ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 12, Issue 4, 2019

Online - 2455-3891
Print - 0974-2441

Research Article

"EVALUATION OF *GALPHIMIA GLAUCA* STEM METHANOL EXTRACT FRACTIONS FOR ANALGESIC AND ANTI-INFLAMMATORY ACTIVITIES"

GARIGE BABA SHANKAR RAO[1]*,SRISAILAM K[2], V UMA MAHESHWARA RAO[3], VASUDHA B[1]

[1]Department of Pharmacognosy and Phytochemistry, School of Pharmacy, Anurag Group of Institutions, Medchal - 501 301, Telangana, India. [2]University College of Pharmaceutical Sciences, Satavahana University, Karimnagar - 505 002, Telangana, India. [3]Department of Pharmacy, Teegala Ram Reddy College of Pharmacy, Meerpet - 500 079, Hyderabad, Telangana, India. Email: babaphd2010@gmail.com

Received: 26 January 2019, Revised and Accepted: 26 March 2019

ABSTRACT

Objective: This current investigation assesses *in vivo* central and peripheral analgesic effects and anti-inflammatory properties of fractions obtained from *Galphimia glauca* (GG) stem methanol extract.

Methods: The laboratory models such as Swiss albino mice and Wistar albino rats were employed in the studies. The GG stem methanol extract was subjected to fractionation with solvents such as hexane, chloroform, ethyl acetate, and methanol. Orally, the dose range of 100, 200, and 400 mg/kg was given for 1 day for evaluating analgesic (hotplate test, tail clip test, writhing test, and formalin test) and weekdays for assessing anti-inflammatory activity (carrageenan and cotton pellet test methods), respectively. The experimental studies were further conducted for determining the involvement of central and peripheral receptor actions in the analgesic activity of the extract by prechallenging it with naloxone and acetic acid, respectively. The *in vivo* anti-inflammatory studies were conducted using carrageenan-induced rat paw edema model and cotton pellet granuloma test.

Results: The LD_{50} of the extract was found to be >2000 mg/kg b.w. The methanol fraction of 400 mg/kg dose exhibited significant ($p \leq 0.001$) and dose-dependent analgesic and anti-inflammatory activity. It also exhibited central and peripheral analgesic actions when treated with naloxone and acetic acid, respectively.

Conclusion: The results revealed that the stem methanol fraction has more potential in terms of analgesic and anti-inflammatory properties.

Keywords: *Galphimia glauca*, Analgesic activity, Anti-inflammatory activity, Formalin test, carrageenan.

 DOI: http://dx.doi.org/10.22159/ajpcr.2019.v12i4.31866

INTRODUCTION

Today, natural remedies are relevant globally, due to their holistic and comprehensive approach to health. The daily schedule of natural remedies enhances the total health of human beings, both mentally and physically, and also helps to bring about peace and harmony in personal life. The WHO estimates that the non-communicable diseases kill about 38 million people each year, and almost 28 million occur in middle- and low-income nations [1]. Herbal drugs offer solutions to these problems. Among the natural sources of drugs, plants, in particular, serve as the primary source of lead molecules of therapeutic significance [2].

Galphimia glauca Cav. (GG) is a striking appearance shrub with bright yellow-colored petals which belong to the family of the Malpighiaceae [3,4]. This plant is seen growing in most of the districts of Telangana state. The plant is familiar as "*Calderonaamarilla*" and "*Florestrella*" [5,6]. The plant GG is in use to treat pain, inflammation, and nervous excitement. The tea made from yellow-colored leaves is consumed to cure fever and coronary pain and also to soothe the nerves. Tortoriello *et al*., 2011, cited the chemistry of the novel isolated nor-seco-triterpene molecules from the methanol extract of GG aerial parts [7].

Galphimines: Galphimine-A, galphimine-B, and galphimine-C were reported by dCardoso Taketa *et al*., 2004 [8]. To confirm the GG traditional uses, in our previous research, we have disclosed that the GG leaf and stem methanol extract exhibited significant analgesic and anti-inflammatory activities [9,10]. In other research carried out, we have disclosed the central nervous system depressant effects and muscle relaxant effects of GG stem and leaf methanol extract [11,12]. Observing the substantial results of the GG stem methanol extract with analgesic and anti-inflammatory properties through our earlier work, the current work is mainly focused on fractionation of the stem methanol extract to explore the fraction with potent analgesic and anti-inflammatory activity.

MATERIALS AND METHODS

Plant material

The GG was collected from the lawn existing in the campus of the Anurag Group of Institutions. The stems were collected in June 2018, shade dried, and powdered. The GG was identified and authenticated by famous taxonomist, Dr. E. Narsimha Murthy, Satavahana University, Karimnagar, Telangana state. A voucher copy is stored with the reference number No. 333, in the Department of Pharmacognosy and Phytochemistry, School of Pharmacy.

Preparation of the extract

The GG stem powder (0.20 kg) was subjected to Soxhlet extraction employing 0.6 L of methanol. The GG stem methanol extract (GGSME) was collected, dried, and stored. The yield obtained was 0.030 kg.

Animals

The laboratory mice strain (Swiss albino) of 42–56 days of age with average body weight 0.0225±0.0025 kg of either sex and the laboratory rat strain (Wistar albino) of 84–98 days of age with average body weight 0.234±0.0248 kg of either sex were purchased from the National Institute of Nutrition, Hyderabad. All animals were adapted for 1 week to the laboratory environment (60–70% relative humidity, 720 min light/dark conditions, and 22±2°C temperature) with regular diet and water *ad libitum.* Animals were maintained on interim fasting at night before the study but unopposed to water. Entire studies were performed using 6 animals of either sex in individual group.

