IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
March 2019 Vol.:14, Issue:4

Analgesic and Anti-Inflammatory Activities of Hydroalcoholic Extract of *Cassia alata* Roxb. Leaves in Experimental Animals

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Sanjaya Kumar. Y.R*, Srikanth Ala, Vasanthakumar K.G, Deep V.C, Sudesh Gaidhani N, Divya A, Swamy G.K.

National Ayurveda Research Institute for Panchakarma, CCRAS, Ministry of AYUSH, Cheruthuruthy(PO), Thrissur (Dist) , Kerala 679 531

Submission: 28 February 2019
Accepted: 3 March 2019
Published: 30 March 2019

Keywords: *Cassia alata*, rats, mice, analgesic, anti-inflammatory

ABSTRACT

Hydro-alcoholic extract of *Cassia alata* Roxb. leaves was evaluated for anti-inflammatory and analgesic activities in Wistar rats and Swiss mice. The extract was found to be safe up to 2000 mg/kg body weight when assessed through OECD 423 acute toxicity study. The extract showed significant anti-inflammatory activity at doses 250 and 500 mg /kg body weight in rats. The extract also showed significant analgesic activity and the effect on peripheral analgesia was found to be more prominent than centrally mediated analgesia.

HUMAN JOURNALS
www.ijppr.humanjournals.com

INTRODUCTION

Cassia alata (L). Roxb. Commonly known as Candle tree/Candle bush (Fig. 1) belongs to leguminosae family and is used as ornamental and medicinal plant (1). It is known as ergag/prapunnal in hindi, seemeagase in Kannada and aanatagara in Malayalam.

Fig. 1 *Cassia alata* tree

In traditional medicine, the leaves, roots and stem bark are widely used for the treatment of skin infections (2). *Cassia alata* is often called ringworm bush owing to its effective fungicidal properties. The leaves have been found to have antioxidant property due to their high polyphenol and flavonoid content (3). Hexane extract of *Cassia alata* leaves has showed anti-inflammatory activity against complete Freund adjuvant induced arthritis in rats. The leaves as decoction are used to treat bronchial asthma and its broncho relaxant activity has been demonstrated by experimental studies in rats (4). Phytochemical analysis of hydroalcoholic extract of *Cassia alata* leaves revealed the presence of alkaloids, flavonoids, saponins, tannins, steroids, glycosides and saponins (5).

MATERIALS AND METHODS

Animals

Adult male and female Swiss albino Mice and Wistar albino rats were used in the study were procured from Small Animal Breeding Station, Veterinary College, Mannuthy, Thrissur, India. Animals were quarantined for 10 days and were caged individually with access to standard pelleted feed and water ad libitum.

Ethical clearance

Necessary permission for conducting the present trial was obtained during Institutional Animal Ethics Committee (IAEC) meeting held at National Ayurveda Research Institute for Panchakarma, Cheruthuruthy, Thrissur, Kerala.

Test drug

Leaves of *Cassia alata* Roxb. were procured from the local areas of Cheruthuruthy, Thrissur, Kerala and were authenticated at PS & GR Division, Centre for Medicinal Plants Research, Arya Vaidyasala, Kottakkal, Kerala. Powdered leaves of the test drug were extracted in 50 percent aqueous alcohol and the process was repeated for extract residue. The filtrate of *Cassia alata* hydro-alcoholic extract (CAHAE) was concentrated, stored at -20ºC. (6).

Test groups

The trial comprised of 5 groups viz., one control group, three test drug groups (low dose group, average dose group & high dose group) and one standard drug group, each comprising of 3 male and 3 female animals. Animals in control group received distilled water and those in standard drug group received ibuprofen (40 mg/kg. B.W.). Animals in low dose, average dose and high dose group received hydroalcoholic extract of *Cassia alata* Roxb. at doses 100 mg, 250 mg and 500 mg /kg body weight respectively.

