ORIGINAL ARTICLE

Evaluation of anti-inflammatory and antinociceptive activity of methanol extract of *Calotropis gigantea* root

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Abstract

Objective: Extracts of the root of *Calotropis gigantea* (family: *Apocynaceae*) have been used as a natural therapeutic agent in traditional medicine to treat inflammation and pain. This study aims to evaluate the anti-inflammatory and analgesic activity of methanol extract of *C. gigantea* root (MECG). **Materials and Methods:** The analgesic activity of MECG was evaluated using formalin-induced pain and glutamate-induced paw licking models. Antagonism of opioid receptors using naloxone was used to determine the involvement of central pathways of pain. The acute inflammation was measured by carrageenan and dextran-induced paw edema models. Cyclooxygenase (COX) assay was carried out to determine the action of MECG on prostaglandins (PGs). **Results:** MECG 200 mg/kg dose was found to produce a significant ($P < 0.001$) and dose-dependent analgesic activity in the models used. MECG caused significant inhibition of edema in the carrageenan and dextran-induced inflammation tests. MECG was found to reduce the expression of COX, thus confirming the inhibitory action of MECG on PGs. **Conclusion:** The findings suggest that MECG possesses analgesic and anti-inflammatory activity mediated through peripheral and central mechanisms. The results justify its traditional use in the treatment of inflammation and pain.

Key words: Analgesic, anti-inflammatory, central analgesia, oxidative stress, paw edema, traditional medicine

INTRODUCTION

Inflammation is a defense system against infection and tissue repair caused by injury or trauma. It protects from injurious stimuli and initiates the process of healing, but inability to control the process can cause damage to own tissue or organ function of a host. Chronic inflammation and pain have assumed great importance in global scientific research due to their presence in numerous human diseases.[1,2] Current anti-inflammatory drugs are not ideally suited for long-term use because they interfere with important biochemical pathways and associated toxicities. The same is true for long term use of analgesics, which have many adverse effects.

Inflammation is a complex pathophysiological process mediated by a variety of signaling molecules produced by leukocytes, macrophages, and mast cells. Inflammatory mediators such as nitric oxide (NO), prostaglandins (PG), and tumor necrosis factor (TNF-α) are produced which amplify the inflammatory response and induce extravasations of fluids leading to edema formation.[1,3-5] Many inflammatory mediators such as platelet-activating factor, PGs, kinins, NO, and cytokines are involved in the recruitment of circulating leukocytes.[1,4] An inflammatory response is also related

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to reactive oxygen species (ROS) produced by neutrophils and activated macrophages.[6,7] PGs induce hyperalgesia by increasing sensitivity of free nerve endings and induce pain. Aδ and C fibers are the primary nociceptive neurons involved in transmission of pain stimuli.[8-10]

Recent studies with a number of herbal extracts have shown promising results. It has been shown that these compounds isolated from various medicinal plants express their anti-inflammatory activities by down regulating expression of several crucial proinflammatory mediators like inducible NO synthase (iNOS), PGs, interleukin-1 β (IL-1β), TNF-α and IL-10.[3,9,10] Due to the adverse effects of nonsteroidal anti-inflammatory drugs and opioids, the search is on for new drugs with lesser side effects. Many valuable drugs of today (e.g., atropine, ephedrine, tubocurarine, digoxin, reserpine, aspirin, vincristine, morphine, and quinidine) came into use through the study of herbal and indigenous remedies.

Calotropis gigantea belongs to the family *Apocynaceae*. The family has a worldwide distribution in tropical and warm climates and is found abundantly in tropical forests. It is commonly called as "crown flower" or "giant milk weed" is a well-known weed to many cultures for treating various disorders related to central nervous system, skin diseases, digestive system, respiratory system, reproductive system, etc.[11] It is used as a traditional medicinal plant and is used to treat common disease such as fevers, rheumatism, indigestion, cough, cold, eczema, asthma, elephantiasis, nausea, vomiting, and diarrhea. According to Ayurveda, dried whole plant is a good tonic, expectorant, depurative, and anthelmintic.[12] The root bark is febrifuge, anthelmintic, depurative, expectorant, and laxative. The powdered root used in asthma, bronchitis, and dyspepsia. Flowers of *C. gigantea* have been found to have hepatoprotective activity against carbon-tetrachloride-induced liver damage in mice.[13] The leaves are useful in the treatment of paralysis, arthralgia, swellings, and intermittent fevers. The leaves have been found to have sedative and anxiolytic effect.[14] The flowers are bitter, digestive, astringent, stomachic, anthelmintic, and tonic. The alcoholic extract of the flowers of *C. gigantea* was found to possess analgesic activity in chemical and thermal models in mice.[15] The traditional practitioners use the leaf extract for the treatment of inflammatory painful conditions and rheumatic pain. The root extract is used for the treating inflammation and as an analgesic by many folk tribes of state of West Bengal, India. To validate the traditional claims of *C. gigantea*, this study is designed to investigate the *in vivo* anti-inflammatory and analgesic activity of the methanol extract of *C. gigantea* (MECG) in animal models.

