

Anti-inflammatory and Anti-nociceptive Effects of Ethyl Acetate Fraction of Root Bark of *Cassia sieberiana* D. C. in Murine Models

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ABSTRACT

Background: The anti-inflammatory and anti-nociceptive effect of the ethyl acetate fraction of the root bark of *Cassia sieberiana* D.C. (Caesalpinaceae), a plant used locally in Ghana for treating pain associated with ulcer and menstruation, was evaluated in various rodent models. **Method:** The anti-inflammatory property was assessed using carrageenan-induced rat paw edema test while the anti-nociceptive effect was evaluated in the formalin, mechanical hyperalgesia, hot plate and acetic acid writhing tests. The possible involvement of the opioid, muscarinic cholinergic, adenosinergic, NO-cGMP and ATP-sensitive K⁺ channels receptor systems in the anti-nociceptive effect of *C. sieberiana* extract (CS-Ea) was also studied in the acetic acid writhing test. **Results:** Results indicate that CS-Ea possesses significant ($p < 0.05$) anti-inflammatory and anti-nociceptive effects in all the test models used. Systemic administration of naloxone, atropine and theophylline significantly blocked the anti-nociceptive effect of the extract. However, systemic N^o-Nitro-L-arginine methyl ester (L-NAME) and glibenclamide did not effects the effects of the extract significantly ($p > 0.05$). **Conclusion:** These results show that CS-Ea has anti-nociceptive and anti-inflammatory activities. The anti-nociceptive effects might be partly attributed to interactions with the opioidergic, muscarinic cholinergic and adenosinergic systems.

Key words: Adenosinergic, muscarinic, carrageenan, formalin test, hot plate

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INTRODUCTION

Cassia sieberiana is a woody plant of the family Fabaceae. The fruit pulps have been used traditionally in North-eastern Nigeria for the treatment of inflammatory conditions, tiredness and joint pains¹. Extracts of the plant are used to treat fever, malaria, diarrhoea, leprosy, bilharzias, stomach pains, lactation after child birth, rheumatic condition, jaundice and diuretic^{1,2}. The Centre for Scientific Research into Plant Medicine (CSRPM), Mampong-Akwapim, Ghana uses the powdered root bark of the plant for the treatment of various pain conditions including pain associated with stomach ulcer and menstruation in its clinic for the past 20 years.

Inflammation is an intricate pathophysiological condition mediated by several signaling molecules produced by leucocytes, macrophages and mast cells and the activation of complement factors that bring about

edema formation as a result of extravasation of fluid and proteins and the accumulation of leucocytes at the inflammatory site³. These molecules also mediate nociception thus the inflammatory process is normally characterized by pain, redness and swelling⁴.

Analgesic and anti-inflammatory therapies for acute and chronic conditions currently rely on three major classes of drugs: Non-Steroidal Anti-Inflammatory Drugs (NSAIDs), opioids and a group of drugs with diverse pharmacological actions collectively called adjuvant. All the steroidal anti-inflammatory agents and NSAIDs cause serious, undesired adverse effects necessitating the development of novel potent and safer alternatives. It has been argued that natural drug substances provide less toxic and more affordable drug molecules⁵.

Earlier works have shown that *C. sieberiana* extracts possess antimicrobial activity against *Neisseria gonorrhoeae*, Herpes simplex virus type 1 and African swine fever virus^{6,7}. Ethanolic and aqueous root extracts of *C. sieberiana* also has analgesic, anti-inflammatory, antiparasitic,

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myorelaxant and antispasmodic activities^{8,9}. Previous studies^{8,9} on the analgesic and anti-inflammatory effects of *C. sieberiana* have focused on the whole root extract. However, preliminary work in our laboratory had shown that the root bark is more effective than the whole root in the treatment of painful conditions. Following fractionation of crude ethanolic root bark extract with various solvents, ethyl acetate produced the highest yield (more than 80% of crude extract). Thus, the present work seeks to investigate the anti-nociceptive and anti-inflammatory effects of the ethyl acetate fraction of the root bark of *C. sieberiana* in murine models with the view to determining its possible mechanism of action.

MATERIALS AND METHODS

Plant material: The root bark of *Cassia sieberiana* was collected from the CSRPM's arboretum (5°55'05.44"N; 0°07'58.95"W) at Mampong-Akwapim, Ghana. They were authenticated by Mr. Ofori Lartey of the Plant Development Department (PDD) of the CSRPM and a voucher specimen (CSRPM No. 300) kept in the Herbarium of the PDD of the CSRPM, Mampong-Akwapim, Ghana.

Preparation of ethyl acetate fraction: Root barks were airdried, crushed and 1.5 kg was cold macerated with 3.5 L of absolute 99% ethanol for 75 h and filtered. Another 1 L of absolute ethanol was added to the residue and left to macerate for 16 h and then filtered. Both filtrates were mixed to yield a volume of 3.1 L, which was dried at 60°C under reduced pressure using rotary evaporator to remove all the ethanol to yield 244.25 g of a dark-brown paste. A volume of 300 mL of 90% ethanol was added and defatted with 300 mL of petroleum ether three times. The aqueous layer obtained was diluted with 300 mL of distilled water after evaporating the ethanol with rotary evaporator and the residue was partitioned with 300 mL of ethyl acetate three times. The ethyl acetate was evaporated from this fraction using rotary evaporator to yield 211.23 g (14.1% w/w of raw plant material) of dry powder and labelled ethyl acetate extract (CS-Ea).