Acute Toxicity study

Acute toxicity studies for hydroalcoholic extracts of leaves *Cassia alata* Roxb. were carried out in Wistar Rats as per OECD guideline 423 (7). Test extracts were given at dose rate of 2000 mg per kg, body weight to 3 female Wistar rats once orally and animals were observed for mortality and signs of toxicity for next 14 days. Body weight and feed consumption of each animal were recorded at weekly intervals. The Study was repeated with another batch of 3 female Sprague Dawley (SD) rats.

Analgesic activity

Radiant Heat method

Effect of *Cassia alata* hydroalcoholic extract on threshold tail flick response in rats was noted using analgesiometer (8). Tail flick response was measured in rats before and at 30, 60, 120

& 180 minutes post drug administration and compared with response in animals of control group.

Hot plate method

Analgesic effect of the *Cassia alata* hydro-alcoholic extract was studied in mice using hot plate method (9). Basal reaction time in terms of paw licking and jump response was noted by keeping the animals individually on hot plate maintained at a constant temperature of 55^0C. A cut off period of 15 seconds was observed to avoid damage to the paws. The animals were retested at 30, 60, 120 and 180 minutes post test drug administration and difference in reaction time was noted and compared with that of control.

Acetic acid induced writhing

Cassia alata hydro-alcoholic extract was administered in different doses and 45 minutes later 3% acetic acid (v/v – 0.1 ml per 10 g. b.w. I.P.) was used to induce writhing in mice (10). No. of stretching episodes (writhings) exhibited by the animal were counted for a duration of 30 minutes post acetic acid injection. Percent reduction in writhing syndrome was calculated and compared with the standard drug. Percent reduction indicates the percentage protection against abdominal constriction which was taken as an index of analgesia.

It was calculated as:

$$\{(W_c - W_t) \times 100\}/W_c$$

Where, W_c = number of writhing of the control group

W_t = number of writhing of the test drug groups/standard drug group.

Formalin licking test

Swiss mice were pre-treated with *Cassia alata* extracts 45 minutes before injection of 20 microlitres of 1% formalin underneath dosrsal surface of left paw. The animals were observed for licking response and the time spent in licking at 0-5 minutes (initial phase) and 20- 30 minutes (late phase) intervals post formalin injection was recorded (11).

Carrageenan induced hind paw oedema

Hind paw oedema was induced by injecting 0.05 ml of 1% carrageenan in normal saline beneath plantar aponeurosis of right hind paw of rats. Control, test drug and standard drugs (Ibuprofen 40 mg per kg. body weight) were administered 45 minutes prior to Carrageenan injection were injected. The increase in paw volume was noted 3 hours post carrageenan administration by fluid displacement method using plethysmometer and compared between the groups (12).

Statistical analysis

The data generated during the study was analysed through ANOVA with post tests.

RESULTS AND DISCUSSION

Single administration of *Cassia alata* hydro-alcoholic extract to female Wistar rats did not cause any mortality or signs of toxicity and the test drug at dose of 2000 mg /kg body weight was found to be safe during the experimental trial.

CAHE at dose of 500 mg/kg b.w. significantly increased the threshold of tail flick response in rats and paw licking/jumping response in mice by hot plate method (tables 1 & 2). The tail flick model and hot plate method are well-validated model for analysis of analgesics through spinal origin.

The test drug at doses 250 and 500 mg /kg body weight significantly reduced the number of writhing in response to acetic acid injection in mice (Table 3). Prostaglandin and arachidonic acid released in response to localized inflammation caused by acetic acid causes pain and writhing inhibiting substances inhibits the release of these mediators and suppress pain (13).

The test drug at doses 250 and 500 mg /kg body weight significantly reduced duration of time spent by mice in licking post formalin injection (Table 4). Early phase of licking is due to effect of formalin on nociceptors and that in the late phase is a sequel to inflammation. The early phase (immediately after injection) seems to be caused by C-fiber activation due to the peripheral stimulus. The late phase appears to depend on the combination of an inflammatory reaction.