MATERIALS AND METHODS

Chemicals and Reagents

Methanol, formalin procured from LOBA Chemicals, Mumbai; pentazocine, diclofenac, from Sun Pharma, naloxone and indomethacin from Cipla. All the chemicals and reagents procured were of analytical grade and obtained from Hi-Media Research Laboratories Pvt. Ltd., Mumbai, India.

Collection and Authentication

For the study, whole plants of *C. gigantea* were collected from the Local Market, Town, West Bengal. It is identified and authenticated by Dr. V. Sampath Kumar, Botanical Survey of India, Government of India, Howrah, West Bengal, and a voucher specimen was also deposited for future reference.

Preparation of the Plant Extract

Dried and powdered root of *C. gigantea* (100 g) was subjected to solvent extraction in a Soxhlet extractor using methanol solvent. The extract was vacuum dried using rotary evaporator to obtain methanol extract. The weight of the dried methanol extract was 17.62 g. The percentage yield was 17.62%.

Yield (%) = (Weight of dried extract/Weight of plant starting material) × 100

Phytochemical Screening

Phytochemical screening of the methanol extract of bark of *C. gigantea* was carried out using standard procedures.[16]

Animals

Albino rats of Wistar strain of 8-10 weeks of age weighing between 100 and 120 g were used for the study. The animals housed in polypropylene cages were fed on pellet diet and water *ad libitum*. Animals were kept under standard laboratory conditions in 12 h light-dark cycle at 25 ± 2°C. Animals were acclimatized for at least 1 week before using them for experiments. All the experiments were performed according to current guidelines for the care of the laboratory animals, and the ethical guidelines for the investigation of experimental pain in conscious animals according to Committee for Control and Supervision of Experiments on Animals (CPCSEA) guidelines (Registration Number: 1805/Go/Re/S/15/CPCSEA).

Acute Toxicity

Toxicity study of MECG was determined according to the OECD guidelines No. 423. Wistar rats 100-150 g (n = 5×4) were used for this study. The extract was administered orally at the single dose of 5, 50, 300, 2000 mg/kg, bw. After the administration of test substance, food for the rat was withheld for 2 h. Animals were observed individually after at least once during the first 30 min, with special attention given the first

4 h, for a total of 14 days. All treated animals were monitored for at least twice daily for behavioral and mortality changes.

Formalin Test

Mice fasted overnight were divided into five groups of six animals each. The different groups of animals were treated orally with distilled water (10 ml/kg); MECG (50, 100, and 200 mg/kg); and pentazocine (30 mg/kg). Formalin (20 µL of 1% solution) injected subcutaneously into the right hind paw of each mouse after 60 min of drug administration is the pain inducing stimuli, which is elicited by licking and biting responses of the injected paw. The time (in seconds) spent in doing so was recorded for each animal. The responses of the mice were observed for 5 min (first phase) and 15-30 min (second phase) post-formalin injection.[17,18] The first 5 min after formalin injection (early phase) and 15-30 min after formalin injection (late phase) were represented as neurogenic and inflammatory pain, respectively.[19]

Inhibition (%) = {(Reaction time [Control] − Reaction time [Treated])/Reaction time [Control]} × 100

Acetic Acid Induced Writhing Model

Mice used in this study were fasted overnight. The mice were divided into five groups of six animals each. The animals were then treated with distilled water (10 ml/kg, p.o.) (control group); MECG (50, 100, and 200 mg/kg, p.o.); and diclofenac sodium (10 mg/kg, p.o.). 60 min after treatment was carried out; mice were administered with acetic acid (0.6%, v/v in saline, 10 ml/kg, i.p.). The number of writhes (characterized by contraction of the abdominal musculature and extension of the hind limbs) was then counted for 20 min.[9,10]