Chemicals and drugs: Diclofenac sodium salt, aspirin, atropine, theophylline, naloxone, N^o-Nitro-L-arginine methyl ester (L-NAME), yeast from *Saccharomyces cerevisiae* Type II and carrageenan were obtained from Sigma-Aldrich Inc. (St. Louis, MO, USA). Other drugs include indomethacin (Cayman Chemical Company, Michigan, USA), glibenclamide (Sanofi-Aventis, Guildford, UK) and tramadol (KRKA, d.d., Novo mesto, Slovenia). All other chemicals were purchased in their purest form available from British Drug House (BDH) Ltd. (Poole, UK).

Animals: Male Sprague-Dawley rats (180-200 g) and male C3H mice (28-32 g) were obtained from the Animal Unit, CSRPM, Mampong-Akuapem, in the Eastern Region of Ghana. The animals were fed on feed obtained from Ghana Agro Food Company (GAFCO), Tema, Ghana. They were also allowed free access to clean water. The research was conducted in accordance with the internationally accepted principles for laboratory animal use and care (NIH, No.85-23, revised 1985). Ethical clearance was obtained from the CSRPM's ethics committee and the paper was written in accordance with the ARRIVE guidelines.

Phytochemical screening of *C. sieberiana* extract:

Phytochemical tests were performed on the extract using methods described by¹⁰ Trease and Evans to determine the presence or absence of saponins, reducing sugars, phenolics, cynogenic glycosides, polyamides, phytosterols, triterpenes, anthracenosides, flavonoids and alkaloids.

Carrageenan-induced edema test: The anti-inflammatory activity of extracts was determined by the carrageenan-induced edema test by a slight modification of the method of¹¹ using a plethysmometer (Model 7150, Ugo basile, Comerio- Varese, Italy). Rats were grouped into 7 of 5 animals. Groups 1-3 received oral administration of CS-Ea extract at doses of 3, 30 and 100 mg kg⁻¹, groups 4-6 received indomethacin (3, 10 and 30 mg kg⁻¹, i.p) as positive control, while group 7 received an equivalent volume of distilled water. Thirty minutes after administration of extract/drug, 0.1 mL of 1% (w/v) carrageenan in normal saline was injected into the sub-plantar region of the right hind paw of the rats. The paw volume (mL) of the right hind limbs were measured prior to the induction of edema (baseline) and three after readings were taken hourly until the 4th h post extract/drug administration. The anti-inflammatory activity was calculated as the degree of paw edema (e) using the formula:

$$e = \frac{E_t - E_o}{E_o} \times 100(\%)$$

where, E_o and E_t are paw volume at baseline and at a particular reading time of the right hind paw.

Formalin test: The formalin test was carried out in rats using the method first described by Dubuisson and Dennis¹² and slightly modified by Woode *et al.*¹³ and Wood and Abotsi¹⁴. Each animal was placed in one of 20 test chambers (Perspex chamber, 18×18×18 cm³) to acclimatize for 30 min before the experiment. The animals, 5 per group, were then administered orally with the extracts at doses of 3, 30 and 100 mg kg⁻¹ and three other groups administered with diclofenac (3,10 and 30 mg kg⁻¹,

i.p.). A control group was administered an equivalent volume of distilled water. Thirty minutes after administration of extract/drug each animal was injected intraplantarly with 50 μL of 5% formalin and returned to their respective observation chamber. A mirror was mounted at 45° behind the test chamber to allow for an unobstructed view and recording of the behavior of the animals by a camcorder placed in front of the mirror. Nociceptive response was quantified for 60 min post formalin administration in 5 min time intervals by counting the incidents of spontaneous biting/licking of the injected paw using the public domain software JWatcher, version 1.0 (University of California, LA, USA and Macquarie University, Sidney, Australia, available at <http://www.jwatcher.ucla.edu/>). In the present study, the neurogenic phase was defined as 0-5 min and the Inflammatory phase 10-60 min post-formalin administration.

Yeast-induced mechanical hyperalgesia: The mechanical nociceptive thresholds were measured in the rat paw pressure by a slight modification of the method of¹⁵ Randall and Selitto, using an analgesimeter (7200, Ugo Basile, Comerio-Varese, Italy). Rats received three training sessions prior to the testing day. Pressure was gradually applied to the right hind paw, and Paw Withdrawal Thresholds (PWTs) were assessed as the pressure (g) eliciting paw withdrawal. Animals that had a PWT of less than 30 g and more than 250 g were excluded. A 250 g cut-off was used to avoid possible tissue damage. Animals that passed the selection test were grouped into 7 of 5 animals. On the test day a baseline measurement was taken before animals were treated with the extract/drug. Groups 1-3 received oral administrations of CS-Ea extract at doses of 3, 30 and 100 mg kg^{-1} , groups 4-6 were administered aspirin (3, 10 and 100 mg kg^{-1} , p.o.) as positive control, and group 7 received an equivalent volume of distilled water. Thirty (30) minutes after the administration of the extract/drug, 0.1ml of yeast (20% w/v in distilled water) was injected into the sub-plantar region of the right hind paw of the rats. The PWTs were measured at 0.5, 1, 2 and 4 h post-yeast administration. The anti-nociceptive activity of the extract/drug was calculated as analgesic coefficient (k) using the formula:

$$\kappa = \frac{a+b+c+d}{e \times 4} \times 100\%$$

where, e is the baseline PWT and a, b, c and d are the PWTs at 1st, 2nd, 3rd and 4th readings.

