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SCREENING OF ANALGESIC AND ANTI-INFLAMMATORY ACTIVITY OF *GLORIOSA SUPERBA* IN RODENTS

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ABSTRACT: Background: Traditionally *Gloriosa superba* is being used as a medicinal plant in outskirts of belagavi, we evaluated and compared the analgesic and anti-inflammatory activities of hydroalcoholic extract of dried aerial parts (rhizomes) of *G. superba* with standard drugs. **Methods:** This experimental animal study was carried out in the Research Laboratory, Department of Pharmacology, BIMS, Belagavi. Prior permission from institutional ethical committee and institutional animal ethics committee was taken. Albino rats and Albino mice were used. Acute toxicity study was also done according to OECD guideline no. 425 and test doses were decided accordingly. The experimental models of Eddy's hot plate method was used to study the analgesic activity whereas formalin induced peritonitis and cotton pellet granuloma models were used for anti-inflammatory action. Statistical analysis was performed using Mann-whitney U test and chi-square test (SPSS software version 22). **Results:** In eddy's hot plate method, for *G. superba* extract of 400mg/kg, percentage increase in reaction time of at 120 mins was 60.49%, in formalin induces peritonitis method, for *G. superba* extract of 400mg/kg, percentage inhibition of peritoneal exudate was 47.14%. In cotton pellet induced granuloma method, for *G. superba* extract of 400mg/kg, percentage inhibition of granuloma formation was 50.32%. All the results of 4 different groups in each method showed significant difference in their respective pharmacological activities (Chi Square values: 14.4, 14.4, 75.2). **Conclusion:** Hydroalcoholic extract of *G. superba* has shown significant analgesic and anti-inflammatory activity in our study.

INTRODUCTION: Pain is an unpleasant sensation and emotional experience associated with actual or potential tissue damage or described in terms of such damage¹. The pathophysiology of pain involves 2 components, peripheral nociception and central mechanism.

There are 2 main classes of pain - Integumental pain and visceral pain². Treatment available to reduce the pain includes NSAIDs, opioids, anticonvulsants and muscle relaxants and these drugs also have adverse effects which are common nowadays.

Inflammation is a normal protective response to tissue injury caused by physical trauma, noxious chemicals or microbiologic agents³. Drug therapy of inflammation has always been debatable. The drugs most commonly used now in the treatment of inflammation are glucocorticoids and non-steroidal

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Anti-inflammatory drugs (NSAIDs). Most of these drugs are known to cause more or less deleterious side effects even leading to hospital admissions due to ADRs. Hence there is an always scope for continuous research to identify more effective and safer agents in the therapy of inflammation which are potent and nontoxic. Indian traditional system of medicine uses herbs that have anti-inflammatory property. *Gloriosa superba* is one of many medicinal plants extensively used in southern parts of India for treatment of diverse conditions predominantly pertaining to pain and inflammation. *Gloriosa superba* is a good abortifacient⁴. Its seeds and tubers are used mainly for treating gout and rheumatism⁵. Roots are purgative, cholagogue, antihelminthic, astringent and germicidal. It also cures leprosy, swelling, piles, chronic ulcers and colic pain in bladder. Powder is used for treatment of rheumatic fever. Various parts of the plant are used in sores, tumours and syphilis. Extract of plant is also used as CNS depressant⁶. Paste is an antidote in snake bite⁷.

Scientific basis for traditional use of this plant is not much explored. Hence, the study is chosen to screen the analgesic and anti-inflammatory activity of hydro alcoholic extract of rhizome of *Gloriosa superba* through analgesic model, acute and subacute inflammatory models and to provide pharmacological basis for its use in traditional medicine for various analgesic and inflammatory disorders.



FIG. 1A:

MATERIALS AND METHODS: Our study was carried out in the Research Laboratory, Department of Pharmacology, Belagavi Institute of Medical Sciences, Belagavi. Ethical clearance was taken before commencing the study and CPCSEA number stated below.

