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Studies on the analgesic and anti-inflammatory effects of *Cassia sophera* roots

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ABSTRACT

Objective: To probe the traditional medicinal claims about anti-inflammatory and analgesic potential of *Cassia sophera* Linn. (Caesalpiniaceae) (*C. sophera*) through scientific methodology.

Methods: The analgesic activity of root methanol extract of *C. sophera* was studied using acetic acid-induced writhing, hot-plate and formalin-induced paw licking tests. Carrageenan-induced paw edema and cotton-pellet edema models were used to assess the anti-inflammatory effects of methanol extract of *C. sophera* root. The methanol extract of *C. sophera* root was administered at concentrations of 50, 100 and 200 mg/kg.

Results: In acetic acid-induced writhing model, methanol extract of *C. sophera* root showed significant activity at all doses with maximum inhibition of 76.29%. Methanol extract of *C. sophera* root exhibited marked inhibition of pain stimuli with latency of (12.28 ± 4.60) s in hot-plate test. The methanol extract of *C. sophera* root significantly reduced licking time at all doses with maximum reduction in licking time (30.6 ± 3.1) s in late phase of paw licking in formalin-induced paw licking test. The methanol extract of *C. sophera* root showed highly significant results in carrageenan-induced paw edema model with maximum protection (65.75%) from edema. A significant inhibition of granuloma formation was observed in cotton-pellet edema model with maximum inhibition (57.37%) of granuloma.

Conclusions: The significant inhibitory effect on peripheral and central pain stimuli; marked edema reduction and granuloma inhibition justified the ethnomedicinal use of *C. sophera* in the management of pain and inflammation.

1. Introduction

Inflammation is an intricate host response, which is responsible for elimination of harmful internal and external stimuli to introduce to the host. These harmful stimuli include radiation, chemicals, infections and aggravated immune response. A high percentage of world population is suffering from different types of rheumatic disorders. The presently available synthetic clinical drugs employed in the management of inflammatory disorders and associated pain pose a major disadvantage due to their toxicity and re-emergence of symptoms after discontinuation. Therefore reflective research efforts with plant drugs can definitely offer opening up of new panoramas in inflammation corrective and curative treatment. And new bioactive natural constituents may serve as a lead for synthesis of safer and more effective anti-inflammatory drugs which can be used at clinical level in the management of various inflammatory conditions like rheumatism, arthritis and pain.

Cassia sophera Linn. (Caesalpiniaceae) (*C. sophera*) is a small to medium sized shrub with 1-3 m tall. Leaves with 14-21 cm lenghth are compound, having petiole near the base which is glandular. Leaflets are arranged in 4-12 pairs, 3-9 cm long, 1.5-2.8 cm wide, oblong to lanceolate with acute tip. It is pantropical in occurrence, existing wildly or cultivated occasionally in some regions of Punjab, Pakistan. Detailed literature survey showed that the whole plant extract is used traditionally for treatment of rheumatic disorders, joint pain, inflammatory fevers, diabetes, dyspnoea, apositia[1], itching, syphilitic sores and liver diseases[2]. According to published material on *C. sophera*, antioxidant activities[1], hepatoprotective activity[2], antiasthmatic activity[3] and hypoglycemic activity[4] have been reported. Mondal *et al.*[5] investigated the anti-inflammatory effects of ethanolic extract of *C. sophera* leaves. It was reported that ethanolic extract effectively inhibited inflammatory process in dextran and carrageenan-induced rat paw edema. The chemical constituents isolated from *C. sophera* include flavonoids and anthraquinones[2]. The isolated constituents include cyclosophoside A, 1,8-dihydroxy-2-methylanthraquinone

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3-neohesperidoside, chrysophanol, physcion, 1,2,7-trihydroxy-6,8-dimethoxy-3-methylanthraquinone, 1,2,6-trihydroxy-7, 8-dimethoxy-3-methyl anthraquinones, 1,8-dihydroxy-3, 6-dimethoxy-2-methyl-7-vinylanthraquinone, 1,3-dihydroxy-5,7,8-trimethoxy-2-methylanthraquinone, emodin and 5,7,3',4'-tetrahydroxy-3-methoxyflavone-5-O-Rha-7-O-Glc-Xyl. Owing to various ethnomedicinal properties of *C. sophera* and no report is available in the literature on the screening of *C. sophera* L. roots for their analgesic and anti-inflammatory properties, the present study was carried out to study the analgesic and anti-inflammatory potential of roots of *C. sophera* by employing several *in vitro* and *in vivo* models in order to validate its traditional medicinal use.