Hot plate test: Test for thermal anti-nociception was carried out using a hot/cold plate system (Model 35100, Comerio-Varese, Italy) with slight modification of previously described methods^{16,17}. Prior to the treatment,

the reaction time of each mouse (licking, retraction of hind paw or jumping) was determined by gently dropping the animal on the hot plate system maintained at 55°C. Only animals that had a reaction time of 4-9 s were used for the experiment. Selected mice were divided into 7 groups of 5. Groups 1-3 received oral administrations of CS-Ea extract at doses of 3, 30 and 100 mg kg^{-1} , groups 4-6 were administered Tramadol (3, 10 and 30 mg kg^{-1} , i.p.) as positive control and group 7 received an equivalent volume of distilled water. The reaction time for each animal was recorded after 30 min and hourly thereafter till the 4th hour post-extract/drug administration. A cut-off time of 40 s was chosen to avoid possible tissue damage. The anti-nociceptive activity was calculated as Maximum Possible Effect (MPE%) using the formula:

$$\text{MPE}(\%) = \frac{L_t - L_o}{40 - L_o} \times 100\%$$

where, L_o and L_t represent the pre-drug and post-drug latencies respectively in seconds.

Acetic acid writhing test: The acetic acid writhing test was carried out in mice by slightly modifying a previous method¹³. Test animals were put into 7 groups of 5. Groups 1-3 received oral administrations of CS-Ea extract at doses of 3, 30 and 100 mg kg^{-1} , groups 4-6 were administered diclofenac (3, 10 and 30 mg kg^{-1} , i.p.) as positive control, and group 7 received an equivalent volume of distilled water. Thirty min after administration of extract/drug, 0.1 mL of acetic acid (0.6% v/v in distilled water) was injected intraperitoneally. The contraction of abdominal muscles together with stretching of the hind limbs was recorded cumulatively over a period of 30 sec 5 min time interval post-acetic acid injection.

Analysis of the possible mechanisms of action of CS-Ea in the acetic acid writhing test: To study the possible mechanisms in the acetic acid-induced nociception, mice were pre-treated with selected receptor antagonists at doses based on preliminary studies in our laboratory. The acetic acid test was chosen because of its sensitivity and it is a less laborious assay which allows for rapid assessment of anti-nociceptive activity in several groups within a short time thus permitting assessments to be done under fairly constant environmental conditions.

To evaluate the possible involvement of adenosinergic, muscarinic cholinergic, opioid, ATP-sensitive K^+ channels and nitric oxide-cGMP systems in the anti-nociceptive effect of the extract, mice were pre-treated with various antagonists. Theophylline, (5 mg kg^{-1} , i.p., a non-selective adenosine receptor antagonist), atropine (2 mg kg^{-1} , i.p., a non-

selective muscarinic receptor antagonist), naloxone, (2 mg kg⁻¹, i.p., a non-selective opioid receptor antagonist), glibenclamide (10 mg kg⁻¹, i.p., an ATP-sensitive K⁺ channel inhibitor) and L-NAME, (10 mg kg⁻¹, i.p., a NO synthase inhibitor) were administered to separate groups of mice. After 15 min mice were administered orally with 100 mg kg⁻¹ of the extract and a control group given equivalent volume of distilled water. Thirty min after administration of extract, 0.1 mL of acetic acid (0.6% v/v in distilled water) was injected intraperitoneally. The contraction of abdominal muscles together with stretching of the hind limbs was recorded cumulatively over a period of 30 at 5 min time interval post-acetic acid injection.

Statistical analysis: GraphPad Prism for Windows version 4.03 (GraphPad Software, San Diego, CA, USA) was used for all data and statistical analyses. $p < 0.05$ was considered statistically significant. The time-course curves were subjected to two-way (treatment \times time) repeated measures analysis of variance (ANOVA) with Bonferroni's *post hoc* test. Total nociceptive score for each treatment was calculated in arbitrary unit as the area under the curve (AUC). Differences in AUCs were

analysed by ANOVA followed by Student-Newman-Keuls *post hoc* test. Doses for 50% of the maximal effect (ED₅₀) for each drug were determined by using an iterative computer least squares method, with the following nonlinear regression (three-parameter logistic) equation:

$$Y = \frac{a - (b - a)}{\left(1 + 10^{(\text{LogED}_{50} - X)}\right)}$$

where, X is the logarithm of dose and Y is the response. Y starts at a (the bottom) and goes to b (the top) with a sigmoid shape.

RESULTS

Phytochemical analysis: The phytochemical screening of ethyl acetate fraction of the root bark *C. sieberiana* revealed the presence of saponins, flavonoids, anthraquinones and phenolics.

Carrageenan-induced edema: The effects of CS-Ea and indomethacin on carrageenan-induced paw edema are shown in Fig. 1. The sub-plantar injection of