Collection of Plant Product: Rhizomes of *G. superba* were collected from the local ayurvedic pharmacy of Belagavi, Karnataka, India and authenticated by an authorized person in botany. The dried parts of rhizomes were powdered and stored in an air tight container.

Preparation of Extract: The powder (2kg) was soaked in 50% ethanol and 50% distilled water (i.e. hydroalcoholic extract) at 50-55°C for 7 days. After 7days, 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 dryness⁸.

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 BIMS animal house registered under the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and maintained under good laboratory conditions. These animals were used for the acute toxicity, anti-inflammatory and analgesic activities. The study was approved by Institutional Ethics Committee for animal experimentation (Approval no: BIMS/IAEC/PG/06/2018 Dated- 13/11/2018).

The animals were stabilized for 1 week, maintained under standard laboratory conditions and were given standard pellet diet and water *ad libitum* throughout the course of the study. The animals were handled gently to avoid undue stress, which could result in an increased adrenal output.

Acute Toxicity Studies: Toxicity study for hydroalcoholic extract of *G. superba* was carried out as per OECD guideline number 425⁹. Healthy adult albino wistar rats (200-250 g) were used. Animals were fasted overnight prior to dosing. The body weight of each animal was determined to calculate the dose. Hydroalcoholic extract of *G. superba* was administered in the dose of 2000 mg/kg body weight orally to one animal which survived. Thereafter, four other animals were dosed sequentially. All the animals were closely observed for 14 days. As no fatality was observed, LD₅₀ was estimated to be greater than 2000 mg/kg. After performing a pilot study, we decided to use 200 mg/kg and 400mg/kg of hydroalcoholic extract *G. superba* for all the experiments.

Screening Methods for Analgesic Activity:

Eddy's Hot Plate Method: Mice of either sex were weighed and divided into 4 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 and IV were treated with extracts at a dose of 200 and 400 mg/kg body weight, respectively. The reaction time of animals was noted down on hot plate at 0, 30, 60, 90, 120 and 150 minutes after the treatment. The basal reaction was the time taken by observing hind paw licking or jump response (whichever appeared 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 calculated¹⁰.

$$\text{Percentage increase in reaction time} = \{(R_t / R_c) - 1\} \times 100$$

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

Screening Methods for Anti-Inflammatory Activity:

Rats were randomly divided into four groups of 6 each. Group I served as control. Group II (diclofenac 13.5 mg/kg body weight) served as standard and groups III and IV were treated with *G. superba* extracts at a dose of 200 and 400 mg/kg body weight, respectively. Each rat was fed with respective drug one hour prior to the administration of phlogestic agent.

Formalin Induced Peritonitis in Rats: The method described by Teotino *et al*¹¹ was used to study the acute inflammatory reaction induced by intraperitoneal injection of formalin in rats. Under aseptic precautions, 1ml of 1% formalin was injected intraperitoneally in each rat. At the end of four hours, peritoneal exudates was collected by opening the ventral abdominal wall. Volume of the peritoneal exudates in diclofenac (13.5mg/kg) and *G. superba* treated group was compared with the control group.

Percentage inhibition of peritoneal exudate formation in diclofenac and *G. superba* treated group was determined by the following formula.

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

Where, V_c - Mean volume of exudates in control group.

V_t - Mean volume of exudates in treated test groups.

Cotton Pellet Induced Granuloma in Rats: The method described by Meier *et al*¹² was used to study the chronic inflammatory reaction induced by subcutaneous cotton pellet implantation. Sterile cotton pellets weighing 20 mg each was implanted subcutaneously in the axillary region of both forefeet and groin under anaesthesia¹³.

Each rat was orally administered with respective drug once daily for 7 consecutive days.

On 8th day, the animals were sacrificed, cotton pellet with granulation tissue was removed, dried at 60°C in hot air oven for 24 hours and dry weight was determined.