2. Materials and methods

2.1. Plant material

The plant material was collected in December 2012, from surrounding area of Sargodha City, Pakistan and authenticated by Prof. Altaf Dasti, the director of Institute of Pure and Applied Biology, Bahauddin Zakariya University, Multan, Pakistan. A specimen bearing voucher number SWT-366 was preserved in the herbarium. The plant materials were air-dried under shade for 15 days at room temperature. The roots of the dried plant material were pulverized to consistent coarse powder and stored in air tight jars under refrigeration for further research.

2.2. Extraction

The maceration of roots powder (500 g) was done in methanol for a period of 24 h and then filtered. The procedure was repeated thrice using 1.0 L methanol in each step. The filtrates from each step were combined and concentrated *in vacuo* at 35 °C. The roots yielded crude methanol extract weighing 25.3 g, 5.06% of the dry weight.

2.3. Animals

In vivo studies were conducted on adult Wistar rats and Swiss albino mice of either sex. Animals were housed in steel cages (10 per cage) with 8 am to 8 pm light-dark cycle. Free access to food and water under prescribed environmental conditions was ensured. The animal studies were performed in compliance with protocols and the policies approved by the Institutional Animal Ethical Committee of Department of Pharmacy, Bahauddin Zakariya University, Multan, Pakistan (Voucher No: IAEC/Approval/01/2014/Pharm).

2.4. Acetic acid-induced writhing

The acetic acid writhing test was used with slight modifications[6]. Five groups of mice (five mice per group) were employed. All mice were kept under starving for 18 h prior to the administration of extract. Saline (10 mL/kg, *p.o.*) was used as the negative control while the positive control group received aspirin (100 mg/kg, *p.o.*). The test groups received doses of 50, 100 and 200 mg/kg of crude methanol extracts *p.o.* After thirty minutes, all mice were injected 0.7% acetic acid aqueous solution (10 mg/kg) *i.p.* and abdominal constrictions were counted for 10 min.

2.5. Hot-plate test

To evaluate central analgesic effect of test extracts hot-plate latency test was performed by formerly reported method[7]. All test animals were placed twice on the hot-plate in advance in order to habituate them with experimental conditions. During the test, the hot plate was maintained at (55.0 ± 0.5) °C. The mice were placed on the hot plate and the time taken by the mice until hind paw licking or jumping off. And then, the time was recorded as the hot plate latency which is the time for reaction to heat. Mice showing baseline latencies (< 5 s or > 30 s) were not included in the study. The determination of baseline heat response was followed by redetermination of hot-plate latencies at 0.5 h, 1 h and 2 h after oral administration of test extracts. Saline was used as negative control while tramadol was used as positive control.

2.6. Formalin-induced paw licking

The formalin-induced paw licking was performed by previously reported method[8]. After one hour of oral administration of extracts, normal saline and indomethacin to respective groups, subcutaneous injection of 0.1 mL of 3% formalin was administered into the plantar surface of the left hind paw of rats. The time spent in licking injected paw by each rat in early phase (0-5 min) and in late phase (20-30 min) was recorded. The mean time of paw licking was determined.

2.7. Carrageenan-induced edema

In order to evaluate anti-inflammatory potential of methanol extract, its effect on inhibition of carrageenan-induced hind paw edema in rats was assessed by a method which was reported earlier[9]. Rats were divided into five test groups (n = 6). The respective groups received 50, 100 and 200 mg/kg extract doses, *i.p.*, negative control group received normal saline at dose of 100 mg/kg via oral route and the positive control group received aspirin (100 mg/kg, *i.p.*). After one hour, subplantar injection of 1% carrageenan (0.1 mL) suspended in 2% acacia gum in normal saline was administered to induce acute inflammation in the right hind paw of the rats. After the injection of carrageenan, the paw volume was determined by plethysmometer (Ugo Basile, Italy) at 1, 2, 3, 4 and 5 h. Percentage inhibition of the inflammation of each group was determined statistically. The percentage inhibition of each extract treated group was determined by comparing with the control group according to the formula: %I = 1 - (dt / dc) × 100, where, dt is the difference in paw volume in the extract treated group, dc

is the difference in paw volume in control group, I is inhibition of inflammation.

