

Anti-nociceptive and Antipyretic Activities of Hydroalcoholic Extract of *Cassia sophera* Linn. Leaves

Debasish Roy¹, Manik Chandra Shill¹, Shrabanti Dev¹, Debashish Deb², Masum Shahriar³,
Asish Kumar Das¹ and M. Shahabuddin Kabir Choudhuri³

¹Pharmacy Discipline, Khulna University, Khulna, Bangladesh

²Biotechnology and Genetic Engineering Discipline, Khulna University, Khulna, Bangladesh

³Department of Pharmacy, Jahangirnagar University, Savar, Dhaka, Bangladesh

Abstract

The crude hydroalcoholic extract of *Cassia sophera* Linn. leaves were investigated for its possible analgesic and antipyretic activities in rodents. The peripheral analgesic activity of *C. sophera* leaves was studied using acetic acid-induced writhing in mice and central analgesic activity was evaluated by using heat-induced pain in mice. The antipyretic activity of HECS was studied in Brewer's yeast-induced pyrexia in rats. All these experiments were done at the doses of 300 and 600 mg/kg body weight. Intraperitoneal administration of *C. sophera* hydroalcoholic extract (HECS) produced significant ($P < 0.01$) reduction in number of writhing induced by acetic-acid. Moreover, in hot-plate test, HECS significantly ($P < 0.01$) raised the pain threshold at different time of observation (0-180 min) in comparison with control. The extract also showed significant as well as dose-dependent antipyretic activity in Brewer's yeast-induced pyrexia in rats throughout the observation period of 4 h.

Key words: *Cassia sophera*; Analgesic; Antipyretic.

Introduction

Cassia sophera L. is a medicinal plant belonging to the family Caesalpiniaceae. As a medicinal plant it is very popular among the traditional medicine practitioners of Bangladesh and India. Locally the plant is known as Kasunda and in Homeopathy it is used as a potent remedy from plant kingdom for allergic rhinitis, asthma and osteoarthritis. Even ethnobotanical literature refers it to be effective in treating acute bronchitis, cough pityriasis, psoriasis, asthma, diabetes and convulsions of children (Dutt, 1995; Drury, 1873; Agharkar, 1991; Chopra, 1956; Kirtikar *et al.*, 2000; Nadkarni, 1982; Dymock *et al.*, 1890). Traditionally the plant is also used for treating microbial infections, remedy of fever and also against fungal infections (Ghani, 1998). Its leaves have free radical scavenging activity (Rahman *et al.*, 2008). Leaves of *C. sophera* also reported to have anti-insecticidal activity (Kestenholz, 2007). Its seeds can be used for a wide range of therapeutic purpose as analgesic, sedative and anticonvulsant agent (Bilal *et al.*, 2007). Despite the ethno-medicinal properties attributed to *C. sophera* very little pharmacological information about this plant has been reported to the best of our knowledge. Thus, we decided to evaluate the analgesic, anti-inflammatory and

antipyretic effect of the HECS obtained from the leaves of *Cassia sophera* L., by using classical pharmacological assays for the purpose of validating its ethnomedical use.

Materials and Methods

Plant materials collection and extraction: *C. sophera* leaves were collected from different places of Jhikargacha upzilla, Dist-Jessore, Division-Khulna, Bangladesh, in February, 2008. The plant was taxonomically identified by the experts of Bangladesh National Herbarium, Mirpur, Dhaka and a voucher specimen (accession no. DACB-32099) was also deposited there. Leaves were shed dried and powdered with a suitable grinder. The powdered material was cold extracted in flat-bottomed glass container using 70% ethanol for 15 days at room temperature. The extract was filtered through Whatman filter paper number 1 and remaining portion of the extract was re-extracted for 7 days. The filtrate (ethanol extract) was dried using a rotary evaporator followed by freeze drier.

Animals: Swiss albino mice (male), weighting 23-25 g and wister rats of either sex weighing 180-200 g bred in the Animal House of the Department of Pharmacy, Jahangirnagar University, Savar, Dhaka, Bangladesh were

used for the present experiments. All the animals were acclimatized for one week prior to the experiments. The animals were housed under standard laboratory conditions (relative humidity 55-65 %, temperature $25.0 \pm 2^\circ\text{C}$, and 12 h light dark cycle). The animals were fed with standard diet from International Center for Diarrheal Diseases and Research, Bangladesh (ICDDR, B) and had free access to tap water.

Drugs: Acetic acid (Sigma chemicals, USA), diclofenac sodium (Square Pharmaceuticals Ltd, Bangladesh), paracetamol (Square Pharmaceuticals Ltd, Bangladesh), nalbuphine (Incepta Pharmaceuticals Ltd.) were collected from the mentioned sources. All other chemicals were analytical grade.