The study protocol was authorized by the Institutional Animal Ethics Committee of the Institute, Anurag Group of Institutions (Formerly Lalitha College of Pharmacy), Hyderabad, Telangana state (Protocol No: I/IAEC/LCP/032/2018/18).

Grouping of animals for pharmacological studies

The below-mentioned grouping procedure is adopted for the fractions for investigating analgesic and anti-inflammatory activities with low, medium, and high doses, respectively.

Mice and rats, as cited in section: Animals, employed were grouped separately. Twelve groups of animals (n=6) received orally 100, 200, and 400 mg/kg of fractions (GGH, GG stem methanol extract hexane fraction; GGC, GG stem methanol extract chloroform fraction; GGE, GG stem methanol extract ethylacetate fraction and GGM, GG stem methanol extract methanol fraction). Remaining animal groups for studies on fractions include negative control and standard groups (morphine [10 mg/kg]/diclofenac sodium [20 mg/kg]). The morphine was employed as a standard drug in the hot plate test and Haffner's tail clip test. The diclofenac sodium was employed as a standard drug in writhing test, carrageenan-induced paw edema test, and cotton pellet-induced granuloma test, whereas both morphine and diclofenac sodium were employed as a standard in the formalin test.

Similarly, as cited above, two groups of animals, each was examined for opioid/peripheral receptors participation in the mechanism of analgesic activity of the active fraction [GGM (400 mg/kg)].

Chemicals and drugs

Analytical grade chemicals used in the studies were supplied from SD Fine Chemicals, India. The morphine was obtained from Troikaa Pharmaceuticals, Ahmedabad. Carrageenan was purchased from Sigma-Aldrich, USA. Naloxone and diclofenac sodium were obtained as gift samples from Samarth Pharma Limited, Mumbai, and Sri Disha Biotech, Hyderabad.

Acute toxicity studies

OECD, 423-2d guidelines were followed for this study [13]. The fractions of GG extract were subjected to acute toxicity studies.

Phytochemical Screening

Phytochemical screening of the GG methanol extract fractions was carried out using standard procedures [14].

Statistical analysis

Numerical data were expressed as mean±SEM (standard error of mean). Statistical analysis was performed with one-way analysis of variance (ANOVA), followed by a Tukey's multiple comparison test. $p \leq 0.05$ was considered to be statistically significant. The statistical analysis was carried out with GraphPad Prism 5.0 Software (GraphPad Software, Inc. La Jolla, USA) available in the Department of Pharmacognosy and Phytochemistry, Anurag Group of Institutions.

Analgesic activity

Hot plate test

The procedure stated by Baba Shankar *et al*., was employed to study the antinociceptive activity employing hot plate test. The laboratory mouse strain (Swiss albino) engaged in this distinct study was first screened initially by putting the mice on Eddy's analgesiometer (V. J Instruments, Maharashtra) set at a steady temperature of 55±1°C. Animals which failed to give nociceptive responses (withdrawing their paws/licking the hind paws/jumping) <15 s were removed, and remaining animals were grouped randomly (n=6) as mentioned in above section. Grouping of animals for pharmacological studies and pre-treatment reaction time was registered [9].

After 0.5 h morphine (10 mg/kg; i.p.) and 1 h oral treatment of the fractions as mentioned in above section grouping of animals for pharmacological studies, the post-treatment reaction time was registered with break time of 0.5 h, 1 h, and 1.5 h, respectively.

Exploration for opioid receptors participate in the antinociceptive activity of the fraction

The procedure stated by Baba Shankar *et al*. was employed to study opioid receptor participation in the analgesic activity. Based on the above results, further studies were conducted for the active fraction [GGM (400 mg/kg)] to examine the opioid receptor participation in its mechanism.

Separately, two groups of mice, each were prechallenged intraperitoneally with naloxone (5 mg/kg), 15 min before administration of morphine (10 mg/kg; i.p.), and active fraction, respectively. The time of response was registered ahead and following the treatment, as cited in the method mentioned above [9].

Haffner's tail clip test

The procedure stated by Baba Shankar *et al*. was employed to study Haffner's tail clip test for testing analgesic drugs. The laboratory rat strain (Wistar albino) used in this study was screened initially for inducing the pain at the root of the tail by applying mechanical stimulus using a metal artery clip. Animals which failed to give quick responses (biting the clip/tail) within 10 s were rejected. The remaining rats were grouped and pre-treatment responding time was registered. After 0.5 h morphine (10 mg/kg) and 1 h oral treatment of the fractions as mentioned in above section grouping of animals for pharmacological studies, the post-treatment responding time was registered. The cutoff time period of 60 s was used for accessing the post-treatment effect [10].

$$\text{Inhibition}(\%) = \frac{(\text{Post treatment Latency}) - (\text{Pre treatment Latency})}{(\text{Cut off time} - \text{Pre treatment Latency})} \times 100$$

Formalin test

The formalin test was conducted in mice as described by Murray *et al*., 1988. The laboratory mouse strain (Swiss albino) fasted overnight of groups (n=6) was used for this study. Grouped mice were treated orally with fractions as mentioned in above section. Grouping of animals for pharmacological studies. The standard groups received intraperitonial administration with diclofenac sodium (20mg/kg)/ morphine (10 mg/kg). After 1 h of fractions administration and after 0.5 h treatment with diclofenac sodium/ morphine, 20 µL of 2.5% formalin was introduced subcutaneously to the right hind paw for all animals. The early phase and late phase pain responses were registered for individual mice [15].

$$\text{Inhibition}(\%) = \frac{\text{Reaction time(Control Group)} - \text{Reaction time(Treated Group)}}{\text{Reaction time(Control Group)}} \times 100$$

Writhing test

The writhing test was conducted in Swiss albino mice using the method described by Koster *et al*., 1959 [16]. The animals were kept on fast overnight and grouped. Animals were treated orally with fractions as mentioned in above section grouping of animals for pharmacological studies and intraperitoneal administration with diclofenac sodium (20 mg/kg; i.p.). After 0.5 h of fractions/diclofenac sodium (20 mg/kg; i.p.) administration, 10 ml/kg of 0.7% acetic acid solution was given to all study animals through intraperitoneal route and abdominal writhings were registered for a span of 0.5 h.

$$\text{Inhibition}(\%) = \frac{\text{Number of writhes(Control Group)} - \text{Number of writhes(Treated Group)}}{\text{Number of writhes(Control Group)}} \times 100$$

Exploration for peripheral receptors participation in the antinociceptive activity of the fractions

The procedure stated by Baba Shankar *et al*., was employed to study peripheral receptor participation in the antinociceptive activity [10].