2.8. Cotton-pellet edema model

The effect of test extracts on chronic inflammation was determined employing cotton pellet edema model according to the standard method[10]. Five groups of male rats were formed with five rats in each group. Each test animal was implanted with cotton pellet (18 ± 1 mg) on each side of abdomen subcutaneously. During pellet implantation, the animals were kept under light ether anesthesia. Controls and test extracts were administered to the respective animal groups once daily for 7 d. On Day 8 after implantation, cotton pellets were dissected after induction of anesthesia with pentobarbital sodium (50 mg/kg, *i.p.*). The dissected cotton pellets were dried at 55 °C for 15 h, and weighed after cooling. Following the determination of mean granuloma weights, the percentage granuloma inhibition was calculated. Saline and indomethacin were used as negative and positive controls respectively.

2.9. In vitro lipoxygenase inhibition assay

Lipoxygenase inhibition was determined by spectrometric method that was reported earlier[11]. Different concentrations of test extracts were used in enzyme inhibition assay. Linoleic acid and lipoxygenase (EC 1.13.11.12) type I-B (soybean) were procured from Sigma (St Louis, MO, USA). All other chemicals used in this experiment were of analytical grade and provided by Sigma (St Louis, MO, USA). Sodium phosphate buffer (pH 7.0, 0.1 mmol/L, 160 μL), test sample solutions (10 mL) and lipoxygenase solution (20 μL) were mixed and subsequently incubated for 5 min at 258 °C. Linoleic acid substrate solution (10 μL) was added to initiate the reaction and the absorption change with the formation of (9Z, 11E)-13S)-13- hydroperoxyoctadeca-9, 11-dienoate was followed for 10 min. The test sample and the positive control baicalein were dissolved in 50% ethanol. All the reactions were performed in triplicate. The inhibitory concentration (IC_{50}) values were calculated by using the EZ-Fit Enzyme-Kinetics Program (Perrella Scientific Inc., Amherst, USA).

2.10. Phytochemical analysis

Preliminary phytochemical screening of powdered roots was carried out according to previously reported standard methods[12].

2.11. Acute toxicity

Rate of mortality of animals subjected to carrageenan induced paw edema study was recorded during 48 h.

2.12. Data analysis

All results were expressed as mean ± SEM. Student's *t*-test was used to analyse data between the groups. The analysis of variance (ANOVA) among groups was followed by Dunnet's test for multiple comparisons. Values of $P < 0.05$ were taken as significant.

3. Results

3.1. Acetic acid-induced writhing

The the methanol extract of *C. sophera* root exhibited significant analgesic activity at all doses. When compared with group receiving saline, the methanol extract reduced the acetic acid induced abdominal constrictions by 76.29% at 200 mg/kg dose ($P < 0.01$) (Table 1). The analgesic activity produced by methanol extract of *C. sophera* root at the dose of 200 mg/kg was comparable with that of aspirin (87.84%).

Table 1
Analgesic effect of *C. sophera* on acetic acid-induced writhing model.

Group	Dose (mg/kg)	Writhing count	% Inhibition
Saline	-	30.20 ± 2.23	-
CSRM	50	18.10 ± 0.87*	40.06
CSRM	100	13.90 ± 1.31*	53.97
CSRM	200	7.16 ± 1.08**	76.29
Aspirin	150	3.67 ± 1.88**	87.84

*: $P < 0.05$, **: $P < 0.01$ compared with negative control group receiving saline. CSRM: methanol extract of *C. sophera* root.

3.2. Hot-plate test

The results of hot plate latency test are presented in Table 2. In hot plate latency test, the methanol extract of *C. sophera* root revealed significant analgesic activity when compared with the animals treated with saline. The peak activity occurred after one hour interval at concentrations of 100 and 200 mg/kg. The hot plate latency showed by the *C. sophera* root extract at 200 mg/kg was (12.28 ± 4.60) s which was in close comparison with that of tramadol (11.60 ± 4.30) s.