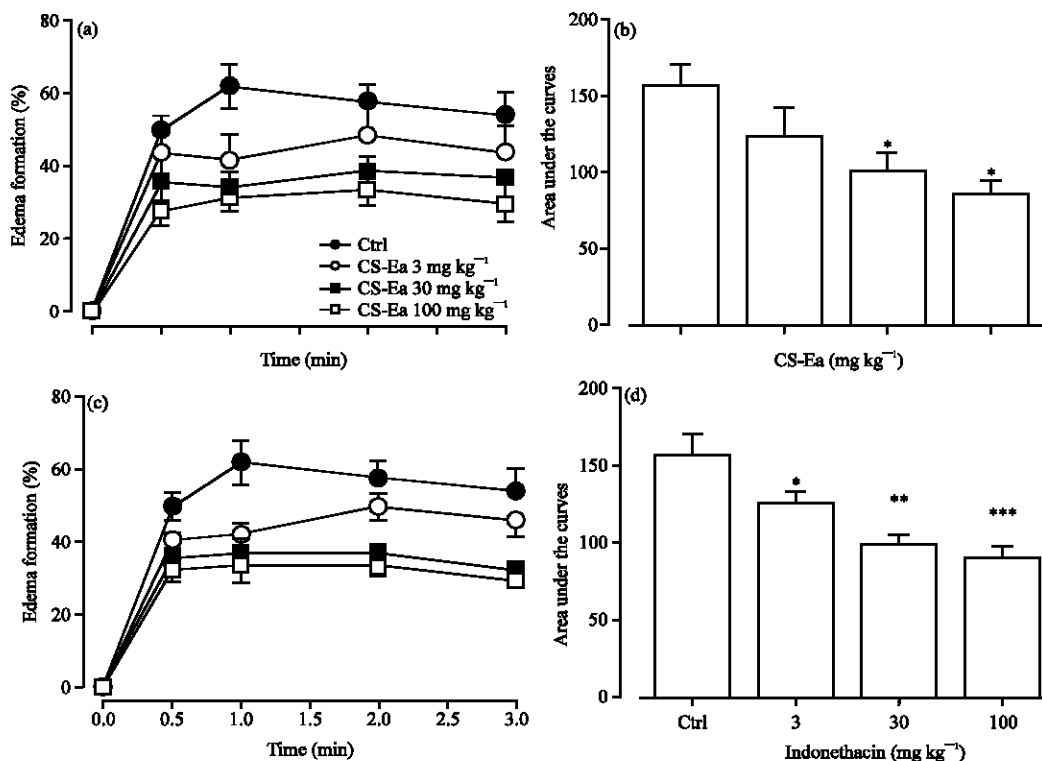


Fig. 1(a-d): (a) Effect of CS-Ea (3-100 mg kg⁻¹) and (c) indomethacin (3-30 mg kg⁻¹, i.p.) on the time course of carrageenan-induced paw edema in rats; (b) and (d) are AUCs determined from (a) and (c). Each point/column represents Mean \pm SEM (n=5). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared to untreated controls.

carrageenan caused an increase in paw volume of all experimental rats with controls experiencing a sustained increase in paw volume over the 3 h study period which peaked after an hour post-edema induction (Fig. 1a and 1c). Both the extract and indomethacin caused significant (CS-Ea: $F_{3,19} = 4.59$, $p < 0.05$; Indo: $F_{3,19} = 11.72$, $p < 0.001$) apparent dose-dependent reduction in the percentage edema formation compared to the controls. The degree of edema inhibition over the treatment period (AUC) for the extract (21.2-45.1 %) and indomethacin (20.2-42.4 %) at 3-100 mg kg⁻¹ and 3-30 mg kg⁻¹, respectively were comparable. The potency of the extract ($ED_{50} = 11.66 \pm 15.56$ mg kg⁻¹) and indomethacin ($ED_{50} = 15.93 \pm 19.35$ mg kg⁻¹) were also comparable (Table 1).

Formalin test: The anti-nociceptive effects of the extract and diclofenac in formalin-induced nociceptive

test are shown in Fig. 2. Sub-plantar administration of formalin evoked biphasic nociceptive behaviour (flinching, lifting, shaking and licking of injected paw) in test animals. The extract and diclofenac produced significant [Phase 1 (CS-Ea: $F_{3,19} = 30.87$, $p < 0.0001$; Diclo: $F_{3,19} = 15.06$, $p < 0.0001$); Phase 2 (CS-Ea: $F_{3,19} =$

Table 1: ED_{50} values of CS-Ea and various standard drugs

Test	ED_{50} values (mg kg ⁻¹)			
	CS-Ea	Indomethacin	Diclofenac	Aspirin Tramadol
Anti-inflammatory	11.66±15.56	15.93±19.35		
Formalin	3.80±0.65 ^a		6.45±1.54 ^a	
		12.38±2.51 ^b	26.28±3.21 ^b	
Mechanical	6.99±1.78			
Hyperalgesia				53.2±9.67
Hot plate	5.21±0.87			4.13±0.77
Writhing	9.34±2.01		5.66±0.59	