Based on the above results, further studies were conducted for the active extract active fraction (GGM [400 mg/kg]) to examine the peripheral receptor participation in its mechanism.

Separately, two groups of mice, each were pre-challenged intraperitoneally with naloxone (5 mg/kg), 15 min before administration of diclofenac (20 mg/kg), and active fraction, respectively. The time of response was registered ahead and following the treatment, as cited in the method mentioned above.

Anti-inflammatory activity

Carrageenan-induced paw edema test

The procedure stated by Winter *et al*., 1962, was used for studying the acute and subacute phases of inflammation. This test is performed in digital plethysmometer (V. J. instruments, Maharashtra). The Wistar albino rats were fasted overnight and grouped. Animals were treated for 1 week with fractions as mentioned in above section grouping of animals for pharmacological studies and with saline (for the standard group). On day 8, after 1 h of treatment with fractions/diclofenac sodium (20 mg/kg, i.p.), 0.1 ml volume of carrageenan (1% in saline solution) was introduced to the right hind paw for all rats to persuade edema. An identification mark is kept at the ankle joint, and a paw volume up to the mark was measured in fractions/diclofenac sodium treated groups, ahead and following the treatment with carrageenan challenge. The variation in paw volume was registered at 1st h, 2nd h, 3rd h, and 4th h, respectively [17].

$$\text{Reduction in Edema}(\%) = \frac{(\text{Mean edema in control group}) - (\text{Mean edema in drug treated group})}{(\text{Mean edema in control group})} \times 100$$

Cotton pellet-induced granuloma experiment

The granuloma test was carried out as mentioned by Meier *et al*., 1950. Wistar albino rats were fasted overnight and grouped. Cotton pellets (Johnson & Johnson) weighing each 0.02 g were sterilized and used for the experiment. Urethane (1.5 g/kg) was administered intraperitoneally to anesthetize the animals. Sterilized cotton pellets were put subcutaneously through small cuts made on the upper side of animals; after that, the cut openings were closed with absorbable surgical suture (Ethicon).

Animals were treated for 1 week with fractions/diclofenac sodium as mentioned in above section grouping of animals for pharmacological studies. On day 8, after anesthetizing the animals, cotton pellets were separated and the adhering foreign tissue was removed off, dried for 1 day at 60°C, and then weighed. The transudative weights and granuloma weights were registered. The inhibition percentage of granuloma tissue that has been formed was resolved with the formula mentioned below [18].

$$\text{Inhibition}(\%) = \frac{\text{Granuloma Tissue Weight}\left[\text{Control Group}\right] - \text{Granuloma Tissue Weight}\left[\text{Treated Group}\right]}{\text{Granuloma Tissue Weight}\left[\text{Control Group}\right]} \times 100$$

RESULTS

Analgesic activity

Thermal stimulus model

The analgesic effects of various fractions of GGSME are cited in Table 1. The activity of GGM administered orally with low, moderate, and high dose was significant ($p \leq 0.05$) when correlated with morphine (10 mg/kg) and control group ($p \leq 0.001$), while there was no change in the latency period of other fractions when correlated with the negative control group.

Exploration for opioid receptor participation in the analgesic activity of the GGSME fraction

The GGM exhibited its central actions ($p \leq 0.05$) with its highest dose (400 mg/kg). It was proved when naloxone-administered groups reversed the pain inhibition property. The results are cited in Table 1.

Haffner's tail clip test

The results are tabulated in Table 2. The activity of GGM administered orally with 100, 200, and 400 mg/kg dose was significant ($p \leq 0.001$) and dose-dependent when correlated with the control group (Fig. 1).

Writhing Test

The results are illustrated in Fig. 2 and Table 3. When correlated with negative control group, GGM administered orally with 100, 200, and 400 mg/kg was significant ($p \leq 0.001$) in decreasing the number of writhing in mice.

Exploration for peripheral receptors participation in the analgesic activity of the GGSME fraction

The GGM exhibited its peripheral actions with its highest dose (400 mg/kg). It was proved when naloxone administered groups exhibited negative response on abdominal constriction in mice. The results are cited in Table 3.

Table 1: Analgesic effect of GG stem methanol extract fractions on thermal stimulus-induced pain in mice - hot plate method