Inhibition (%) = {(Number of writhes [control] − Number of writhes [treatment])/Number of writhes [control]} × 100

Glutamate-induced Paw Licking Test

Mice were divided into five groups of six animals each. The different groups of animals were treated with distilled water (10 ml/kg, p.o.), MECG (50, 100, and 200 mg/kg, p.o.), and diclofenac sodium (10 mg/kg, p.o.). 30 min post-treatment, 20 µl of glutamate (in phosphate-buffered saline solution) was injected intraplantary in the right hind paw. The animals were then observed for 15 min following glutamate injection and the time spent licking or biting the injected paw was recorded as an indication of nociception.[20,21]

Involvement of Opioid Receptors Using Tail Flick Test

The possible involvement of the opioid receptors in the antinociceptive effect caused by MECG was investigated using the tail flick test. The animals were divided into five groups ($n = 6$) and pretreated with vehicle (10 ml/kg, p.o.), MECG (200 mg/kg, p.o.), pentazocine (30 mg/kg, s.c.), MECG + naloxone (5 mg/kg, i.p.), and pentazocine (30 mg/kg, s.c.) + naloxone (5 mg/kg, i.p.) 30 min before carrying out the tail flick test. The tail flick latency is measured for the various test groups.[19,22]

Carrageenan-induced Paw Edema

Paw edema is induced by administration of the phlogistic agent, carrageenan. Rats used in this experiment were divided into five groups, and the respective groups were treated with distilled water (10 ml/kg, p.o.), MECG (50, 100 and 200 mg/kg, p.o.), and indomethacin (5 mg/kg, p.o.) ($n = 6$). 1 h after administration of the various agents, edema was induced by injection of carrageenan (0.1 ml, 1%, w/v in saline) into the subplantar tissue of the right hind paw (Winter *et al.,* 1962). Paw volume was measured plethysmographically at 0^{th}, 1^{st}, 2^{nd}, 3^{rd}, 4^{th} and 6^{th} h after carrageenan administration.[23,24]

Inhibition (%) = (Increase in paw edema, control − Increase in paw edema, treated)/Increase in paw edema, control) × 100

Dextran-induced Paw Edema

In the case of dextran-induced paw edema, the procedure was the same as used for carrageenan, and the edema measurements were carried out at 30, 60 and 120 min after the injection.[25]

Cyclooxygenase (COX) Assay

The COX assay was conducted using the Cayman COX assay kit. The COX activity assay utilizes the peroxidase component of COX. The peroxidase activity is assayed colorimetrically by monitoring the appearance of oxidized N,N,N',N'-tetramethyl-p-phenylenediamine at 590 nm. The measurements were performed according to the manufacturers' protocols. The reaction mixture contains, 150 ml of assay buffer, 10 ml of heme, 10 ml of enzyme (either COX-1 or COX-2), and 10 ml of plant sample (1 mg/ml). Indomethacin (30 mM) was used as a standard drug.[26] The percent COX inhibition was calculated using following equation:

COX inhibition activity (%) = $(1-T/C) \times 100$

Where, T = Absorbance of the inhibitor well at 590 nm.

C = Absorbance of the 100% initial activity without inhibitor well at 590 nm.

Statistical Analysis

All the values were expressed as mean ± standard error of mean. Statistical significance was analyzed by one-way

analysis of variance followed by student Newman-Keuls multiple comparison test. GraphPad InStat (version 3.06) software was used for all statistical analysis.

RESULTS AND DISCUSSION

Phytochemical Screening

Phytochemical screening showed the presence of alkaloids, carbohydrates, proteins, phenolic compounds, glycosides, and flavonoids in MECG. The amount of phenolic components is calculated as pyrocatechol equivalents. It is found to be 127.57 ± 0.73 µg/mg pyrocatechol equivalents. The total flavonoid concentration was measured as quercetin equivalents and is found to be 44.71 ± 0.43 µg/mg quercetin equivalents.

Acute Toxicity Study

MECG did not show any mortality at 2000 mg/kg, and thus was considered experimentally non toxic.