Anti-nociceptive activity of HECS: Anti-nociceptive activity was assessed by abdominal writhing test using acetic acid and hot plate reaction time applied to male albino mice. Animals of the negative control received the vehicle only (1% Tween 80, 10 ml/kg, i.p).

Acetic acid-induced writhing assay: Anti-nociceptive activity of *C. sophera* was tested using the acetic acid-induced writhing method as described by Ahmed *et al.*, (2004) with minor modifications. In the writhing test, 0.6% acetic acid (10 mg/kg, i.p.) was injected and the number of writhes were counted for a period of 10 min. Diclofenac sodium (10 mg/kg, i.p.) or the *C. sophera* leaf extract (HECS, 300 and 600 mg/kg) were administered intraperitoneally 30 min before acetic acid injection.

Hot plate reaction time assay: The hot plate method was used to measure analgesic activity as described by Shanmugasundaram and Venkataraman (2005) with minor modifications. The temperature of the metal surface was maintained at $55 \pm 0.2^\circ\text{C}$. Latency to a discomfort reaction (licking, shaking or jumping) was determined before and after drug administration. The cut-off time was fixed to 15s to avoid the damage to the paw of the animals (Hasan *et al.*, 2009). The latency was recorded at 0, 30, 60, 120, 180 min following i.p. administration of the agents. The prolongation of the latency times compared with the values of the control was used for statistical comparison.

Antipyretic activity of HECS: The antipyretic activity of HECS was evaluated using Brewer's yeast-induced pyrexia in rats as described by Loux *et al.*, (1972) with minor modifications. Pyrexia was induced by subcutaneously injecting 20% w/v Brewer's yeast suspension (10 ml/kg). Seventeen hrs after the injection,

the rectal temperature of each rat was measured using a thermometer. Only rats that showed an increase in temperature of at least 0.7°C were employed for the experiments. Prior to the experiment, the rats were maintained in separate cages for 7 days and the animals with approximately constant rectal temperature were selected for the study. Paracetamol (200 mg/kg, p.o.) was used as standard drug for comparing the antipyretic action of HECS.

Statistical analysis: Student's t-test was used to determine a significant difference between the control group and experimental groups. Experimental values were expressed as Mean \pm SEM (Standard Error of Mean). $P < 0.05$ was considered as significant.

Results

Antinociceptive activity

Writhing assay: The HECS leaves produced significant writhing inhibition on acetic acid-induced writhing mice. The extract produced about 20.90% and 42.47% inhibition of writhing at the doses of 300 and 600 mg/kg body weight respectively, which was comparable to that of standard drug diclofenac sodium where the inhibition was about 61.41% ($p < 0.05$) at the dose of 10 mg/kg body weight respectively (Table 1).

Table 1. Effect of hydroalcoholic extract of the leaf of *Cassia sophera* on acetic acid induced writhing in mice

Groups	Dose (mg/kg)	Number of writhing	Inhibition (%)
Control	10	25.5 \pm 1.41	
Standard drug (Diclofenac sodium)	10	9.84 \pm 0.72	61.41**
HECS	300	20.17 \pm 1.14	20.90*
HECS	600	14.67 \pm 0.87	42.47**

All administrations were made intraperitoneally. Values expressed as mean \pm SEM, (n=6); **: $p < 0.01$, *: $p < 0.05$ significant compared to control.

Hot plate method: HECS when intraperitoneally administered with a dose of 300 and 600 mg/kg in mice has shown significant analgesic activity in hot plate method as supported by increase in latency time. The increase in latency time was dose dependant. Both doses of the extract showed significant analgesic activity; however, it was the maximum for the dose of 600 mg/kg and this was comparable with standard (Table 2).

Table 2. Effect of hydroalcoholic extract of *Cassia sophera* leaves on the latency of mice exposed to hot plate

Group	Dose mg/kg	Mean latency period before and after drug administration					Percentage of inhibition			
		0 min	30 min	60 min	120 min	180 min	30 min	60 min	120 min	180 min
Control	10	3.18 ± 0.17	3.21 ± 0.42	3.16 ± 0.5	3.06 ± 0.44	2.98 ± 0.56				
Standard	10	3.12 ± 0.06	5.51 ± 0.48	7.95 ± 1.16	11.19 ± 1.38	12.38 ± 1.56	43.38	60.76	72.13	74.79
Dose1	300	3.19 ± 0.08	4.10 ± 0.25	4.71 ± 0.28	5.1 ± 0.41	5.36 ± 0.39	22.21	32.32	37.52	40.58
Dose2	600	3.17 ± 0.10	4.81 ± 0.32	6.12 ± 0.42	7.05 ± 0.43	7.88 ± 0.61	34.25	48.31	55.12	59.85

All administrations were made intraperitoneally. Values represented as mean ± SEM, (n=6); *: P<0.05, **: P<0.01, significant compared to control.

