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Evaluation of Antinociceptive and Anti-inflammatory Activity of Leaves of *Cassia grandis* Linn.

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ABSTRACT
The aim of the present study was to evaluate the antinociceptive and anti-inflammatory activity of the methanolic extract of leaves of *Cassia grandis* (MECG). The analgesic activity of the extract was evaluated for its central and peripheral pharmacological actions using Eddy's hotplate method and acetic acid-induced writhing respectively. The anti-inflammatory activity was evaluated by using Digital plethysmometer (UGO Basil, Italy 7140). The study was carried out using dose of 100 mg/kg p.o. The pharmacological screening of the extract showed significant analgesic activity with good anti-inflammatory profile.

Keywords: Analgesic, anti-inflammatory, *Cassia grandis.*

INTRODUCTION

Inflammation is a local response of living mammalian tissues to injury. It is a body defence reaction in order to eliminate or limit the spread of injurious agent. There are various components to an inflammatory reaction that can contribute to the associated symptoms and tissue injury. Oedema formation, leukocyte infiltration and granuloma formation represent such components of inflammation. [1] Oedema formation in the paw is the result of a synergism between various inflammatory mediators that increase vascular permeability and/or the mediators that increase blood flow. [2] Several experimental models of paw oedema have been described. Carrageenan-induced paw oedema is widely used for determining the acute phase of inflammation. Histamine, 5-hydroxytryptamine and bradykinin are the first detectable mediators in the early phase of carrageenan-induced inflammation, [3] whereas prostaglandins are detectable in the late phase of inflammation. [4]

A large number of Indian medicinal plants are attributed with various pharmacological activities because they contain a diversified class of phytochemicals. It is believed that current analgesia-inducing drugs such as opoids and non-steroidal anti-inflammatory drugs are not useful in all cases, because of their side-effects and potency. [5] As a result, a search for other alternatives seems necessary and beneficial. Medicinal plants having a wide variety of chemicals from which novel anti-inflammatory agents could be discovered. Scientific studies are required to judge their efficacy. Traditional and folklore medicines play an important role in health services around the globe. About three quarters of the world population relies on plants and plant extracts for healthcare. India has an extensive forest cover, enriched with plant diversity. Several plants have been used in folklore medicine. [6-7] The rational design of novel drugs from traditional medicine offers new prospects in modern healthcare. Ayurveda the traditional medicinal system in India; describes certain plants which strengthen the host immune system.

Cassia grandis L. (Family: *Leguminosae*) is a deciduous or semi-deciduous spreading tree. It is well known as a Pink shower. [8] Several studies on the various parts of this plant have been reported as *in-vitro* antioxidant, purgative and in treatment of skin disorders etc. The pulp from the pods is very strong smelling with a bitter and astringent taste, which has laxative properties. It is sometimes used in veterinary practices also hence known as Horse Cassia. The juice from the pods is reported to strengthen the blood. The phytochemical studies revealed the presence of flavonoids, anthraquinones and sterols. [9-12]

MATERIALS AND METHODS

Plant material

The leaves of *Cassia grandis* were collected from Nashik, India, in the month of July. The plant was identified with the help of available literature and authenticated by Department of Pharmacognosy, N. D. M. V. P. Samaj's College of Pharmacy, Nashik.

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Preparation of methanolic extract of leaves

The powdered leaves (500 g) were packed in soxhlet apparatus. The drug was defatted with petroleum ether (60-80°C) for about 30-35 complete cycles. Defatted material was extracted with two liters of methanol by soxhlet apparatus and then extracted material successively extracted with methanol followed by maceration at room temperature, then extract were dried by rotary vaccum dryer. The methanol extract of *Cassia grandis* was designated as MECG and the percentage of yield was found to be 12.75 %.

Animals

Adult male mice (20-35 g) were used for the antinociceptive experiments. Adult male wistar rats (150-200 g) were used to study the anti-inflammatory activity. The animals (five per cage) were maintained under standard laboratory conditions (light period of 12 h/day and temperature 27 ± 2°C), with access to food and water *ad libitum*. Animal experiments were approved by the Institutional Animal Ethical Committee.

Acetic Acid-induced Writhing method

The antinociceptive activity of MECG was assessed using writhing test (abdominal constriction test). Acetic acid solution (10 ml/kg) was injected intraperitoneally, and the contraction of abdominal muscles together with stretching of the hind limbs was 30 and 60 min beginning 5 min after acetic acid injection. The MECG extract (100 mg/kg, p.o.) was administered 0.5 h before the acetic acid injection. Antinociceptive activity was expressed as the percentage inhibition of abdominal constrictions mice pre-treated (n= 5) with the extract. In an attempt to investigate the participation of the opioid system in the antinociceptive effect of this plant extract, separate groups of mice (n= 5) were pretreated with non-specific opioid receptor antagonist, pentazocin (5 mg/kg, i.p.), injected 15 min before the administration of the acetic acid. [13]

Hotplate Test

The hotplate test was performed to measure response latencies according to the method previously described. [14] The hotplate was maintained at 55.0 ± 0.2°C and the animals were placed into the perspex cylinder on the heated surface and the time (sec) to discomfort reaction (licking paws or jumping) was recorded as response latency, prior to and 30, and 60 min after administration of the extract (100 mg/kg, p.o.). A latency period of 20 sec was defined as complete analgesia and the measurement was terminated if it exceeded the latency period in order to avoid injury.