Percentage inhibition of granuloma formation in diclofenac (13.5mg/kg) and *Gloriosa superba* treated rats was determined by the following formula:

$$\text{Percentage inhibition of granuloma formation} = \{1 - (T_t / T_c)\} \times 100$$

Where, T_c - Weight of granuloma tissue in control group

T_t - Weight of granuloma tissue in treated test groups

RESULTS: Results were analyzed using Mann-whitney U test and chi-square test (SPSS software version 22). **Table 1** and **Figure 1** indicate that hydroalcoholic extract of rhizomes of *Gloriosa superba* in dose of 200mg/kg and 400mg/kg shows significant analgesic activity with percentage increase in reaction time of 47.84% & 60.84% respectively in eddy's hot plate method. Further, above two test doses of 200mg/kg and 400mg/kg of *Gloriosa superba* when compared with standard drug – Pentazocine (79.78%), showed no significant difference in analgesic activity ($P=0.18 - P > 0.05$, $P = 0.748 - P > 0.05$). Thus, test drug activity is not comparable with the standard drug pentazocine ($P > 0.05$). Analgesic activity of two test doses of test drug (200mg/kg and 400mg/kg) was not comparable ($P = 0.407 - P > 0.05$).

All the results of four groups showed significant difference in analgesic activity (Chi square = 14.412).

Table 2 and **Figure 2** indicate that hydroalcoholic extract of rhizomes of *Gloriosa superba* in dose of 200mg/kg and 400mg/kg shows significant anti-inflammatory activity with percentage inhibition in peritoneal exudates of 40.35% & 47.14% respectively in acute model of inflammation. Further, above two test doses of 200mg/kg and 400mg/kg of *Gloriosa superba* when compared with standard drug Diclofenac (49.28%), showed no significant difference in anti-inflammatory activity ($P=0.145 - P > 0.05$, $P = 0.63 - P > 0.05$). Thus, test drug activity is not comparable with the standard drug diclofenac ($P > 0.05$).

Anti-inflammatory activity of two test doses of test drug (200mg/kg and 400mg/kg) was not comparable ($P = 0.256 - P > 0.05$). All the results

of four groups showed significant difference in anti-inflammatory activity (Chi square = 14.441). **Table 3** and **Fig. 3** indicate that hydroalcoholic extract of rhizomes of *Gloriosa superba* in dose of 200mg/kg and 400mg/kg shows significant anti-inflammatory activity with percentage inhibition in granuloma formation of 35.54% & 50.32% respectively in sub-acute model of inflammation. Further, above two test doses of 200mg/kg and 400mg/kg of *G. superba* when compared with standard drug – Diclofenac (55.98%), showed significant difference in anti-inflammatory activity ($P=0.001 - P < 0.05$, $P = 0.004 - P < 0.05$). Thus, test drug activity is comparable with the standard drug diclofenac ($P < 0.05$). Anti-inflammatory activity of two test doses of test drug (200mg/kg and 400mg/kg) was comparable or statistically significant ($P = 0.001 - P < 0.05$). All the results of four groups showed significant difference in anti-inflammatory activity (Chi square = 75.174).

TABLE 1: ANALGESIC EFFECT OF VARIOUS DRUGS ON MICE USING EDDY'S HOT PLATE METHOD

Groups	Dose	Reaction time in seconds				Mean reaction time in seconds +/-SEM	Percentage (%) increase in reaction time
		0 min	30 min	60min	120 min		
Control	-	3.0 ± 0.29	3.3 ± 0.19	3.17 ± 0.38	3.5 ± 0.22	3.2 ± 0.27	-
Standard (Pentazocine)	46.8 mg/kg	2.8 ± 0.25	4.5 ± 0.37	6.8 ± 0.52	9.1 ± 0.25	5.8 ± 0.35	79.78
Test 1	200 mg/kg	3.0 ± 0.29	4.0 ± 0.29	5.8 ± 0.25	6.3 ± 0.19	4.7 ± 0.26	47.84
Test2	400 mg/kg	3.2 ± 0.24	4.6 ± 0.29	5.9 ± 0.12	7.1 ± 0.39	5.2 ± 0.17	60.49