Antipyretic activity: This study was performed on yeast-induced pyrexia in rats. HECS significantly reversed hyperthermia at either dose (300 mg/kg and 600 mg/kg). Time of peak effect was obtained between 1 to 4h after

oral administration. The standard drug, Paracetamol (200 mg/kg) also suppressed hyperthermia induced by yeast significantly (P<0.01) during all the observation times when compared with control values (Table 3).

Table 3. The antipyretic effect of the hydroalcoholic extract of *Cassia sophera* leaves

Groups	Dose (mg/kg)	Average rectal temperature (°C)				
		0 hr	1 hr	2 hr	3 hr	4 hr
Control	10	36.95±0.2	38.07±0.32	38.47±0.28	38.46±0.22	38.57±0.3
Paracetamol	200	37.15±0.25	35.87±0.17**	35.84±0.24**	35.88±0.28**	35.93±0.26**
HECS	300	37.01±0.21	36.82±0.11*	36.8±0.41**	36.74±0.45**	36.79±0.38*
HECS	600	37.08±0.1	36.4±0.41*	36.23±0.56**	36.37±0.55**	36.38±0.44**

Values are expressed as mean ± S.E.M; (no. of animal n=6). **: p < 0.01, *: p < 0.05 significant compared to control.

Discussion

Pain can be distinguished as two types, peripheral or neurogenic pain which may involve the following pathological states: peripheral nociceptive afferent neurons which are activated by noxious stimuli and central mechanism which is activated by afferent inputs pain sensation (Rang *et al.*, 2011). Here the antinociceptive activity of HECS was tested by both acetic acid-induced writhing in mice and hot plate method where hot plate method was considered to be selective for screening of the compound acting through the opioid receptor and acetic acid-induced writhing assay is useful in detecting both central as well as peripheral analgesics (Mate *et al.*, 2008). Acetic acid-induced writhing model represents pain sensation by triggering localized inflammatory response. It induces algesia by liberation of endogenous substances, which in turn excite the pain nerve endings (Taesotikul *et al.*, 2003). Increased levels of PGE₂ and PGF_{2α} in the peritoneal fluid have been reported to be responsible for pain sensation caused by intraperitoneal administration of acetic acid (Derardt *et al.*, 1980). The hydroalcoholic extract of *C. sophera* leaves showed statistically significant inhibition on acetic acid-induced writhing in

mice. Therefore, the possible mechanism behind the analgesic effect of HECS may be the inhibition of synthesis or action of prostaglandin. Generally the centrally acting analgesics elevate the pain threshold of mice towards heat. Here, in hot plate method, HECS increased the mean basal latency which shows that the extract also acts like centrally acting analgesics. Narcotic analgesic inhibits both the types of pain, while NSAIDs such as paracetamol and aspirin inhibit only the peripheral pain (Elisabetsky *et al.*, 1995; Pal *et al.*, 1999). HECS has inhibited both types of pain which suggests that the extract may act as a narcotic analgesic. Alkaloids, glycosides and tannins have been reported for analgesic activity (Deb *et al.*, 2010; Starec *et al.*, 1988). Phytochemical analysis reveals that the leaf extract of *C. sophera* contains alkaloid, steroid, tannin, and reducing sugar (Roy *et al.*, 2010).

Most of the anti-inflammatory and analgesic drugs show antipyretic activity. In this context, the HECS was also tested for possible antipyretic activity in Brewer's yeast-induced febrile rat model. Infection or one of the sequelae of tissue damage, inflammation, graft rejection, or other disease states can cause fever. Antipyretic agents

reduce the elevated body temperature. Body temperature can be raised due to several other normal reasons like exercise or increase in ambient temperature. But a standard antipyretic drug like paracetamol does not influence body temperature in such cases (Paschapur *et al.*, 2009). Yeast-induced fever is a pathogenic fever. Its etiology includes production of prostaglandins, which set the thermoregulatory center at a lower temperature (Bentley *et al.*, 1983; Chandrasekharan *et al.*, 2002). The present result shows that HECS possesses significant as well as dose dependent antipyretic effect in yeast-induced pyrexia which is comparable to that of the standard antipyretic drug paracetamol. Suppression of prostaglandin as do paracetamol may be the possible means of antipyretic effect of HECS though many others mediators or processes can be the reason for the pathogenesis of fever.

Based on the results of the present studies, it can be concluded that the hydroalcoholic extract of *C. sophera* leaves possesses significant analgesic and antipyretic potential. However, further detail studies are essential to find out the underlying mechanisms and also to isolate the active compound(s) responsible for these pharmacological properties.

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