^aFirst Phase of the formalin test, ^bSecond Phase of the formalin test

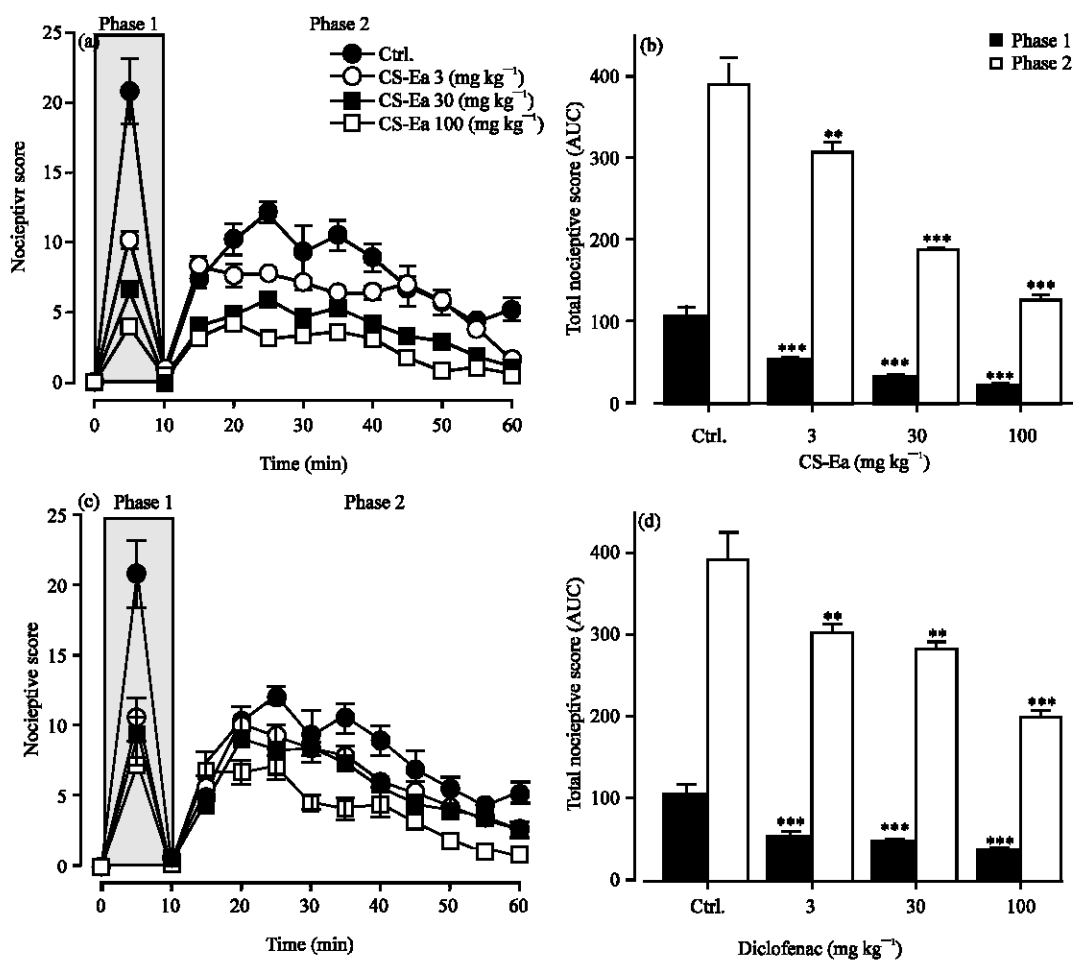


Fig. 2(a-d): (a) Effect of CS-Ea (3-100 mg kg⁻¹, p.o.) and (c) diclofenac (3-30 mg kg⁻¹, i.p.) on the time course of formalin-induced nociception in rats; (b) and (d) are total cumulative nociceptive responses determined from (a) and (c) respectively. Values are Mean±SEM (n=5). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared to untreated controls

42.49, $p < 0.0001$; Diclo: $F_{3,19} = 17.48$, $p < 0.0001$] dose-dependent inhibition of the nociception caused by the injection of formalin in mice compared to controls. CS-

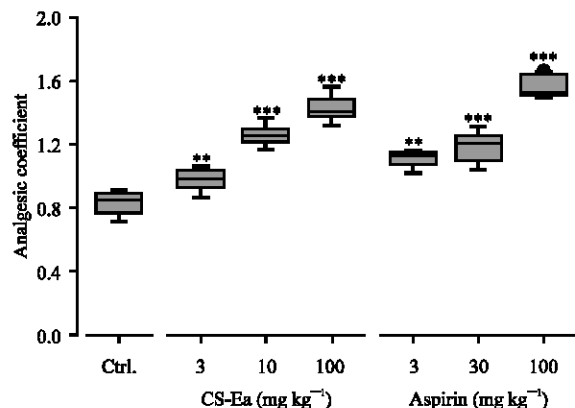


Fig. 3: Effect of CS-Ea (3-100 mg kg⁻¹, p.o.) and aspirin (3-100 mg kg⁻¹, p.o.) on yeast-induced hyperalgesia in rats. Values are Mean ± SEM (n=5). ** $p < 0.01$, *** $p < 0.001$ compared to untreated controls

Ea (3-100 mg kg⁻¹, p.o.) more potent in both the neurogenic phase ($ED_{50} = 3.80 \pm 0.65$ mg kg⁻¹) and the inflammatory phase ($ED_{50} = 12.38 \pm 2.51$ mg kg⁻¹) than the diclofenac (3-30 mg kg⁻¹, i.p.) in the neurogenic phase ($ED_{50} = 6.45 \pm 1.54$ mg kg⁻¹) and the inflammatory phase ($ED_{50} = 26.28 \pm 3.21$ mg kg⁻¹) (Table 1).

Yeast-induced mechanical hyperalgesia: The effects of the extract and aspirin on the yeast-induced mechanical hyperalgesia are represented in Fig. 3. An hour after sub-plantar injection of yeast, the ipsilateral paw showed marked hyperalgesia in all experimental rats which was significantly reduced in both the extract- ($F_{3,19} = 57.95$, $p < 0.0001$) and aspirin-treated ($F_{3,19} = 52.77$, $p < 0.0001$) rats in a dose-related manner compared to controls. CS-Ea ($ED_{50} = 6.99 \pm 1.78$ mg kg⁻¹) was more potent than aspirin ($ED_{50} = 53.20 \pm 9.67$ mg kg⁻¹) (Table 1).