TABLE 2: ANTI-INFLAMMATORY EFFECT OF VARIOUS DRUGS ON RATS USING FORMALIN INDUCED PERITONITIS METHOD

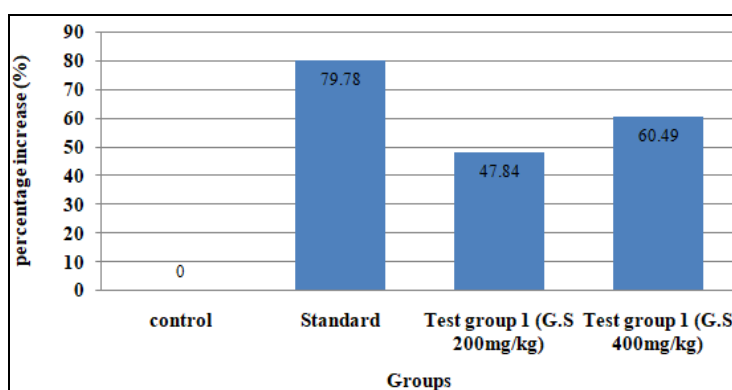
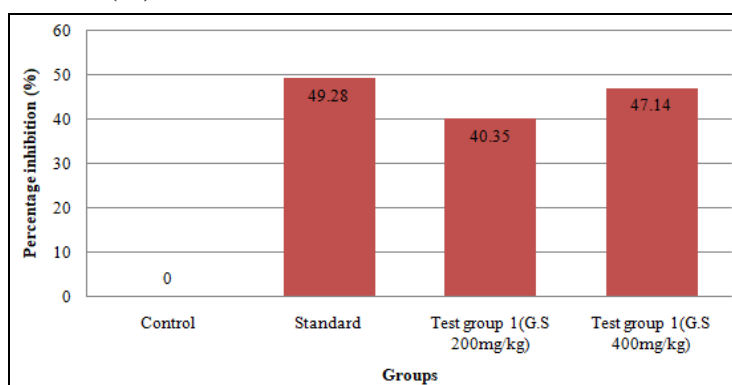
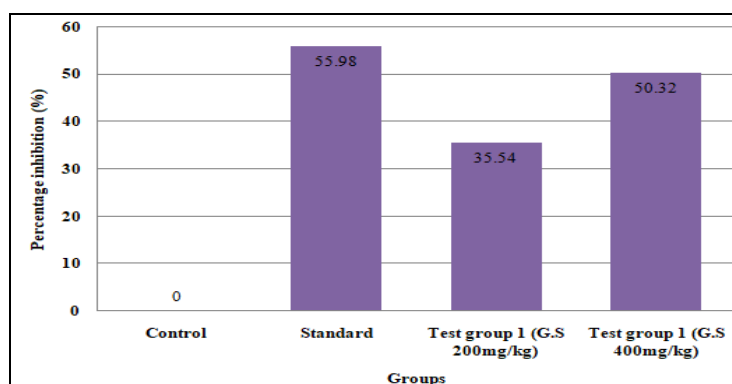
Groups	Dose	Volume of peritoneal exudates (ml)	Mean peritoneal exudates volume (ml) ± SEM	% Inhibition
Control	-	2.6, 2.8, 3.2, 3.0, 2.7, 2.5	2.80 ± 0.09	0
Standard (Diclofenac)	13.5 mg/kg	1.6, 2.0, 1.4, 1.3, 1.2, 1.0	1.42 ± 0.11	49.28
Test 1	200mg/kg	1.4, 1.4, 1.8, 1.8, 2.0, 1.6	1.67 ± 0.09	40.35
Test 2	400mg/kg	1.7, 1.8, 1.5, 1.5, 1.2, 1.2	1.48 ± 0.08	47.14