Group (s)	Dose (mg/kg)	Reaction time after administering control/standard/GGSME fractions (s)			
		0 min	30 min	60 min	90 min
I (Distilled water)	10 (ml/kg)	3.4±0.1	3.5±0.1	3.4±0.1	3.4±0.1
II (Morphine)	10	3.4±0.1	8.2±0.3[a]	10.0±0.1[a]	13.0±0.2[a]
III (GGH)	100	3.6±0.2	3.5±0.1[b]	3.8±0.1[b]	3.9±0.1[b]
IV (GGH)	200	3.6±0.2	3.9±0.08[b]	4.1±0.09[b]	4.2±0.07[b]
V (GGH)	400	3.7±0.3	4.0±0.2[b]	4.3±0.1[b]	4.3±0.1[b]
VI (GGC)	100	3.6±0.2	4.0±0.1[b]	4.4±0.2[b]	4.7±0.3[a,b]
VII (GGC)	200	4.0±0.2	4.4±0.2[b]	4.5±0.2[a,b]	4.9±0.1[a,b]
VIII (GGC)	400	3.9±0.2	4.5±0.2[b]	5.0±0.3[a,b]	5.2±0.4[a,b]
IX (GGE)	100	3.9±0.1	4.1±0.1[b]	4.6±0.2[a,b]	4.6±0.1[b]
X (GGE)	200	3.6±0.2	4.4±0.1[b]	6.1±0.1[a,b]	6.1±0.1[a,b,c]
XI (GGE)	400	3.8±0.3	5.6±0.2[a,b,c]	6.5±0.2[a,b,c]	6.5±0.2[a,b,c]
XII (GGM)	100	3.6±0.1	5.5±0.1[a,b]	7.2±0.1[a,b]	10.8±0.3[a,b]
XIII (GGM)	200	3.9±0.2	6.2±0.1[a,b,c]	8.1±0.2[a,b,c]	11.5±0.3[a,b]
XIV (GGM)	400	3.8±0.2	7.6±0.2[a,c]	9.6±0.3[a,b,c]	12.7±0.2[a,b,c]
XV (Naloxone+Morphine)	(5+10)	3.7±0.8	3.6±0.2[b]	3.5±0.8[b]	3.5±0.1[b]
XVI (Naloxone+GGM)	(5+400)	3.8±0.1	3.7±0.7[b]	3.7±0.6[b]	3.7±0.4[b]

GGH, GG stem methanol extract hexane fraction; GGC, GG stem methanol extract chloroform fraction; GGE, GG stem methanol extract ethyl acetate fraction; GGM, GG stem methanol extract methanol fraction. Values are expressed as mean±SEM.; n=6; the statistical significance was done by one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison tests and is represented by a symbol. [a]$p<0.001$ indicates comparison with Group I. [b]$p<0.05$ indicates comparison with Group II. [c]$p<0.05$ indicates the dose-dependent activity in comparison of the high dose with respective low doses of the GGSME fractions.

Table 2: Analgesic effect of *G. glauca* stem methanol extract fractions on tail clip induced pain in rats (Haffner's tail clip test)

Group (s)	Dose (mg/kg)	Pre-treatment reaction latency (sec)	Post-treatment reaction latency (sec)	Inhibition (%)
I (Distilled water)	10 (ml/kg)	1.48±0.3	1.49±0.8	0.01
II (Morphine)	10	1.52±0.2	56±2.3[a]	93
III (GGH)	100	1.83±0.1[a,b]	1.95±0.1[b]	0.20
IV (GGH)	200	1.69±0.3[a,b]	1.84±0.2[b]	0.25
V (GGH)	400	1.62±0.3[a,b]	1.83±0.2[b]	0.3
VI (GGC)	100	1.76±0.3[a,b]	1.80±0.2[b]	0.06
VII (GGC)	200	1.69±0.5[a,b]	1.74±0.3[b]	0.08
VIII (GGC)	400	1.84±0.7[a,b]	1.97±0.3[b]	0.22
IX (GGE)	100	1.68±0.2[a,b]	5±0.9[b]	5.6
X (GGE)	200	1.68±0.4[a,b]	7±±0.9[a]	9.2
XI (GGE)	400	1.72±0.2[a,b]	10±1.3[a,b]	14.20
XII (GGM)	100	1.71±0.3[a,b]	36±1.9[a,b]	58.82
XIII (GGM)	200	1.67±0.5[a,b]	45±1.8[a,b,c]	74.28
XIV (GGM)	400	1.62±0.1[a,b]	54±1.8[a,c]	89.00

GGH, GG stem methanol extract hexane fraction; GGC, GG stem methanol extract chloroform fraction; GGE, GG stem methanol extract ethyl acetate fraction; GGM, GG stem methanol extract methanol fraction. Values are expressed as mean±SEM.; n=6; the statistical significance was done by one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison tests and is represented by a symbol. [a]p<0.001 indicates comparison with Group I. [b]p<0.05 indicates comparison with Group II. [c]p<0.001 indicates the dose-dependent activity in comparison of the high dose with respective low doses of the GGSME fractions.

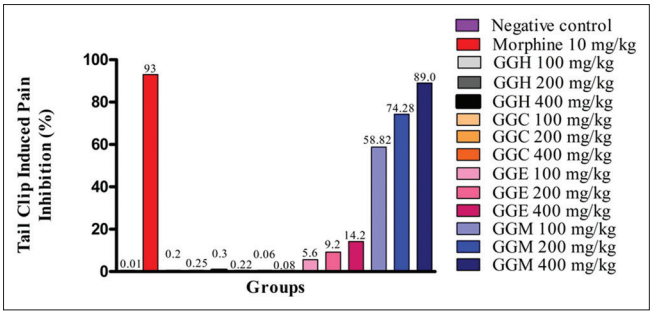

Fig. 1: Effect of *Galphimia glauca* stem methanol extract fractions on tail clip-induced pain in rats (Haffner's tail clip test)
GGH, GG stem methanol extract hexane fraction; GGC, GG stem methanol extract chloroform fraction; GGE, GG stem methanol extract ethyl acetate fraction; GGM, GG stem methanol extract methanol fraction.

