract exhibited maximum percentage protection or percentage increase in reaction time at 90 minutes after drug administration. The percentage increase in reaction time at 90 minutes was 21.02%, 79.96% and 158.05% for extract at a dose of 100, 200 and 400 mg/kg body weight, respectively. However, the extract exhibited dose-dependent analgesic action [Table 1].

Effect of the Hydroalcoholic Extract of *G. supberba* in the Acetic Acid-induced Writhing Method

Pretreatment of mice with the extract of *G. superba* at a dose 100, 200, 400 mg/kg body weight produced a very significant (P <0.01) reduction in writhes induced by acetic acid when compared to control. The % inhibitions of writhes or % protection were found to be 64.09%, 78.56% and 81.45% for extract at a dose 100, 200 and 400 mg/kg body weight, respectively. However, it exhibited dose-dependent analgesic activity [Table 2].

Effect of the Hydroalcoholic Extract of *G. supberba* in Carrageenan-induced Paw Edema (acute inflammatory model)

The rats treated with oral administration of the extract of *G. superba* reduced acute paw edema volume as compared to control. The percentage inhibition of paw edema was increased with time and gave maximum effect at 2 hours, then declined in case of standard extract 400 mg/kg body weight. Only the 200 and 400 mg/kg body weight extract exhibited a significant result (P <0.05) when compared with

Table 1: Analgesic effect of hydroalcoholic extract of *Gloriosa superba* on Eddy's hot plate method

Treatment	Reaction time in minutes at					
	0 minute	30 minutes	60 minutes	90 minutes	120 minutes	150 minutes
Control	3.343 ± 0.14	3.352 ± 0.19	2.972 ± 0.19	2.610±0.50	2.710 ± 0.16	2.577 ± 0.07
Standard pentazocine 46.8 mg/kg	2.977 ± 0.07	5.673 ± 0.22** (90.59)	9.972 ± 0.02** (234.99)	9.592 ± 0.18** (222.23)	7.520 ± 0.29** (152.63)	4.553 ± 0.24** (52.95)
Extract 100 mg/kg	3.512 ± 0.14	3.562 ± 0.16 (1.43)	4.085 ± 0.20 (16.33)	4.250 ± 0.28 (21.02)	3.552 ± 0.09 (1.14)	3.080 ± 0.18 (-12.29)
Extract 200 mg/kg	2.862 ± 0.14	3.867 ± 0.06 (35.12)	4.528 ± 0.15** (54.74)	5.150 ± 0.09** (79.96)	4.490 ± 0.28 (56.90)	3.197 ± 0.23 (11.71)
Extract 400 mg/kg	3.207 ± 0.08	4.907 ± 0.9** (53.01)	5.632 ± 0.15** (75.62)	8.275 ± 0.37 (158.05)	6.747 ± 0.30** (110.39)	4.0 ± 0.15 (24.74)

Values are expressed as mean ± SEM; n = 6 animals in each group; numbers in parenthesis indicate percentage increase in reaction time *P<0.01, **P<0.001 when compared to control

control. The hydroalcoholic extract of G. superba exhibited dose-dependent anti-inflammatory activity. [Table 3]

Effect of the Hydroalcoholic Extract of *G. supberba* in Cotton Pellet Granuloma Method (sub-acute inflammatory model)
The rats were treated with the oral administration of the extract of *G. superba* that significantly reduced ($P < 0.05$) the granuloma mass formation when compared to control. The rats exhibited 9.59%, 28.72% and 45.8% inhibition of granuloma mass formation after 7 days treatment with 100, 200 and 400 mg/kg body weight of the extract when compared with control. However, it exhibited dose-dependent anti-inflammatory activity [Table 4].

DISCUSSION

The extract significantly ($P < 0.05$) reduced the number of abdominal writhings induced by acetic acid in mice. Abdominal constriction induced by acetic acid is used to screen the peripheral analgesic effect.[9] The results support the hypothesis of participation in the inhibition of prostaglandin synthesis since the nociceptive mechanism of abdominal writhing induced by acetic acid involves the process or release of arachidonic acid metabolites via cyclo-oxygenase (COX) and prostaglandin biosynthesis. The effect of the extracts on acetic acid-induced abdominal writhing suggested that they might inhibit or modify responses to pain mediated by noiceptors peripherally.[10]

The extract increased reaction latency to thermal pain induced by the hot plate method in mice, which is a specific central antinociceptive test.[11] Inhibition of histamine or kinin pathway may reduce pain. The results of the present study also showed that extract exhibited a comparable magnitude of antinociceptive activity in both models of pain which suggested that the phytochemical constituents are responsible for the analgesic effect. The analgesic activity of some flavonoids[12] and terpenoids already has been reported suggesting that these or similar constituents may be responsible for the analgesic effect of the extract. In conclusion, the results of the present study indicated that the hydroalcoholic extract of *G. superba* might contain constituents capable of relieving or modifying responses to pain caused by either thermal or chemical stimulation of the noiceptors mediated by both central and peripheral mechanisms.