Formalin Test

MECG had significant analgesic effects on both early (0-5 min) and late (15-30 min) phases of formalin-induced pain as shown in Table 1. The formalin-induced paw licking test is a well-described model of nociception, which is illustrated by the presence of separate biphasic nociceptive responses. The first phase (0-5 min) of pain is the neurogenic pain while the second phase (15-30 min), involves inflammatory processes.[17,18] Substance P and bradykinin are believed to participate in the first-phase responses while histamine, serotonin, PG, and bradykinin are involved in the second-phase responses.[27] In the first phase, injection of formalin into the subplantar tissue of the right hind paw of control mice produced nociceptive response of biting and licking of the treated paw with a total duration of 124.74 ± 4.3 s. MECG produced a significant ($P < 0.001$) dose-dependent inhibition of nociceptive reaction with peak effect produced at the highest dose of 200 mg/kg. This effect was less but not significantly different ($P > 0.05$) from that produced by 30 mg/kg pentazocine. In second phase, the total duration of nociceptive reaction in the control group was 184.26 ± 5.6 s. The effect of MECG in inhibiting the biting and licking response was significant ($P < 0.001$). The greatest inhibition was produced at the highest dose of 200 mg/kg. The results indicate that MECG has inhibitory actions on both the inflammatory and neurogenic components of pain. Since MECG inhibited both phases, it may be inferred that the analgesic activity results from combination of peripheral and central mechanisms of action.

Table 1: The antinociceptive effect of MECG as observed in the formalin test in mice

Treatment	Early phase (0-5 min)	Late phase (15-30 min)
Control	124.74±4.3	184.26±5.6
Pentazocine 30 mg/kg	73±2.671***	44.83±2.4***
MECG 50 mg/kg	53.33±3.051***	27.5±2.37***
MECG 100 mg/kg	23.56±2.603***	17.83±1.9***
MECG 200 mg/kg	19.2±2.236***	12.16±1.13***

Values are presented as the mean±SEM (n=6). The asterisks denote the significance levels as compared to control, ***P<0.001, by one-way ANOVA followed by student Newman-Keuls multiple comparison test. ANOVA: Analysis of variance, MECG: Methanol extract of *Calotropis gigantea*, SEM: Standard error of mean

Acetic Acid Induced Writhing Model

As shown in Figure 1, intraperitoneal injection of acetic acid resulted in writhing responses in control mice with 52.23 ± 2.3 writhes counted in 20 min. MECG produced a significant dose-dependent ($P < 0.001$) reductions in the number of writhes with peak effect produced at the highest dose of 200 mg/kg. This effect was comparable and not significantly different ($P > 0.05$) from that produced by 10 mg/kg diclofenac. It has been suggested that acetic acid injection into peritoneal cavity leads to an increased levels of COX products in peritoneal fluids and the release of many inflammatory mediators such as PGs, bradykinin, substance P, TNF-α, IL-1β, IL-8 (Ikeda *et al*., 2001). The dose-dependent inhibition of writhings induced by acetic acid in this study by MECG suggest the mechanism of MECG may be linked partly to the inhibition of COX and other inflammatory mediators in peripheral tissues.[28]

Glutamate-induced Paw Licking Test

The results presented in Figure 2 shows that MECG (50, 100, and 200 mg/kg), caused a significant inhibition of

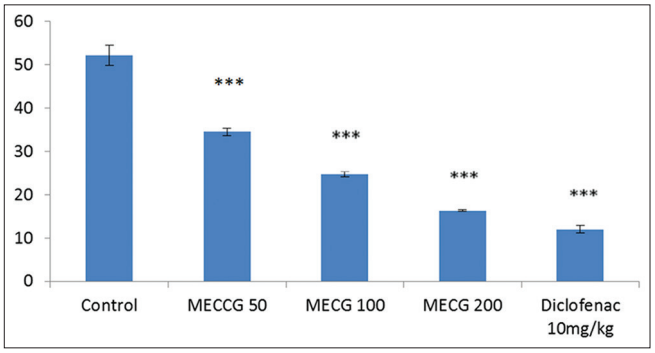

Figure 1: Analgesic effect of methanol extract of *Calotropis gigantea* (MECG) as observed in the acetic acid-induced writhing test. Values are presented as the mean ± standard error of mean (n=6). MECG: Methanol extract of *Calotropis gigantea*. The asterisks denote the significance levels as compared to control, ***$P < 0.001$, by one-way analysis of variance followed by student Newman-Keuls multiple comparison test