Table 2
Analgesic effect of *C. sophera* on hot plate latency test (mean ± SEM).

Group	Dose	Hot-plate latency (s)			
	(mg/kg)	0 h	0.5 h	1 h	2 h
Saline	-	21.36 ± 4.90	21.16 ± 4.30	21.57 ± 5.20	21.32 ± 6.20
CSRM	50	21.91 ± 5.70	19.20 ± 5.50	18.21 ± 4.90	18.06 ± 5.30
CSRM	100	21.56 ± 4.80	17.34 ± 4.60	14.26 ± 4.40*	14.39 ± 5.10*
CSRM	200	21.78 ± 4.70	16.38 ± 5.10	12.28 ± 4.60*	13.28 ± 4.70*
Tramadol	5	21.72 ± 5.10	13.75 ± 4.50*	11.60 ± 4.30*	11.12 ± 4.10*

*: $P < 0.05$ compared with negative control group receiving saline. CSRM: methanol extract of *C. sophera* root.

3.3. Formalin-induced paw licking

The root methanol extract presented highly significant ($P < 0.01$) activity in this model at all dose levels and produced analgesia more rapidly than positive control in both early and late phases. The root extract showed the maximum reduction in licking time (30.6 ± 3.1) s in late phase of paw licking at the dose of 200 mg/kg (Table 3).

Table 3

Analgesic effect of *C. sophera* on formalin-induced paw licking (mean ± SEM).

Group	Dose (mg/kg)	Licking time (s)	
		Early phase	Late phase
Saline	-	82.6 ± 3.4	73.8 ± 4.1
CSRM	50	47.2 ± 3.1**	36.4 ± 2.7**
CSRM	100	43.1 ± 2.6**	33.2 ± 3.2**
CSRM	200	40.4 ± 3.3**	30.6 ± 3.1**
Indomethacin	5	52.2 ± 2.8*	41.3 ± 2.9**

*: $P < 0.05$, **: $P < 0.01$ compared with negative control group receiving saline. CSRM: methanol extract of *C. sophera* root.

3.4. Carrageenan-induced edema

The results of the carrageenan-induced paw edema of *C. sophera* are presented in Table 4. The *C. sophera* root extract demonstrated highly significant ($P < 0.01$) anti-inflammatory activity at test doses of 100 and 200 mg/kg after 3 h of drug administration. Paw edema was predominantly reduced more or less in dose dependent manner during the dose assessment time. The *C. sophera* root extract exhibited 54.79% and 65.75% protection against inflammation after 3 h of drug administration at the dose of 100 and 200 mg/kg body weight respectively.

3.5. Cotton-pellet edema model

The *C. sophera* root extract exhibited significant ($P < 0.05$) inhibition of edema induced by cotton pellets at all doses. Highly significant ($P < 0.05$ and $P < 0.01$) inhibition was observed at the dose of 100 and 200 mg/kg of extract with granuloma inhibition of 48.39% and 57.37% respectively (Table 5).

Table 5

Anti-inflammatory effect of *C. sophera* on Cotton-pellet oedema model

Group	Dose (mg/kg)	Increase in weight of pellets (mg)	% Inhibition
Saline	-	62.4 ± 3.7	-
CSRM	50	37.9 ± 2.7*	39.27
CSRM	100	32.2 ± 2.2*	48.39
CSRM	200	26.6 ± 2.3**	57.37
Indomethacin	5	23.1 ± 1.7**	62.98

*: $P < 0.05$, **: $P < 0.01$ compared with negative control group receiving saline. CSRM: methanol extract of *C. sophera* root.

3.6. In vitro lipoxygenase inhibition assay

The methanol extract of *C. sophera* root did not show any observable inhibition of lipoxygenase. However, the standard (baicalein) exhibited inhibition of lipoxygenase at IC_{50} value being (21.20 ± 0.04) mmol/L.

3.7. Phytochemical analysis

According to the results, root of *C. sophera* revealed presence of different pharmacological active groups including saponins, anthraquinones, phenols, flavonoids, sterols, terpenoids and tannins while alkaloids were not present.