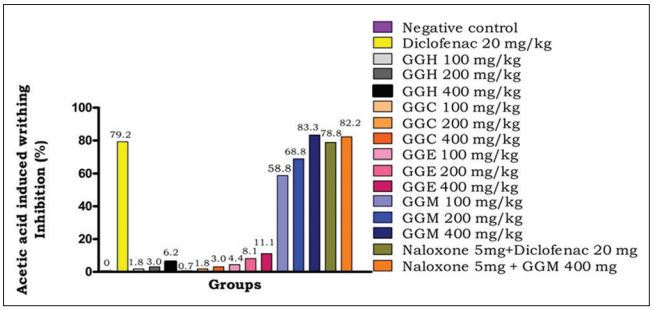

Fig. 2: Effects of *Galphimia glauca* stem methanol extract fractions on acetic acid-induced pain in mice

Formalin Test

This test disclosed the dose-dependent actions (p≤0.001) of GGM acting at the two phases. The GGM at all tested doses showed significant activity in comparison with standard drugs. The results are cited in Table 4 and Fig. 3.

Anti-inflammatory activity

Carrageenan-induced paw edema model

The accessed results are cited in Table 5. The GGM at higher dose of 400 mg/kg inhibited paw edema dose-dependently (p<0.001)

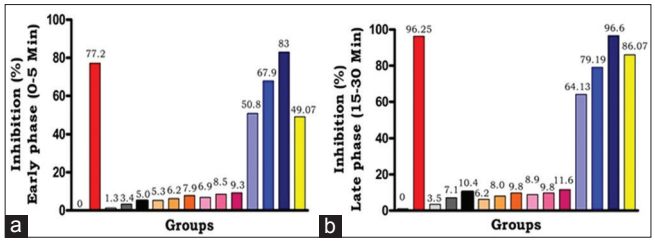

Fig. 3: Effects of *Galphimia glauca* stem methanol extract fractions on formalin-induced pain in mice: (a) Early phase (0–5 min); (b) Late phase (15–30 min)
GGH, GG stem methanol extract hexane fraction; GGC, GG stem methanol extract chloroform fraction; GGE, GG stem methanol extract ethyl acetate fraction; GGM, GG stem methanol extract methanol fraction.

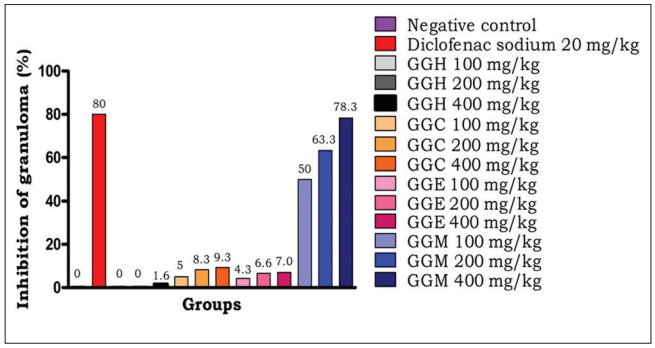

Fig. 4: Effects of *Galphimia glauca* methanol extract fractions on cotton pellet-induced granuloma test in rats
GGH, GG stem methanol extract hexane fraction; GGC, GG stem methanol extract chloroform fraction; GGE, GG stem methanol extract ethyl acetate fraction; GGM, GG stem methanol extract methanol fraction.

with percentage inhibition of 75.1% and 81.91% at 3rd and 4th h, respectively.

Cotton pellet-induced granuloma test

The GGM at higher dose reduced the transudative weight to 74.2 mg and granuloma formation to 78.3% when correlated with diclofenac sodium, which exhibited 93.5 mg and 80% reduction in transudative

Table 3: Analgesic effect of GG stem methanol extract fractions on acetic acid-induced pain in mice (writhing test)

Group (s)	Dose (mg/kg)	Acetic acid induced writhing	
		Number of writhing's	Inhibition (%)
I (Distilled water)	10 (ml/kg)	27±1.5	-
II (Diclofenac sodium)	20	5.6±0.2[a]	79.2
III (GGH)	100	26.5±1.4[b]	1.8
IV (GGH)	200	26.1±1.4[b]	3.07
V (GGH)	400	25.3±1.4[b]	6.2
VI (GGC)	100	26.8±1.2[b]	0.7
VII (GGC)	200	26.5±1.2[b]	1.8
VIII (GGC)	400	26.1±1.5[b]	3.07
IX (GGE)	100	25.8±1.5[b]	4.4
X (GGE)	200	24.8±1.6[b]	8.1
XI (GGE)	400	24±0.8[b]	11.1
XII (GGM)	100	11.1±1.4[a]	58.8
XIII (GGM)	200	8.4±0.8[a]	68.8
XIV (GGM)	400	4.5±0.4[a,c]	83.3
XV (Naloxone+Diclofenac sodium)	(5+10)	5.7±0.2	78.8
XVI (Naloxone+GGM)	(5+400)	4.8±0.4	82.2

GGH, GG stem methanol extract hexane fraction; GGC, GG stem methanol extract chloroform fraction; GGE, GG stem methanol extract ethyl acetate fraction; GGM, GG stem methanol extract methanol fraction.Values are expressed as mean±SEM.; n=6; the statistical significance was done by one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison tests and is represented by a symbol. [a]p<0.001 indicates comparison with Group I. [b]p<0.001, indicates comparison with Group II.

Table 4: Analgesic effect of *G. glauca* stem methanol extract fractions on formalin-induced pain in mice