glutamate-induced paw licking ($P < 0.001$, compared with control). The maximum inhibition of paw licking was observed with 200 mg/kg MECG which was comparable to diclofenac ($P < 0.05$). Administration of MECG produced a significant and dose-dependent suppression of the nociceptive response caused by intraplantar injection of glutamate. The nociceptive response induced by glutamate appears to involve the peripheral, spinal and supraspinal sites of action and is greatly mediated by the activation of N-methyl-d-aspartate (NMDA) receptors, as well as by NO release or by some NO derived substances. NO is an important neurotransmitter involved in the nociceptive process and contributes to the development of central sensitization in the dorsal horn of the spinal cord. Inhibition of glutamate induced nociception suggests that inhibition of NO release or blockade of NMDA receptors could be contributing to the analgesic effect of MECG.[20,21,28]

Involvement of Opioid Receptors

The tail flick latency observed for MECG + naloxone group is significantly lower than that observed for MECG group after both 30 and 60 min of treatment ($P < 0.001$). This suggests that the nociception provided by MECG is inhibited by the addition of naloxone. The tail flick latency observed for MECG + naloxone group is comparable and similar to that of pentazocine + naloxone group ($P < 0.05$ at 30 min posttreatment and $P > 0.05$ (nonsignificant) at 60 min posttreatment, respectively). This result suggests that opioid system might be involved in the antinociceptive effect of MECG. The results are presented in Table 2. The central antinociceptive effect of the MECG is supported by the results observed in the tail flick test which uses heat stimuli to induce pain.[29] Opioid analgesics exhibit their effects both via supraspinal (μ1, κ3, δ1, σ2) and spinal (μ2, κ1, δ2) receptors.[30] The tail flick response appears to be a spinal reflex, which is modulated by supraspinal neuronal networks. The supraspinal systems that regulate the spinal reflexes could involve fibers that descend through the dorsolateral funiculus. The central antinociceptive effect of the MECG is supported by the results observed in the tail flick test. In addition, the results also showed that pretreatment with naloxone, an opioid receptor antagonist significantly antagonized the antinociceptive effect of MECG and pentazocine in the tail flick test. This confirms the central mode of action of MECG, which could be due to stimulation of opioid receptors.[19,29,31]

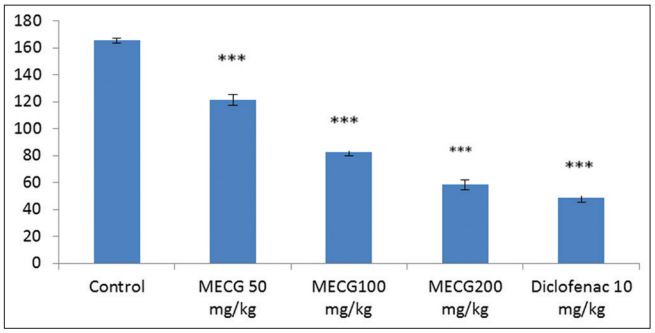

Figure 2: The antinociceptive effect of methanol extract of *Calotropis gigantea* as observed in glutamate-induced paw licking test. Values are presented as the mean ± standard error of mean ($n = 6$). MECG: Methanol extract of *Calotropis gigantea*. The asterisks denote the significance levels as compared to control, ***$P < 0.001$, by one-way analysis of variance followed by student Newman-Keuls multiple comparison test

Carrageenan-induced Paw Edema

For the vehicle treated group, volume of the posterior intraplantar left paw progressively increased after being injected with 0.1 ml of 1.0% carrageenan, reaching the peak of edema at the 4th h (1.08 ± 0.03 mL). Administration of MECG at 50, 100 and 200 mg/kg caused a reduction of the edema. The effect of MECG was dose-dependent from the 4th to the 6th h with peak effect produced at the dose of 200 mg/kg at the 6th h ($P < 0.001$). The effect of MECG at 200 mg/kg and indomethacin (10 mg/kg) was equivalent at the 6th h ($P > 0.05$). The results are shown in Table 3. The acute anti-inflammatory activity of MECG was evaluated in this study using the carrageenan induced paw edema. Carrageenan is a phlogistic agent, and when injected locally into the rat paw, produces a severe inflammatory reaction. Carrageenan-induced inflammation consists of three distinct phases including an initial release of histamine and serotonin; a second phase mediated by kinins; and a third phase involving PGs.[32,33] In this study, MECG showed significant inhibitory effect on rat paw edema development in the middle phase and more pronouncedly in the later phase of carrageenan-induced