3.8. Acute toxicity

No significant difference in terms of mortality and morbidity was observed between the negative control and other treatment groups after 48 h of administration.

Table 4

Anti-inflammatory effect of *C. sophera* on carrageenan-induced oedema model (mean ± SEM).

Group	Dose (mg/kg)	Increase in paw volume (mL)				
		1 h	2 h	3 h	4 h	5 h
Saline	-	0.740 ± 0.050	0.730 ± 0.060	0.730 ± 0.040	0.690 ± 0.040	0.740 ± 0.030
CSRM	50	0.610 ± 0.040 (17.57%)	0.560 ± 0.050 (23.29%)	0.520 ± 0.050 (28.77%)	0.530 ± 0.040 (23.19%)	0.530 ± 0.060 (28.38%)
CSRM	100	0.490 ± 0.067 (33.79%)	0.480 ± 0.056 (34.25%)	0.330 ± 0.060** (54.79%)	0.360 ± 0.070** (51.35%)	0.360 ± 0.060** (51.36%)
CSRM	200	0.410 ± 0.050* (44.60%)	0.380 ± 0.030* (47.95%)	0.250 ± 0.034** (65.75%)	0.260 ± 0.040** (62.32%)	0.260 ± 0.050** (64.87%)
Indomethacin	5	0.230 ± 0.030** (68.92%)	0.200 ± 0.021** (72.61%)	0.180 ± 0.023** (75.34%)	0.190 ± 0.027** (72.47%)	0.190 ± 0.033** (74.33%)

% Protection is given in parenthesis. *: $P < 0.05$, **: $P < 0.01$ compared with negative control group receiving saline. CSRM: methanol extract of *C. sophera* root.

4. Discussion

Through current study, the analgesic and anti-inflammatory potential of root of *C. sophera* based on folk medicinal uses was investigated. The prime emphasis of the study was to validate traditional medicinal use of *C. sophera* for management of pain and inflammation through a set of standard *in vitro* assay and *in vivo* models. The study yielded significantly ($P < 0.05$) positive outcome

in both analgesic and anti-inflammatory models.

The acetic acid-induced abdominal writhing model is a known visceral pain model. Acetic acid induces nociception owing to release of arachidonic acid derivatives[13]. The results of acetic acid-induced abdominal writhing model illustrated the fact that 100 mg/kg dose produced significant ($P < 0.05$) analgesia with 53.97% inhibition of abdominal constrictions. In addition, the highly significant ($P < 0.01$) analgesic effect was observed at 200 mg/kg dose inhibiting acetic acid-induced writhing by 76.29%. Thus reduction of acetic acid-induced writhing may be correlated to their inhibitory effect on the biosynthetic pathway of arachidonic acid metabolites through possible inhibition of cyclooxygenases[13,14].

In the hot-plate latency model, a well-known animal model to evaluate the centrally acting analgesics[14], the methanol extract of *C. sophera* root exhibited significant ($P < 0.05$) analgesia at dose of 100 and 200 mg/kg.

The formalin-induced paw licking was well established tonic pain model which induced pain resembles closely to clinical human pain conditions[8]. The results of the formalin-induced paw licking illustrated that the methanol extract of *C. sophera* root exhibited highly significant ($P < 0.01$) analgesic effect at all doses when compared with negative control in both phases of paw licking. The *C. sophera* root extract showed the maximum reduction in licking time in late phase of paw licking at the dose of 200 mg/kg. The analgesic profile was better than indomethacin in terms of time taken for paw licking.

The results of the all animal pain models clearly exhibited the mechanism of analgesia produced by *C. sophera*. It could have caused reduction of pain associating with peripheral stimuli by possible inhibition of cyclooxygenases as inferred from the results of acetic acid induced abdominal writhings in the current study[13,14]. The results of hot plate latency model and the formalin-induced paw licking are suggestive of analgesic effect most probably of opioid type because significant inhibition of thermal and chemical nociceptive stimuli indicate the opioid type of analgesia[6,8]. These results might also be attributed to a raised endogenous serotonin level and interaction with 5-HT2A and 5-HT3 receptors[15].