Group (s)	Dose (mg/kg)	Paw licking time (s)			
		Early phase (0–5 min)	Inhibition (%)	Late phase (15–30 min)	Inhibition (%)
I (Distilled water)	10 (ml/kg)	173±3.3	-	112±3.0	-
II (Morphine)	10	39.3±1.1[a,c]	77.2	4.2±0.2[a]	96.25
III (GGH)	100	170.7±1.6[b,c]	1.3	108±3.9[b,c]	3.5
IV (GGH)	200	167.0±2.5[b,c]	3.4	104±3.7[b,c]	7.1
V (GGH)	400	164.3±1.8[b,c]	5.0	100.3±2.5[b,c]	10.4
VI (GGC)	100	163.8±2.0[b,c]	5.3	105±2.5[b,c]	6.25
VII (GGC)	200	162.2±2.6[b,c]	6.2	103±3.9[b,c]	8.0
VIII (GGC)	400	159.3±1.8[a,b,c]	7.9	101±3.7[b,c]	9.8
IX (GGE)	100	161.0±1.9[a,b,c]	6.9	102±2.5[b,c]	8.9
X (GGE)	200	158.2±1.9[a,b,c]	8.5	101±5.0[b,c]	9.8
XI (GGE)	400	156.8±2.1[a,b,c]	9.3	98.6±4.6[b,c]	11.6
XII (GGM)	100	85.1±3.7[a,b]	50.8	40.17±1.8[a,b,c]	64.13
XIII (GGM)	200	55.5±2.2[a,b,c,d]	67.9	23.3±2.4[a,b,d]	79.19
XIV (GGM)	400	29.1±1.4[a,c,d]	83	3.7±0.1[a,d]	96.6
XV (Diclofenac sodium)	20	93±2.3[a]	49.07	15.6±0.8[a]	86.07

GGH, GG stem methanol extract hexane fraction; GGC, GG stem methanol extract chloroform fraction; GGE, GG stem methanol extract ethyl acetate fraction; GGM, GG stem methanol extract methanol fraction. Values are expressed as mean±SEM.; n=6; the statistical significance was done by one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison tests and is represented by a symbol. [a]p<0.05 indicates comparison with Group I. [b]p<0.001 indicates comparison with Group II. [c]p<0.001 indicates comparison with Group XV. [d]p<0.001 indicates the dose-dependent activity in comparison of the high dose with respective low doses of the GGSME fractions

weight and granuloma formation. The results are cited in Table 6 and Fig. 4.

DISCUSSIONS

Many of the effective chemical compounds have come from medicinal plants. Natural medicine grew by sharing knowledge both locally as well as across cultures. It is therefore, important that the natural products must continue to retain their quality and efforts have to be made to tap the real potential of natural sources of medicines.

Medicinally, a very little known, GG from the genus *Galphimia* was chosen for this study to explore their pharmacological activities. The plant was chosen based on their traditional uses and there were no references reported for pharmacological research in the area of this study, so there is a need to identify the traditional uses to help the scientific community by sharing the current research work.

Based on our earlier research carried out, the best active extract, GGSME was subjected to fractionation with solvents of varying polarities [9]. The fractions n-hexane (GGH), chloroform (GGC), ethyl acetate (GGE), and methanol (GGM) were again subjected to the above discussed pharmacological studies to explore the active fraction that can be used for the separation and isolation of bioactive phytoconstituent.

The GGM (methanol fraction) exhibited more significant (p≤0.001 and p≤0.05) changes in its latency time than GGH (hexane fraction), GGC (chloroform fraction), and GGE (ethyl acetate fraction) when correlated with the negative control and the morphine group in the hot plate test and in the tail clip test. The central pain-relieving properties of GGM at 400 mg/kg dose were further proved through reversal actions of naloxone [9].

The GGM at all tested doses showed significant (p<0.001) activity in comparison with standard drugs. The results disclose the GGM effects

Table 5: Anti-inflammatory effects of *G. glauca* methanol extract fractions on carrageenan-induced paw edema test in rats

Group (s)	Dose (mg/kg)	Changes in paw edema volume after administration of control/standard/GGSME fractions (hr)							
		1st hr	IPE (%)	2nd hr	IPE (%)	3rd hr	IPE (%)	4th hr	IPE (%)
I (Distilled water)	10 (ml/kg)	1.17±0.01	-	1.25	-	1.57±0.02	-	1.88±0.01	-
II (Diclofenac sodium)	20	0.75±0.02[a]	35.89	0.51[a]	59.2	0.44±0.08[a]	71.9	0.29±0.02[a]	84.57
III (GGH)	100	1.17±0.02[b]	0.00	1.25±0.04[b]	0.00	1.57±0.02[b]	0.00	1.89±0.01[b]	0.00
IV (GGH)	200	1.17±0.02[b]	0.00	1.25±0.04[b]	0.00	1.57±0.02[b]	0.00	1.89±0.01[b]	0.00
V (GGH)	400	1.17±0.02[b]	0.00	1.25±0.04[b]	0.00	1.57±0.02[b]	0.00	1.89±0.01[b]	0.00
VI (GGC)	100	1.15±0.01[b]	1.70	1.22±0.03[b]	2.4	1.52±0.02[b]	3.18	1.79±0.01[b]	4.78
VII (GGC)	200	1.14±0.01[b]	2.56	1.20±0.04[b]	4	1.49±0.02[b]	5.09	1.74±0.06[b]	7.4
VIII (GGC)	400	1.13±0.01[b]	3.41	1.19±0.04[b]	4.8	1.48±0.01[b]	5.73	1.71±0.07[b]	9.04
IX (GGE)	100	1.16±0.01[b]	0.85	1.23±0.02[b]	1.6	1.51±0.02[b]	3.82	1.78±0.02[b]	5.31
X (GGE)	200	1.15±0.02[b]	1.70	1.20±0.04[b]	4	1.49±0.02[b]	5.09	1.75±0.06[b]	6.91
XI (GGE)	400	1.13±0.01[b]	3.4	1.18±0.12[b]	5.6	1.47±0.01[b]	6.36	1.69±0.03[a,b]	10.10
XII (GGM)	100	1.01±0.01[a,b]	13.6	0.96±0.02[a,b]	23.2	0.91±0.02[b]	42.0	0.87±0.02[a,b]	53.72
XIII (GGM)	200	0.93±0.03[a,b,c]	20.5	0.82±0.03[a,b,c]	34.4	0.70±0.01[b,c]	55.4	0.57±0.01[a,b,c]	69.68
XIV (GGM)	400	0.64±0.02[a,c]	45.2	0.45±0.02[a]	64	0.39±0.02[c]	75.1	0.34±0.02[a,b,c]	81.91

GGH, GG stem methanol extract hexane fraction; GGC, GG stem methanol extract chloroform fraction; GGE, GG stem methanol extract ethyl acetate fraction; GGM, GG stem methanol extract methanol fraction; hr: hour; IPE (%), percentage inhibition of paw edema Values are expressed as mean±SEM.; n=6; the statistical significance was done by one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison tests and is represented by a symbol. [a]p<0.05 indicates comparison with Group I. [b]p<0.001 indicates comparison with Group II. [c]p<0.001 indicates the dose-dependent activity in comparison of the high dose with respective low doses of the GGSME fractions.