Hot plate test: The effects of the extract and tramadol on the reaction times of mice (calculated as %MPE) are presented in Fig. 4. Prior to commencement of experiment, mice showed a baseline reaction time of about 5-8 sec which was sustained in control animals

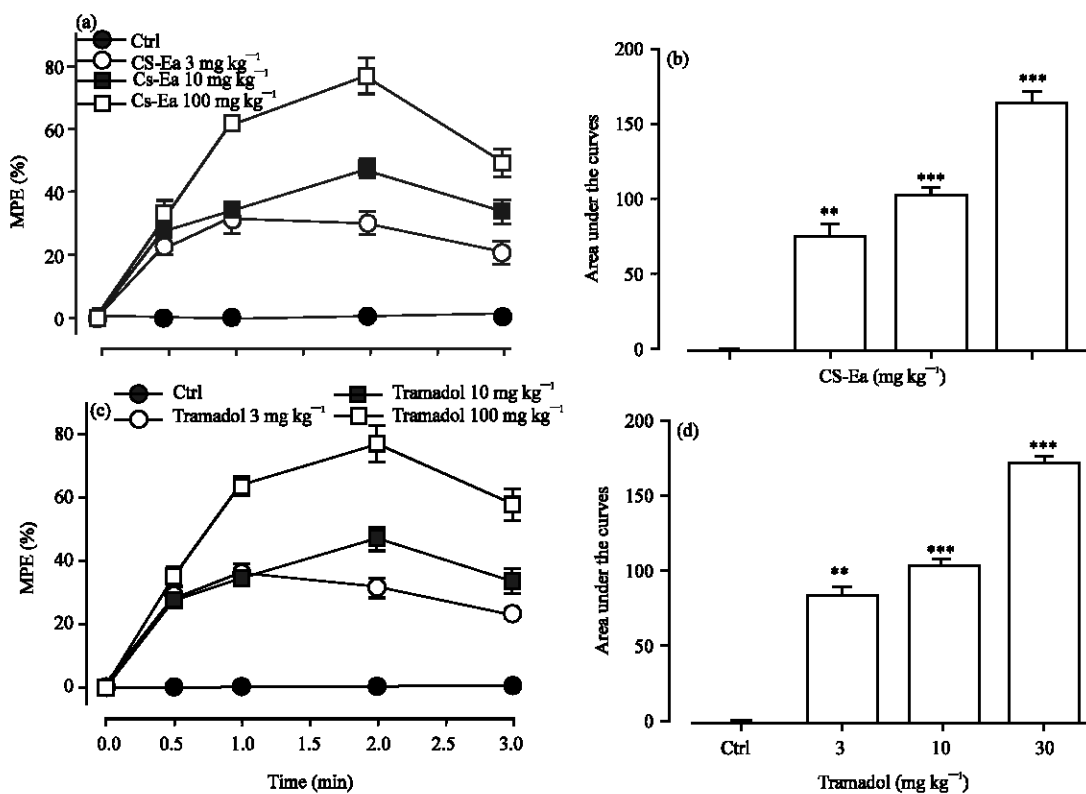


Fig. 4(a-d): (a) Effect of C.S.-Ea (3-100 mg kg⁻¹, p.o.) and (c) tramadol (3-30 mg kg⁻¹, i.p.) on the time course of thermal nociception in mice in hot plate test; (b) and (d) are AUCs determined from (a) and (c) respectively. Each point/column represents Mean ± SEM (n=5). ** $p < 0.01$, *** $p < 0.001$ compared to untreated controls