Table 2: Analgesic effect of MECG as observed in the tail flick test using opioid antagonist in mice

Treatment	Tail flicking latency at 30 min	Tail flicking latency at 60 min
Control	6.45±0.11	6.3±0.1
MECG 200 mg/kg	12.2±0.41***	11.3±0.4***
MECG (200 mg/kg) + naloxone (5 mg/kg)	6.71±0.2	6.5±0.3
Pentazocine (30 mg/kg)	11.41±0.32***	12.12±0.6***
Pentazocine (30 mg/kg) + naloxone 5 mg/kg	6.37±0.2	6.81±0.2

Values are presented as the mean±SEM (n=6). The asterisks denote the significance levels as compared to control, ***P<0.001, by one-way ANOVA followed by student Newman-Keuls multiple comparison test. MECG: Methanol extract of *Calotropis gigantean,* ANOVA: Analysis of variance, SEM: Standard error of mean

Table 3: Anti-inflammatory effect of MELG as observed in the Carragennan-induced edema model

Treatment	Edema volume (ml) 0 h	Edema volume (ml) 1 h	Edema volume (ml) 2 h	Edema volume (ml) 3 h	Edema volume (ml) 4 h	Edema volume (ml) 6 h
Control	0.34±0.03	0.47±0.04	0.65±0.03	0.88±0.06	1.08±0.08	0.92±0.08
MECG 50 mg/kg	0.35±0.01	0.46±0.02	0.65±0.01	0.87±0.02	1.04±0.06	0.88±0.03
MECG 100 mg/kg	0.34±0.03	0.45±0.03	0.62±0.02	0.79±0.03**	0.97±0.02**	0.84±0.03**
MECG 200 mg/kg	0.34±0.02	0.44±0.05	0.57±0.04***	0.61±0.01***	0.54±0.03***	0.47±0.04***
Indomethacin 5 mg/kg	0.33±0.01	0.40±0.03	0.45±0.01***	0.52±0.02***	0.46±0.04***	0.41±0.02***

Values are presented as the mean±SEM (n=6). The asterisks denote the significance levels as compared to control, ***P<0.001, **P<0.01 by one-way ANOVA followed by student Newman-Keuls multiple comparison test. MELG: Methanol extract of *Calotropis gigantea*, ANOVA: Analysis of variance, SEM: Standard error of mean

inflammation. This suggests that the extract possibly acts by inhibiting the release and/or actions of vasoactive substances (histamine, serotonin, and kinins) and PGs.

Dextran-induced Paw Edema

In the vehicle treated group, the intraplantar injection of 0.1 mL of 1.5% dextran promoted an edema characterized by sudden onset and reaching the peak at 30 min (0.94 ± 0.04 mL), as shown in Table 4. All the three doses of MECG produced significant inhibition of paw edema at all the time intervals (P < 0.001 at 30 min, 60 min and 120 min). Maximum inhibition of paw edema was observed with MECG 200 mg/kg dose and was equivalent to that produced by 5 mg/kg indomethacin at 120 min (P > 0.05). Edema produced by subplantar injection of dextran in animals is characterized by a rapid increase in the paw edema and spontaneous decrease after 30 min, with histamine and serotonin being the main mediators.[25,32] Reduction in dextran-induced paw edema by MECG suggests that inhibition of histamine and serotonin releases could be responsible for reducing fluid extravasations caused by dextran.[32]

COX Assay

The results of the COX inhibition using different concentrations of MECG are summarized in Table 5. MECG was found to inhibit both COX-1 and COX-2. At 100 µg/ml concentration of MECG, the COX-1 and COX-2 inhibition was found to be 82.54 ± 2.7% and 74.36 ± 4.6%, respectively. Inhibition of COX produced by indomethacin (30 mM) was 94.34 ± 3.6% and 59.48 ± 2.6% inhibition for COX-1 and COX-2. Inhibition of COX leads to reduced levels of PGs, which are very significant inflammatory mediators and are involved in increased pain sensitivity.[26,34]