Table 6: Anti-inflammatory effects of *G. glauca* methanol extract fractions on cotton pellet-induced granuloma test in rats

Group (s)	Dose (mg/kg)	Granuloma wet weight (mg)	Granuloma dry weight (mg)	Transudative weight (mg)	Granuloma weight (mg) (mg/mg cotton)	Inhibition of granuloma (%)
I (Distilled water)	10 (ml/kg)	184.2±2.1	80.3±2.3	124±5.6	3.3±0.3	-
II (Diclofenac sodium)	20	125.5±1.9[a]	32.6±3.8[a]	93.5±5.1[a]	0.6±0.7[a]	80
III (GGH)	100	186.2±1.7[b]	80.6±2.3[b]	104.0±4.8	3.7±0.1[a]	0.00
IV (GGH)	200	184.5±0.2[b]	80.8±2.9[b]	104.5±4.7	3.2±0.4[a]	0.00
V (GGH)	400	185.2±1.16[b]	79.5±2.4[b]	106.2±4.5	2.95±0.2	1.6
VI (GGC)	100	181.7±0.7[b]	77.8±3.4[b]	104.7±4.8	2.85±0.1[b]	5
VII (GGC)	200	179.0±2.3[b]	75.3±4.3[b]	104.9±4.2	2.75±0.7[b]	8.3
VIII (GGC)	400	174.2±3.7[b]	74.4±3.4[b]	99.8±3.9a	2.72±0.4[b]	9.3
IX (GGE)	100	180.8±1.35[b]	77.8±3.4[b]	103.3±4.2[a]	2.87±0.5	4.3
X (GGE)	200	177.3±3.7[b]	76.5±4.7[b]	101.3±5.2[a]	2.8±0.1	6.6
XI (GGE)	400	172.5±6.13[b]	75.0±4.3[b]	96.7±3.8[a]	2.79±0.4	7.0
XII (GGM)	100	157.5±7.01[a,b]	51.1±4.1[a,b]	106.5±5.8	1.50±0.3[a]	50
XIII (GGM)	200	130.3±3.05[a, c]	42.1±1.9[a]	88.3±3.8[a]	1.1±0.7[a]	63.3
XIV (GGM)	400	110.2±2.45[a, c]	33.5±2.4[a, c]	74.2±2.5[a,b,c]	0.65±0.2[a]	78.3

GGH, GG stem methanol extract hexane fraction; GGC, GG stem methanol extract chloroform fraction; GGE, GG stem methanol extract ethyl acetate fraction; GGM, GG stem methanol extract methanol fraction Values are expressed as mean±SEM.; n=6; the statistical significance was done by one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison tests and is represented by a symbol. [a]p<0.05 indicates comparison with Group I. [b]p<0.05 indicates comparison with Group II. [c]p<0.05 indicates the dose-dependent activity in comparison of the high dose with respective low doses of the GGSME fractions.

through the involvement of central and peripheral mechanisms. In writhing's test, GGM was significant (p≤0.001) in decreasing the number of writhing in mice, whereas the remaining fractions such as GGH, GGC, and GGE were insignificant. The peripheral action of GGM was also proved when naloxone-administered groups exhibited negative response on abdominal constriction in mice.

The anti-inflammatory activity in fractions was assessed by employing acute and chronic models of inflammation [9]. In acute model (carrageenan-induced paw edema test), the GGM at higher dose of 400 mg/kg inhibited paw edema dose-dependently (p<0.001), whereas in a chronic model (cotton pellet induced granuloma test), the GGM reduced the transudative weight and granuloma formation and the results were comparable with diclofenac sodium (at the tested dose). The remaining fractions GGH, GGC, and GGE exhibited insignificant results in both the models of inflammation.

The above results suggest the central analgesic actions of the GGM was perhaps mediated by the inhibition of opioid receptors. Similar kind of results was earlier reported by Zakaria *et al*., 2010 [9,19]. Peripherally acting drugs inhibit COX enzyme in the peripheral tissues by blocking the synthesis and/or release of inflammatory mediators like cell-derived mediators [Vaso active amines (histamine, 5HT and neuropeptide)], eicosanoids (PGD_2, PGE_2, PGF_2-α, PGI_2, and TXA_2), lysosomal components (granules of neutrophils, granules of monocytes, and tissue macrophages), platelet-activating factor, cytokines (IL-1 and IL-6 are active in acute inflammation, while IL-12 and IL-17 are active in chronic inflammation), and free radicals (Mohan, 2015) [10,20].

The process of acute inflammation was studied employing carrageenan-induced paw edema model. Two phases were involved in inducing paw edema, the initial phase, and the late phase. In the initial phase substances such as serotonin, kinins, and histamine were released, whereas prostaglandins were released in the late phase of inflammation (Mohan, 2015) [9,10,20,21].