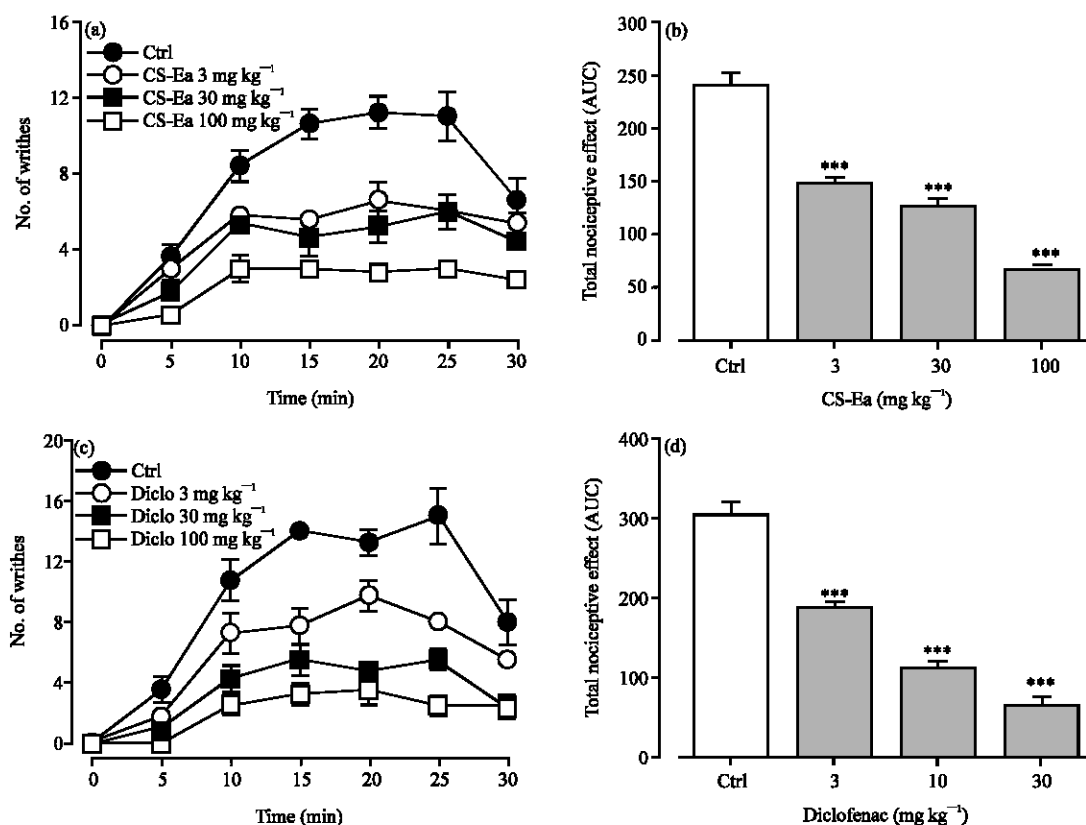


Fig. 5(a-d): (a) Effect of CS-Ea (3-100 mg kg⁻¹, p.o.) and (c) diclofenac (3-30 mg kg⁻¹, i.p.) on the time course of acetic acid-induced writhing in mice; (b) and (d) are AUCs determined from (a) and (c) respectively. Each point/column represents Mean ± SEM (n=5). ***p < 0.001 compared to untreated controls

throughout the 3 h study period. CS-Ea (3-100 mg kg⁻¹, p.o.) and tramadol (3-30 mg kg⁻¹, i.p.) significantly increased the reaction time of mice (CS-Ea: $F_{3,19} = 120.7$, $p < 0.0001$; Tramadol: $F_{3,19} = 234.5$, $p < 0.0001$) with maximum effect at 100 mg kg⁻¹. From the ED₅₀ values obtained (Table 1), CS-Ea (5.21 ± 0.87 mg kg⁻¹) was comparable to tramadol (4.13 ± 0.77 mg kg⁻¹) in potency.

Acetic acid writhing test: Figure 5 represents the cumulative number of writhes in 5 min intervals for 30 min post-acetic acid injection. The writhing activity (contraction of abdominal muscle and stretching of hind limbs) started about 3 min after acetic acid injection in all experimental mice and increased dramatically in controls to peak around the 20-25th min and then receded. The extract (3-100 mg kg⁻¹) and diclofenac (3-100 mg kg⁻¹) significantly (CS-Ea: $F_{3,19} = 89.88$, $p < 0.0001$; diclo:

$F_{3,19} = 68.15$, $p < 0.0001$) reduced the writhing action in a dose-dependent fashion at the tested dose levels compared to controls. The ED₅₀ of CS-Ea (9.34 ± 2.01 mg kg⁻¹) is comparable to that of diclofenac (5.66 ± 0.59 mg kg⁻¹) (Table 1).

Mechanism of action of CS-Ea: Results presented in Fig. 6 show that pre-treatment of mice with theophylline, (5 mg kg⁻¹, i.p.) and atropine (2 mg kg⁻¹, i.p.), 15 min before extract administration significantly ($p < 0.05$) but not completely blocked the anti-nociceptive effect of CS-Ea (100 mg kg⁻¹, p.o.) while naloxone (2 mg kg⁻¹, i.p.) almost completely reversed the anti-nociceptive action of the extract (Fig 6b). However, glibenclamide (10 mg kg⁻¹, i.p., an ATP-sensitive K⁺ channel inhibitor) and L-NAME, (10 mg kg⁻¹, i.p., a NO synthase inhibitor) did not have a significant ($p > 0.05$) effect on the anti-nociceptive effect of the extract (Fig. 6a).

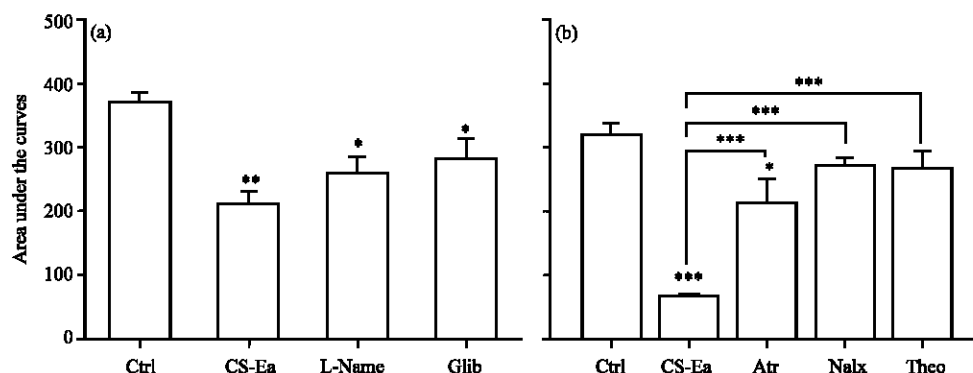


Fig. 6(a-b): (a) Effect of glibenclamide (10 mg kg⁻¹, i.p.) and L-NAME (10 mg kg⁻¹, i.p.) on the anti-nociceptive activity of C.S.-Ea (100 mg kg⁻¹, p.o.) in acetic acid writhing test in mice; (b) Effect of theophylline (5 mg kg⁻¹, i.p.), atropine (10 mg kg⁻¹, i.p.) and naloxone, (2 mg kg⁻¹, i.p.) on the anti-nociceptive activity of C.S.-Ea (100mg kg⁻¹, p.o.) in acetic acid writhing test in mice. Each column represents Mean ± SEM (n=5). *p<0.05, **p<0.01, ***p<0.001 compared to untreated controls

DISCUSSION

Nature has been a source of medicinal agents for thousands of years and nearly 50% of modern drugs have been isolated from natural products and their derivatives¹⁸. The powdered root barks of *C. sieberiana* has been processed and used in our clinic-with anecdotal evidence for its use in the treatment of pain associated with ulcer and menstruation-for the past 20 years. The present study has demonstrated that the oral administration of the ethyl acetate extract of the root bark of *C. sieberiana* has significant analgesic and anti-inflammatory activities.

