

Antinociceptive and Anti-inflammatory Activity of Seeds of *Cassia absus* Linn as a Medicinal plants constituents

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ABSTRACT :- The main purpose and aim of the present study was to evaluate the antinociceptive and anti-inflammatory activity of the methanolic extract of seeds of *Cassia absus* (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. 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 absus*.

Introduction :- We have a large number of Indian medicinal plants are attributed with various Chemical and pharmacological activities because they contain a diversified class of Chemicals and phytochemicals. It is believed that current analgesia-inducing drugs such as opioids and non-steroidal anti-inflammatory drugs are not useful in all cases, because of their side-effects and potency. As a result, a search for other alternatives seems necessary and beneficial. Medicinal plants having a wide variety of chemicals from which novel *Cassia absus* L. (Family: Leguminosae) is a deciduous or semi-deciduous spreading tree. It is well known as a Pink shower. 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 Chemical and phytochemical studies revealed the presence of flavonoids, anthraquinones and sterols.

MATERIALS AND METHODS

The Plant material The seeds of *Cassia absus* were collected from different places of India, in the month of July. The plant was identified with the help of available literature and authenticated by Department of Chemistry, Magadh University, Bodhgaya.

Preparation of methanolic extract of seeds :- The powdered seeds (250 g) were packed in Soxhlet apparatus. The drug was defatted with petroleum ether (60- 70°C) for about 15 - 20 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 vacuum dryer. The methanol extract of *Cassia absus* was designated as MECG and the percentage of yield was found to be 8.75 %.

Animals :- All the adult male mice (10-25 g) were used for the antinociceptive experiments. Adult male Wistar rats (100-150 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 $28 \pm 2^\circ\text{C}$), with access to food and water ad libitum. Animal experiments were approved by the Institutional Animal Ethical Committee.

Acetic Acid-induced Writhing method :-

By the antinociceptive activity of MECG was assessed using writhing test (abdominal constriction test). Acetic acid solution (5 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 (50 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= 6) 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 10 min before the administration of the acetic acid

Hotplate Test: -

In hotplate test was performed to measure response latencies according to the method previously described. The hotplate was maintained at $60.0 \pm 0.2^\circ\text{C}$ 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 20, and 40 min after administration of the extract (50 mg/kg, p.o.). A latency period of 15 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 :-

The MECCG was evaluated for anti-inflammatory activity by carrageenan-induced rat paw oedema method.. Male wistar rats (100-150 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 5 mg/kg, i.p), while the third group received 100 mg/kg, body weight of MECCG 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) every hour till 2 h after carrageenan injection, and mean increase in paw volumes were noted. Thus oedema volumes in control (V_c) and in groups treated with test compounds (V_t) were calculated. The percentage inhibition was calculated by using the formula.

Percentage of inhibition = $100 (1 - V_t / V_c)$:-

Where, V_c = Edema volume in control and V_t = Edema volume in test / standard compound

Statistical analysis :-

Results are expressed as mean \pm SEM The statistical analysis was performed by analysis of variance (ANOVA) test.

RESULTS :- The Acetic Acid-induced Writhing The results of MECCG 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 MECCG against carrageenan-induced paw oedema is shown in Table 3. MECCG (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 51.58* % at the dose of 100 mg/kg after 3 h of drug treatment in carrageenan-induced paw oedema whereas the standard drug showed 54.70*% of inhibition.

DISCUSSION :-

By 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. From the results it is apparent that the MECCG 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. There are also a few reports on the role of tannins in antinociceptive and anti-inflammatory activities. NSAIDs can inhibit cyclo-oxygenase in peripheral tissues, thus interfering with the mechanism of transduction in primary afferent nociceptors. The mechanisms of antinociceptive action of MECCG 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 and the delayed phase is sustained by the leucotrienes and prostaglandins. Flavonoids and tannins are reported to inhibit prostaglandin synthesis. 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 seeds of *Cassia absus* exhibited significant analgesic and anti-inflammatory activity.

Table 1:**The effect of methanolic extract of seeds of *Cassia absus* Linn on latency to acetic acid-induced writhing test S.**

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.	25	76.74±0.69	16.15±0.54*	89.28	3.78±0.52*	95.14
2.	55	67.33±0.52	36.34±0.47*	71.24	7.45±0.75*	93.01

Values are mean±SEM, (n=5), *P

Table 2:**The effect of methanolic extract of seeds of *Cassia absus* 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.	25	8.82±0.41	11.14±0.21*	14.89±0.14*
2.	55	8.25±0.38	10.37±0.59*	13.76±0.44*

Table 3:**The Anti-inflammatory activity of methanolic extract of seeds of *Cassia absus* 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	1.10±0.14	37.98	0.91±0.71	48.72	0.82±0.21	54.70*	0.76±0.42	53.59
3.	Methanolic extract	100 mg/kg	1.11±0.21	35.87	0.92±0.11	46.09	0.91±0.47	51.58*	0.85±0.15	51.75

Values are mean±SEM, (n=5), *P

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

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