TABLE 3: ANTI-INFLAMMATORY EFFECT OF VARIOUS DRUGS ON RATS USING COTTON PELLET INDUCED GRANULOMA METHOD

Groups	Dose	Dry granuloma weight in (mg)	Mean dry granuloma Weight (mg) ± SEM	% Inhibition occurred
Control	-	17, 19, 19, 19, 18, 16, 18, 18, 18, 18, 16, 16, 20, 18, 19, 21, 20, 20, 18, 18, 20, 20, 19, 18	18.46 ± 0.36	0
Standard (Diclofenac)	13.5 mg/kg	5, 7, 6, 6, 6, 8, 7, 6, 7, 9, 8, 9, 7, 6, 9, 9, 8, 8, 9, 6, 11, 9, 10, 9	8.125 ± 0.37	55.98
Test 1	200mg/kg	12, 11, 11, 12, 13, 11, 10, 12, 14, 10, 12, 12, 12, 10, 12, 12, 10, 10, 12, 24, 16, 10, 12, 16	11.9 ± 0.25	35.54
Test 2	400mg/kg	10, 8, 7, 9, 11, 6, 7, 8, 9, 8, 7, 7, 12, 10, 9, 10, 10, 11, 9, 10, 12, 11, 9, 10	9.17 ± 0.48	50.32

TABLE 4: REPORTED ADDITIONAL SPECIAL PHARMACOLOGICAL PROPERTIES OF DIFFERENT PARTS OF *GLORIOSA SUPERBA* WITH DIFFERENT EXTRACTS

Extract	Parts used	Activity	References
Chloroform and N-butanol	Leaves, tubers, seeds	Anti-microbial and Anti-cancer	27
Methanolic, aqueous and petroleum ether	Tubers	Anti-bacterial, Anti-fungal, mutagenic	28
Acetone	Tubers and stem	Anti-fungal	29
Alcoholic	Tubers	Anti-microbial	30
Alcoholic	Tubers	Anti-helminthic	30
Ethanol and water	Whole plant	Anti-helminthic	31
Methanolic	Leaf and stem	Anti-oxidant and Anti-microbial	32
Methanolic	Seeds, tubers and leaves	Anti-oxidant	17
Methanolic, acetone and water	Tubers	Anti-oxidant	33
Methanol, hexone, chloroform	Tubers and seeds	Anti-bacterial	34
Acetone	Tubers and leaves	Anti-bacterial	35
Alcoholic	Tubers	Anti-haemolytic	36
Aqueous	Leaves	Anti-thrombolytic and Anti-coagulant	37

**FIG. 1: SHOWING PERCENTAGE (%) INCREASE IN REACTION TIME IN EDDY'S HOT PLATE MODEL****FIG. 2: SHOWING PERCENTAGE (%) INHIBITION OF MEAN VOLUME OF PERITONEAL EXUDATES IN FORMALIN INDUCED PERITONITIS MODEL****FIG. 3: SHOWING PERCENTAGE (%) INHIBITION OF GRANULOMA FORMATION IN COTTON PELLET INDUCED GRANULOMA MODEL**

DISCUSSION: In the present era there are number of different inflammatory disorders for which there are variety of anti-inflammatory drugs but associated with side effects like gastric ulcers, GI bleeding, renal damage etc. So, anti-inflammatory drugs with minimal side effects to be explored therapeutically in huge magnitudes for variety of inflammatory disorders and used accordingly.

In this context, it is exciting to note the reports of medicinal values of several indigenous plant preparations. Uses of these preparations are also consistently highlighted in ayurveda but without proper scientific knowledge. Hence, some of different extracts of different plants have been tested for pharmacological activities.

Globally there is extensive traditional use of a plant by name "*Gloriosa superba*" as there is significant therapeutically potential like analgesic, anti-rheumatic, anti-pyretic activities etc. Thereby we tried to evaluated the analgesic and anti-inflammatory activities of this plant by specific models respectively as described above to support and provide pharmacological basis in future for the use in our allopathy practice.