Carrageenan-induced rat hind paw edema has been widely used for the discovery and evaluation of anti-inflammatory drugs since the relative potency estimates obtained from most drugs tend to reflect clinical experience¹¹. From the results in the present study, the extract and indomethacin showed significant anti-inflammatory activity in this model. Carrageenan-induced edema is a biphasic event¹⁹. There is marked edema formation resulting from the rapid production of several inflammatory mediators such as histamine, serotonin and bradykinin (first phase), which is subsequently sustained by the release of prostaglandins and nitric oxide (second phase) produced by inducible isoforms of COX (COX-2) and nitric oxide synthase (iNOS), respectively^{20,21}. It is, therefore, likely that CS-Ea exerts anti-inflammatory activity by the inhibition of one or more of the inflammatory mediators.

Oral administration of the extract and diclofenac significantly and dose-dependently inhibited both the neurogenic (first) and inflammatory (second) phases of formalin-induced nociception in rats. Centrally acting drugs, such as opioids, inhibit both phases of the

nociceptive response equally²² while many NSAIDs and corticosteroids inhibit only the inflammatory phase. Therefore, CS-Ea may be acting via both peripheral and central mechanisms to produce anti-nociception. The inhibitory effect of CS-Ea in the second phase also confirms the anti-inflammatory action of the extract.

The Randall-Selitto paw pressure test is an inflammatory pain model widely used for quantification of thresholds of the rat hind paw withdrawal reflex to nociceptive pressure stimulation. The extract, together with aspirin, showed significant anti-nociceptive against yeast-induced inflammatory pain. The results corroborate the observed activity of CS-Ea in the second phase (inflammatory pain) of the formalin test.

The hot plate test measures the complex response to an acute, non-inflammatory nociceptive input and is one of the models normally used to specifically study central nociceptive activity. CS-Ea, as well as tramadol, showed significant, dose-dependent anti-nociceptive activity compared to controls. This confirms the involvement of central mechanisms in the anti-nociceptive effects of the extract since the two behavioral components that were measured in terms of their reaction times-namely paw licking and jumping-are considered to be supraspinally integrated responses²³.

The intraperitoneal injection of acetic acid causes the release of inflammatory mediator which excites pain nerve and it is an assay used to screen for both peripheral and central acting agents. The abdominal constriction is as a result of sensitization of nociceptive receptors to prostaglandins²⁴. The extract and diclofenac showed significant dose-related reduction in the number of writhes of test animals compared to controls. The NSAIDs including diclofenac reduce the number of

writes in this test by inhibiting cyclooxygenase in peripheral tissues thus blocking the release and/or synthesis of inflammatory mediators²⁵. The extract may be acting via a similar mechanism-This needs to be confirmed in further studies.

With the exception of diclofenac in the acetic acid writhing test, the extract had comparable or lower ED₅₀ than all the standard drugs used in the various tests (Table 1). The extract, thus, appears to be a good potential analgesic and anti-inflammatory drug candidate.

In order to assess possible mechanisms of action, the anti-nociceptive activity of the extract was determined in mice pre-treated with theophylline, atropine, naloxone, L-NAME or glibenclamide. Naloxone, a non-selective opioid antagonist, reversed the anti-nociceptive effect of the extract suggesting an opioidergic system involvement in the anti-nociceptive actions of the extract. Atropine, a muscarinic cholinergic antagonist, partially blocked the anti-nociceptive effect of the extract suggesting an interaction at the muscarinic cholinergic system.

Previous studies have shown that blockade of adenosine receptors by theophylline, a non-selective adenosine receptor antagonist at A₁ and A₂ receptors, produced hyperalgesia²⁶. Similarly, adenosine A₁ receptor agonists have been shown to be effective anti-nociceptive agents in neuropathic and inflammatory pain and mice lacking the adenosine A₁ receptor are hyperalgesic^{27,28}. In the present study, the anti-nociceptive effect of the extract was partially blocked by systemic administration of theophylline, suggesting that the anti-nociception may involve the adenosinergic system.

ATP-sensitive K⁺ channels are important in the mechanisms of pain modulation^{29,30}, likewise the NO system. L-NAME, NO synthase inhibitor and glibenclamide, ATP-sensitive K⁺ channels blocker, did not significantly modify the anti-nociceptive effect of the extract suggesting that the NO-cGMP and ATP-sensitive K⁺ pathways may not be implicated in the anti-nociceptive effects of the extract.

Phytochemical analysis of ethyl acetate extract of the root bark of *C. sieberiana* showed the presence of saponins, flavonoids, anthraquinones and phenolics. The presence of flavonoids and tannins has been reported in the aqueous root extract of the plant⁹. Flavonoids and saponins are known for their anti-nociceptive and anti-inflammatory properties^{31,32,33}. Thus, the flavonoids and saponins present in CS-Ea may be responsible for the anti-nociceptive and anti-inflammatory activities reported in the present study.

In conclusion, it may be said that CS-Ea has anti-nociceptive and anti-inflammatory activities. The anti-nociceptive effects might be due, at least partly, to the interaction with the opioidergic, muscarinic cholinergic and adenosinergic systems. These findings thus support

its ethnomedicinal use and the anecdotal claims for its use in the management of pain. Further studies are underway to determine the safety of *C. sieberiana* extracts in animals.

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