In chronic inflammatory conditions, the macrophage stimulation was induced by IL-1α, IL-1β, IL-2, and TNF-α. In addition, macrophage proliferation was induced by multiplication of small blood vessel,

proliferation of fibroblasts, and M-CSF (Macrophage colony stimulating factor). The acute and chronic anti-inflammatory effects of GGM may be arbitrated through the above-discussed mechanisms. The analgesic and anti-inflammatory results of fractions suggest that GGM was more potent than the entire fractions employed to treat the pain and inflammation [11,22,23].

CONCLUSION

The GG stem methanol extract (GGSME) and its methanol fraction (GGM) have a significant analgesic and anti-inflammatory activity.

ACKNOWLEDGMENTS

The authors would like to thank the Chairman, Dr. P. Rajeshwar Reddy, Anurag Group of Institutions for providing the best research facilities.

AUTHOUR'S CONTRIBUTION

Conception and design of the work were done by Dr. K. Srisailam and V. UmaMaheshwaraRao. The entire experimental work and drafting was done by Dr. G. Baba Shankar Rao. The experimental assistance was provided by Dr. Vasudha Bakshi.

CONFLICTS OF INTEREST

We declare that we do not have any conflicts of interest.

REFERENCES

1. World Health Organization. The Top 10 Causes of Death by a Broad Income Group. World Health Organization; 2004. Available from: http://www.who.int/mediacentre/factsheets/fs310/en/index.html. [Last accessed on 2009 Apr 12].
2. Veeresham C. Natural products derived from plants as a source of drugs. J Adv Pharm Technol Res 2012;3:200-1.
3. Anderson C. Revision of *Galphimia* (*Malpighiaceae*). Contr Univ Mich Herbarium 2007;25:1-82.
4. Anderson C. *Galphimia* (*Malpighiaceae*) in South America. Contr Univ Mich Herbarium 2005;24:1-2.
5. Tortoriello J, Lozoya X, Gomez E, Brunner M. Mesenceplalic effects of galphimine-B, a triterpenoid from *Galphimia glauca*. Neurophytopharmaceuticals 1998;3:77-96.
6. Herrera-Ruiz M, González-Cortazar M, Jiménez-Ferrer E, Zamilpa A, Alvarez L, Ramírez G, *et al.* Anxiolytic effect of natural galphimines from *Galphimia glauca* and their chemical derivatives. J Nat Prod 2006;69:59-61.
7. Tortoriello J, Arellano AH, Herrera-Ruiz ML, Zamilpa A, Cortazar MG, Jimenez Ferrer JE. New anxiolytic phytopharmaceutical elaborated with the standardized extract of *Galphimia glauca*. In: Kalinin V, editor. Anxiety Disorders. Croatia: In Tech Publication; 2011.
8. Cardoso Taketa AT, Lozada-Lechuga J, Fragoso-Serrano M, Villarreal ML, Pereda-Miranda R. Isolation of nor-secofriedelanes from the sedative extracts of *Galphimia glauca*. J Nat Prod 2004;67:644-9.
9. Baba Shankar Rao G, Srisailam K, Uma Maheswararao V. Assessment of Anti-nociceptive and anti-inflammatory activities of *Galphimia glauca* stem methanol extract on noxious provocation induced pain and inflammation in *in vivo* models. J Chem Pharm Res 2016;8:1282-9.
10. Baba Shankar Rao G, Srisailam K, Uma Maheswararao V. *In vivo* evaluation of noxious stimulus induced pain and inflammation in mice and rats using a methanol extract of *Galphimia glauca* leaf. Asian J Chem 2016;28:1815-9.
11. Baba Shankar Rao G, Srisailam K, Uma Maheswararao V. CNS depressant effects and muscle relaxant activity of *Galphimia glauca* leaf methanol extract. Int J Pharm Tech Res 2016;9:230-40.
12. Garige BS, Keshetti S, Vattikuti UM. *In vivo* study on depressant effects and muscle coordination activity of *Galphimia glauca* stem methanol extract. Pharmacognosy Res 2016;8:219-25.
13. OECD Guidelines 423. OECD Guideline for Testing of Chemicals. Acute Oral Toxicity- Acute Toxic Class Method. Paris: OECD Publishing; 2001.
14. Khandelwal KR. Practical Pharmacognosy Techniques and Experiments. 21st ed. Pune: Niraliprakashan; 2002.
15. Murray CW, Porreca F, Cowan A. Methodological refinements to the mouse paw formalin test. An animal model of tonic pain. J Pharmacol Methods 1988;20:175-86.
16. Koster R, Anderson M, De Beer EJ. Acetic acid for analgesic screening. Fed Proc 1959;18:412-21.
17. Winter CA, Risley EA, Nuss GW. Carrageenin-induced edema in hind paw of the rat as an assay for anti-iflammatory drugs. Proc Soc Exp Biol Med 1962;111:544-7.
18. Meier R, Schuler W, Desaulles P. On the mechanism of cortisone inhibition of connective tissue proliferation. Experientia 1950;6:469-71.
19. Zakaria ZA, Patahuddin H, Mohamad AS, Israf DA, Sulaiman MR. *In vivo* anti-nociceptive and anti-inflammatory activities of the aqueous extract of the leaves of *Piper sarmentosum*. J Ethnopharmacol 2010;128:42-8.
20. Mohan H. Textbook of Pathology. 4thed. New Delhi: Jaypee Brothers Medical Publishers (P) Ltd.; 2015.
21. Chauhan S, Navpreet K, Lishore L, Randhir S. Pharmacological evaluation of anti-inflammatory and analgesic potential of *Litchi chinensis* Gaertn. (Sonn). Int J Pharm Pharm Sci 2014;6:116-9.
22. Pandeya SN. A Textbook of Medicinal Chemistry. 3rd ed. Varanasi: SG Publishers; 2004.
23. Laaboudi W, Ghanam J, Aissam H, Merzouki M, Benlemlih M. Anti-inflammatory and analgesic activities of olive tree extract. Int J Pharm Pharm Sci 2016;8:414-9.