Table 2: Analgesic effect of hydroalcoholic extract of *Gloriosa superba* on acetic acid-induced writhing in mice

Treatment	Number of writhings	% Inhibition
Control	66.83 ± 0.9	
Standard	10.50 ± 0.5*	84.03
Extract 100 mg/kg	24.00 ± 1.6*	64.09
Extract 200 mg/kg	14.33 ± 1.3*	78.56
Extract 400 mg/kg	12.33 ± 0.7*	81.45

Values are expressed as mean ± SEM; n = 6 animals in each group; *P<0.01 when compared to control.

Table 3: Anti-inflammatory effect of hydro alcoholic extract of *Gloriosa superba* on carrageenan-induced paw edema in rats

Treatment	Dose	Increase in paw volume (ml)			
		30 minutes	60 minutes	90 minutes	120 minutes
Control	-	0.23 ± 0.22	0.36 ± 0.01	0.66 ± 0.03	0.79 ± 0.02
Standard diclofenac	13.5 mg/kg	0.15 ± 0.03*** (34.48)	0.18 ± 0.03*** (50.00)	0.37 ± 0.06*** (59.10)	0.28 ± 0.06*** (64.56)
Extract	100 mg/kg	0.22 ± 0.18 (4.42)	0.30 ± 0.08 (16.65)	0.49 ± 0.08 (25.76)	0.68 ± 0.20 (13.92)
Extract	200 mg/kg	0.19 ± 0.03 (16.95)	0.22 ± 0.01* (38.89)	0.41± 0.07* (37.88)	0.60 ± 0.02 (24.05)
Extract	400 mg/kg	0.17 ± 0.02 (26.09)	0.20 ± 0.02* (43.45)	0.36 ± 0.04** (45.46)	0.46 ± 0.13** (41.77)

Values are expressed as mean ± SEM; n = 6 animals in each group; numbers in parenthesis indicate percentage inhibition in increase in paw volume. *P <0.05, **P <0.01, ***P<0.001 when compared with control

Table 4: Anti-inflammatory effect of hydro alcoholic extract of *Gloriosa superba* on the cotton pellet granuloma model in rats

Treatment	Dose	Weight of cotton pellet (mg)		Weight of granuloma (mg)	% Inhibition
		Before	After		
Control	-	20.16 ± 0.20	100.41 ± 1.81	80.23 ± 1.49	-
Standard diclofenac	13.5 mg/kg	20.14 ± 0.52	50.81 ± 1.08	30.7 ± 2.41**	61.81
Extract	100 mg/kg	20.21 ± 0.87	92.81 ± 1.92	72.61 ± 0.92	9.59
Extract	200 mg/kg	20.19 ± 0.61	78.01 ± 1.85	57.81 ± 1.62*	28.72
Extract	450 mg/kg	20.14 ± 0.81	65.01 ± 1.99	44.87 ± 1.75*	45.78

Values are expressed as mean ± SEM; n = 6 animals in each group; numbers in parenthesis indicate percentage inhibition of increase in granuloma weight. *P <0.05, **P <0.01 when compared with control.

The result of the present investigation revealed that the aerial part of *G. superba* possesses a moderate anti-inflammatory effect that was evidenced by the significant reduction in paw edema and cotton pellet granuloma methods. Carrageenan is a sulphated polysaccharide obtained from seaweed (Rhodophyceae) which is commonly used to induce acute inflammation and is believed to be biphasic.[13] The first phase is due to release of histamine and serotonin. The second phase is caused by the release of bradykinin, protease, prostaglandin and lysosome.[14] It has been reported that the second phase of the edema is sensitive to most clinically effective anti-inflammatory drugs, which has frequently to access the anti-edematous effect of natural products.[15] Prostaglandins play a major role in the development of the second phase of the reaction, which is measured at around 3 hours time. [16] The carrageenan-induced paw edema model in rats is known to be sensitive to cyclo-oxygenase (COX) inhibitors and has been used to evaluate the effects of non-steroidal anti-inflammatory agents against which primarily inhibits the enzyme COX involved in prostaglandin synthesis. Based on the results, it can be inferred that the inhibitory effect of hydroalcoholic extract on carrageenan-induced inflammation in rats may be due to the inhibition of enzyme cyclo-oxygenase. But lypo-oxygenase inhibitors also possess significant anti-inflammatory action against carrageenan-induced paw edema. It was also observed that extract significantly reduce the granuloma formation in rats. Multiplications of small blood vessels as well as proliferation of fibroblast are the characteristic features of the repair phase of inflammation. The extract of *G. superba* effectively reduced the cotton pellet-induced granuloma, suggesting its activity in the proliferative phase of inflammation.

CONCLUSION

An extensive research in ethnopharmacology has taken place throughout the world. The plant *G. superba* was traditionally claimed for a large number of pharmacological action and medicinal uses. In the present investigation, the acute toxicity study in rats revealed that the hydro alcoholic extract of aerial parts of plant is safe up to 2000 mg/kg body weight. The significant analgesic, anti-inflammatory and wound healing action may be attributed to the phytoconstituents present in it. The present study offered a scientific proof to the traditional use of *G. superba*. However, further phytochemical studies are needed to isolate the active compounds responsible for these pharmacological activities.

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