The carrageenan-induced paw edema is an *in vivo* model to investigate effects of natural products on acute inflammation[9,16]. Edema induced by subplantar injection of carrageenan in the rat paw is a progressively increasing event during 1-5 h. The initial phase (1 h or 1.5 h), marked by predominant nonphagocytic edema, is due to release of histamine, 5-hydroxytryptamine and platelet activating factor. Kinin is released from 1.5 to 2.5 h[9]. In the last step (until 5 h), owing to release of prostaglandins, bradykinin and related autacoids resulting migration of large number of poly morphonuclear neutrophils and macrophages to the site of inflammation, the edema attains its maximum volume[17,18]. The study showed significant ($P < 0.05$, $P < 0.01$) anti-inflammatory activity by methanol extract of *C. sophera* root at doses of 100 and 200 mg/kg in carrageenan-induced paw edema model at various assessment times. This activity could be correlated partly with inhibition of inflammatory mediator release and partly because of inhibitory effects on migration and accumulation of neutrophils and macrophages at the area of inflammation[5] as test extract showed peak % protection after 3 h of administration in last phase which was characterized by phagocytic edema. Previously, the inhibitory effects of the leaf extract of *C. sophera* on carrageenan-induced rat paw edema had been reported. The leaf extract (200 and 400 mg/kg body weight) significantly inhibited the edema formation by 23% and 44% of control, respectively[5]. Hence it can be deduced that the methanol extract of *C. sophera* root possessed far better anti-inflammatory profile than that of leaf extract.

Results of acute toxicity described that the methanol extract of *C. sophera* root was safe after 48 h of administration. Statistically, there was no observable difference between the negative control and other treatment groups both in terms of mortality and morbidity.

The cotton pellet granuloma test is a well established animal model for chronic inflammation. This test is based on leukocyte infiltration and formation of granuloma at the site of the inflammation[8]. The results showed that the methanol extract of *C. sophera* root significantly ($P < 0.05$) inhibited leukocyte migration during inflammatory process at all dose levels. Thus it could be deduced that methanol extract of *C. sophera* root inhibited (significant at lower doses with $P < 0.05$ and highly significant at moderate to high doses with $P < 0.01$) leukocyte migration and subsequent granuloma formation in the area of inflammation. The maximum percent inhibition of granuloma formation was observed at the dose of 200 mg/kg. Ethanolic extract of *C. sophera* leaves was reported to significantly inhibit cotton pellet granuloma. The inhibition caused by ethanolic extract of *C. sophera* leaves was 31.84 and 37.47% at doses of 200 and 400 mg/kg, respectively[5]. Thus, the anti-inflammatory response exhibited by the methanol extract of *C. sophera* root in terms of % inhibition of cotton pellet granuloma is superior to that of ethanolic extract of leaves.

Thus, the ability of *C. sophera* to inhibit mediators involved in inflammatory process could be responsible for decreasing the edematous condition. However, root extract failed to show any observable lipoxygenase inhibition, which was indicative of the involvement of lipoxygenase-independent mechanisms responsible for inflammatory process.

The major outcome of the experiments on the both inflammatory animal models was that the *C. sophera* might have caused inhibition of inflammation through the same mechanism as in the case of indomethacin which involved the anti-inflammatory process initiated through carrageenan and cotton pellets[11].

Phytochemical screening based on standard reported methods exhibited presence of flavonoids, phenolics and saponins in the drug. Inhibitory effects of flavonoids and saponins on pain perception and inflammation, due to their ability to inhibit enzymes responsible for production of inflammatory mediators, had been documented[18,19]. Flavonoids can also inhibit neutrophil degranulation resulting in reduced release of arachidonic acid due to inhibitory effects on cyclooxygenases[20]. It was reported that flavonoids can raise endogenous serotonin level and interact with 5-HT2A and 5-HT3 receptors which may produce central analgesic activity[15]. The anti-inflammatory and analgesic activity may be attributed to these bioactive groups. However, in depth studies are most warrant in order to correlate these activities to the outcome of phytochemical screening. Current research regarding analgesic and anti-inflammatory activities of *C. sophera* has justified its ethnomedicinal use and it is recommended that in order to have detailed mechanistic insights further studies at molecular and cellular levels should be conducted.

Conflict of interest statement

We declare that we have no conflict of interest.

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