Anti-inflammatory Activity

MECG was evaluated for anti-inflammatory activity by carrageenan-induced rat paw oedema method. [15-16] Male wistar rats (150-200 g) were randomly distributed into three groups of five animals each. The first group served as a control, second group served as the standard (received aceclofenac sodium 10 mg/kg, i.p), while the third group received 100 mg/kg, body weight of MECG respectively. After 1 h, 0.1 ml of 1% w/v suspension of carrageenan was injected into the sub-plantar region of the right hind paw to all the three groups. The paw volumes were measured using plethysmometer (UGO Basile, 7140 Italy) every hour till 3 h after carrageenan injection, and mean increase in paw volumes were noted. Thus oedema volumes in control (Vc) and in groups treated with test compounds (Vt) were calculated. The percentage inhibition was calculated by using the formula [17]:

$$\text{Percentage of inhibition} = 100\ (1\text{-}Vt / Vc)$$

Where, Vc= Edema volume in control and Vt= Edema volume in test / standard compound

Statistical analysis

The results are expressed as mean ± SEM. The statistical analysis was performed by analysis of variance (ANOVA) test.

RESULTS

Acetic Acid-induced Writhing

The results of MECG on acetic acid-induced writhing test indicated a significant increase ($P < 0.01$) in reaction time, which is comparable to the reference drug pentazocine (Table 1).

Hot-plate Test

The results of the hotplate test indicated a significant increase ($P < 0.01$) in reaction time in 1 h comparable to the reference drug pentazocine (Table 2).

Anti-inflammatory Activity

The result of MECG against carrageenan-induced paw oedema is shown in Table 3. MECG (100 mg/kg, i.p.) gave significant ($P < 0.01$) reduction of rat paw oedema at all assessment times. The methanolic extract showed maximum inhibition of 52.58 % at the dose of 100 mg/kg after 2 h of drug treatment in carrageenan-induced paw oedema whereas the standard drug showed 56.70 % of inhibition.

DISCUSSION

The thermal stimuli in hotplate test and the writhing response of the animals to an intra-peritoneal injection of noxious chemical are used to screen both peripherally and centrally acting analgesic activity. Acetic acid causes analgesia by liberating endogenous substances that excite the pain nerve endings. [18] From the results it is apparent that the MECG showed a significant antinociceptive effect in the hotplate test and writhing response, which is comparable to that of the standard. Studies demonstrate that various flavonoids such as rutin, quercetin, luteolin, hesperidin and biflavonoids produced significant antinociceptive and anti-inflammatory activities. [19-20] There are also a few reports on the role of tannins in antinociceptive and anti-inflammatory activities. [21] NSAIDs can inhibit cyclo-oxygenase in peripheral tissues, thus interfering with the mechanism of transduction in primary afferent nociceptors. [22] The mechanisms of antinociceptive action of MECG could be due to the presence of flavonoids and mediated through central and peripheral mechanisms.

Carrageenan-induced paw oedema was taken as a prototype of exudative phase of acute inflammation. Inflammatory stimuli microbes, chemicals and necrosed cells activate the different mediator systems through a common trigger mechanism. The development of carageenan-induced oedema is believed to be biphasic. The early phase is attributed to the release of histamine and serotonin [23-24] and the delayed phase is sustained by the leucotrienes and prostaglandins. [25] Flavonoids and tannins are reported to inhibit prostaglandin synthesis. [26] Most of the non steroidal anti-inflammatory drugs (NSAIDs) have well balanced anti-inflammatory and ulcerogenic activities, which are considered to be due to PG synthetase inhibitor activity. From the above discussion, the methanolic extract from the leaves of *Cassia grandis* exhibited significant analgesic and anti-inflammatory activity.

Table 1: Effect of methanolic extract of leaves of *Cassia grandis* Linn on latency to acetic acid-induced writhing test

S. No.	Time after administration (min)	Vehicle distilled water (10 ml/kg, i.p.)	Methanolic extract (100 mg/kg, i.p.)	% Inhibition	Pentazocine (5 mg/kg, i.p.)	% Inhibition
1.	30	67.74±0.69	15.15±0.54*	78.28	2.78±0.52*	96.14
2.	60	62.33±0.52	25.34±0.47*	60.24	6.45±0.75*	90.01

Values are mean±SEM, (n=5), *P<0.01

Table 2: Effect of methanolic extract of leaves of *Cassia grandis* Linn on latency to hotplate test

S. No.	Time after administration (min)	Vehicle distilled water (10 ml/kg, i.p.)	Methanolic extract (100 ml/kg, p.o.)	Pentazocine (5 mg/kg, i.p.)
1.	30	9.82±0.41	13.14±0.21*	15.89±0.14*
2.	60	9.25±0.38	12.37±0.59*	14.76±0.44*

Values are mean±SEM, (n=5), *P<0.01

Table 3: Anti-inflammatory activity of methanolic extract of leaves of *Cassia grandis* Linn on carrageenan-induced paw oedema in rats

S. No.	Treatment	Dose	0 h		1 h		2 h		3 h	
			EV (ml)	EI (%)	EV (ml)	EI (%)	EV (ml)	EI (%)	EV (ml)	EI (%)
1.	Control	-	1.79±0.21	-	1.83±0.33	-	1.94±0.24	-	1.82±0.11	-
2.	Aceclofenac sodium	10 mg/kg	1.11±0.14	37.98	0.92±0.70	49.72	0.84±0.21	56.70*	0.79±0.42	56.59
3.	Methanolic extract	100 mg/kg	1.13±0.21	36.87	0.95±0.11	48.09	0.92±0.47	52.58*	0.86±0.15	52.75

Values are mean±SEM, (n=5), *P<0.01; EV=Edema Volume, EI=Edema Inhibition

Further detailed investigation is underway to determine the exact phytoconstituents that are responsible for these activities.

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