Mechanism of Action

Damaged cells releases arachidonic acid which is converted through enzymatic reactions to leukotrienes and PGs which can trigger further inflammatory responses and increase the sensitivity of pain receptors. Concurrently, the production of ROS and proinflammatory cytokines from injured cells also can initiate the activation of nuclear factor kappa (NF-kB-light-chain-enhancer of activated B cells), a transcription factor that is responsible for the activation of genes associated with the transcription of inflammatory mediators, such as ILs, TNF-α and PGs, and inflammatory enzymes, such as iNOS responsible for the synthesis of NO and COXs which finally leads to a complex cascade of signaling events of pain perception. NF-kB and C-jun proteins play a pivotal role in the inflammatory cycle by enhancing the expression of various proinflammatory cytokines and enzymes. Under normal physiological conditions, the intracellular levels of ROS are regulated and normal oxidative status of the body is maintained.

C. gigantea L. belonging to *Apocynaceae* contains a galaxy of phytoconstituents which are thought to exert anti-inflammatory effect through a multi modal approach, hitting multiple therapeutic targets. Some of the possible targets reported for anti-inflammatory activity of natural products by Dulce *et al.* are as follows:[35] Anti-inflammatory effect can be due to the inhibition and the stimulation of the production of cytokines IL-12 and of IL-4, respectively, in addition to the decrease in NO; positive effect over proinflammatory markers, relieving oxidative stress and downregulating COX-2, TNFα, NF-κB, and IL-8; inhibition of the inflammatory cytokine-induced production of PGE2 and NO which ultimately inhibits lipopolysaccharide-induced NO production in a dose-dependent manner by the suppression of iNOS and COX-2 production and TNF-α and PGE2 inhibition.

The plant under investigation is reported to contain glycosides, triterpenoids, saponins, flavonoids which have been duly credited for anti-inflammatory responses through multimodal approach. Possible inhibition of COX synthesis in tandem with downregulation of NF-κB, scavenging of free radicals and inhibition of other proinflammatory mediators besides COX by downregulating their activity or gene expression could be the possible modes of action. However, further studies at molecular level are required to elucidate the exact mechanistic pathway. However, this research shall form an excellent basis for developing the bioactive extract of the plant *C. gigantea* as an excellent drug candidate in the near future.

Table 4: Anti-inflammatory effect of MECG as observed in the dextran-induced edema

Treatment	Edema volume (ml) 0 h	Edema volume (ml) 30 min	Edema volume (ml) 60 min	Edema volume (ml) 120 min
Control	0.34±0.03	0.94±0.04	0.84±0.03	0.63±0.06
MECG 50 mg/kg	0.35±0.01	0.72±0.02	0.65±0.01	0.55±0.02
MECG 100 mg/kg	0.34±0.03	0.74±0.03	0.46±0.02***	0.41±0.03**
MECG 200 mg/kg	0.34±0.02	0.64±0.05***	0.42±0.04***	0.3±0.01***
Indomethacin 5 mg/kg	0.33±0.01	0.40±0.03***	0.32±0.01***	0.28±0.02***

Values are presented as the mean±SEM (n=6). The asterisks denote the significance levels as compared to control, ***P<0.001, **P<0.01 by one-way ANOVA followed by student Newman-Keuls multiple comparison test. MELG: Methanol extract of *Calotropis gigantea*, ANOVA: Analysis of variance, SEM: Standard error of mean

Table 5: The effect of MECG on COX-1 and COX-2 inhibition

Treatment	% COX-1 inhibition	% COX-2 inhibition
MECG (25 µg/ml)	45.96±1.18	37.25±1.3
MECG (50 µg/ml)	66.17±2.5	49.74±2.3
MECG (75 µg/ml)	79.21±1.89	65.64±3.8
MECG (100 µg/ml)	82.54±2.7	74.36±4.6
Indomethacin (30 mM)	94.34±3.6	59.48±2.6

Values are presented as the mean±SEM (n=6).
MECG: Methanol extract of *Calotropis gigantea,*
COX: Cyclooxygenase, SEM: Standard error of mean

CONCLUSION

The present work aimed to evaluate the anti-inflammatory and antinociceptive activities of the MECG. The results obtained indicate that the MECG has significant anti-inflammatory and analgesic activity, which thus validates the traditional claims of *C. gigantea*. The results of this study reveal that administration of MECG produced pronounced antinociceptive effects, and the actions were mediated by both peripherally and centrally acting mechanisms. This particular research can definitely provide a strong scientific evidence for the extract of *C. gigantea* to be developed as possible natural product lead.

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