In Eddy's hot plate model the extract of *G. superba* increased reaction time from 40.84% in test dose 200mg/kg to 60.49% in 400mg/kg to thermal pain, which simulates central antinociceptive test¹⁴ and thereby standard drug pentazocine was used to compare and simulate central antinociception with test drug. 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 flavonoids¹⁵ and terpenoids already has been reported suggesting that these or similar constituents may be responsible for the analgesic effect of the extract. 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 nociceptors mediated by both central and peripheral mechanisms. Both the two doses of extract exhibited maximum percentage protection due to

increase in reaction time (6.30 ± 0.19 , 7.16 ± 0.39) at 90 minutes after drug administration thereby clearly indicating good analgesic activity of *Gloriosa superba*. The results obtained from this study is similar to study done by John et al¹⁶ and support our study.

Formalin induced peritonitis model effectively demonstrates the use of formalin to induce peritonitis in rats by injecting intraperitoneally and results showed significant effect of standard anti-inflammatory drug diclofenac and *Gloriosa superba* as there was significant percentage protection or percentage inhibition in the volume of peritoneal exudates (40.34% with G S 200mg/kg and 47.14% with G S 400mg/kg) when compared to control thereby clearly indicating anti-inflammatory activity of *Gloriosa superba*.

Formalin was chosen as the preferred phlogestic agent to induce acute inflammation because it adequately resembles the chain of events underlying inflammation in patients. Other phlogestic agents which can be used to induce acute inflammation are carrageenan (sulphated polysaccharide obtained from seaweed Rhodophyceae), lipopolysaccharide, zymosan based on varying degree and duration of action needed. As there are no similar studies done of this model for this plant *G. superba*, comparison cannot be done.

Cotton pellet induced granuloma model effectively demonstrates and measures both exudative and proliferative phases of inflammation. 200mg/kg of *G. superba* extract showed statistically significant decrease in dry granuloma weight (35.54%, $P=0.001$ hence $P < 0.05$) when compared to control group. This shows that hydroalcoholic extract of this plant is effective against subacute and chronic inflammation but its activity was inferior in the dose of 200mg/kg when compared to standard drug diclofenac. 400mg/kg dose of this plant extract showed statistically significant decrease in dry granuloma weight (50.32%, $P=0.001$ hence $P < 0.05$) when compared to control group. The activity of *G. Superba* in 400mg/kg was almost similar to standard drug diclofenac. The results obtained from this model for *G. superba* is slightly superior to study done by John, et al¹⁶ and support the study. As there are very few studies done previously of

this model for this plant, our results cannot be compared with other studies. Because medicinal plants are rich in flavonoids and every group of flavonoids has a capacity to act as antioxidants¹⁷, different studies of various parts of this plant has been demonstrated the scientific basis for analgesic and anti-inflammatory activity by isolation of various chemical constituents of this plant. Among them, flavones and catechin components acting as most powerful flavonoids for protecting the body against ROS¹⁸.

The other flavonoid components such as quercetin, kaempferol, myricetin and rutin have antioxidant, anti-inflammatory, antiviral and antiallergic, as well as anticancer activities^{19, 20}, *G. superba* tubers contain colchicines, benzoic and salicylic acid, sterols and resinous substances like as colchicines, 3-demethyl colchicine, 1,2-didemethyl colchicine, 2, 3-didemethyl colchicine, N-formyl, N-deacetyl colchicines, colchicocide, gloriosine, tannins and superbine²¹.

Colchicine is the major compound isolated from the seed and rhizome of this plant²² and other important compound is gloriosine^{23, 24}, In addition, *G. superba* tubers hold 0.25% colchicine apart from containing sitosterol, glucoside, β -and gamma lumicolichicines, β -sitosterol, flucoside and 2-H-6-MeO benzoic acid and flowers contain luteolin and N-formylde-Me-Colchicine^{25, 26}. Apart from these important phytochemical constituents of *G. superba* and their respective pharmacological properties, there are more additional special properties of this plant with different extracts which are extensively explored and documented by various reports of different studies (As mentioned in **Table 4**). In our study we conclude scientifically that rhizomes of *Gloriosa superba* showed good analgesic & anti-inflammatory activity in mice & rats.

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CONFLICTS OF INTEREST: NIL

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