The test drug at doses 250 and 500 mg /kg. body weight prevented the development of oedema by injection of phlogestic agent carrageenan (Table 5). Cyclooxygenase plays an important role in conversion of arachidonic acid into prostaglandins in the later inflammation phase in the carrageenan-induced edema model and this enzyme is considered to be a known target for a variety of NSAIDs. The anti-inflammatory activity of Cassia alata might be attributed to flavonoid content and Kaempferol- 3 O-gentibioside (K3G) flavonoid glycoside isolated from leaves has showed significant anti-inflammatory activity in experimental studies (14).

Table 1.Effect of *Cassia alata* leaves hydro-alcoholic extract on latency of tail flick response in rats

Group	**Reaction time in Sec (Mean ± SEM) at various time intervals post drug administration**				
	Initial	**30 Min**	**60 Min**	**120 Min**	**180 Min**
Control group	7.17 ± 0.4	7.17 ± 0.4	7.17 ± 0.4	7 ± 0.36	7.17 ± 0.3
Low dose group CAHE 100 mg/kg	7.83 ± 0.6	7.83 ± 0.6	8.17 ± 0.4	8.33 ± 0.33	8.5 ± 0.43
Average dose group CAHE 250mg/kg	6.67 ± 0.42	7.17 ± 0.3	7.83 ± 0.3	8.33 ± 0.56	8.33 ± 0.33
High dose group CAHE 500 mg/kg	6.83 ± 0.6	7.17 ± 0.5	8.33 ± 0.55	9 ± 0.52*	9.5 ± 0.43*
Standard drug group (Ibuprofen) 40 mg/kg	8.66 ± 0.21	9.16 ± 0.3 *	10.83 ± 0.3**	11.66 ± 0.33**	12.33 ± 0.33**

*P<0.05, ** P<0.01 as compared to Control group

Table 2. Effect of *Cassia alata* leaves hydro-alcoholic extract on hot plate analgesia in mice

Group	Reaction time in Sec (Mean ± SEM) at various time intervals				
	Initial	30 Min	60 Min	120 Min	180 Min
Control group	4.83 ± 0.48	5 ± 0.45	5 ± 0.26	5.33 ± 0.42	4.83 ± 0.31
Low dose group CAHE 100 mg/kg	6 ± 0.36	5.67 ± 0.42	6 ± 0.36	5.83 ± 0.17	6.33 ± 0.33
Average dose group CAHE 250mg/kg	6.67 ± 0.56	5.83 ± 0.31	6 ± 0.26	6.67 ± 0.21	7.17± 0.4
High dose group CAHE 500 mg/kg	6 ± 0.36	6 ± 0.26	6.5 ± 0.22*	7.17 ± 0.31*	8.5 ± 0.22**
Standard drug group (Ibuprofen) 40 mg/kg	6.17 ± 0.31	6.33 ± 0.33	7 ± 0.36**	8.33± 0.42***	9.17 ± 0.31***

*P<0.05, ** P<0.01, *** P<0.001 as compared to Control group

Table 3. Effect of *Cassia alata* leaves hydro-alcoholic extract acetic acid induced writhing in mice

Group	No. of stretching episodes (Mean ± SEM)	Percentage inhibition of oedema
Control group	50 ±2.87	----
Low dose group CAHE 100 mg/kg	43.83 ±1.7	12.33
Average dose group CAHE 250mg/kg	40.83±0.98*	18.33
High dose group CAHE 500mg/kg	39.5 ±1.94**	21
Standard drug group (Ibuprofen) 40 mg/kg	23 ±1.93**	54

*P<0.05, ** P<0.01 as compared to Control group

Table 4. Effect of *Cassia alata* leaves hydro-alcoholic extract on paw licking test in mice

Group	Duration of Paw licking in seconds (Mean ±SEM)	
	Early Phase	Late Phase
Control group	39.17 ± 1.28	149.16 ±6.02
Low dose group CAHE 100 mg/kg	35 ±1.53	134.5 ±5.08
Average dose group CAHE 250mg/kg	32.5 ±2.03*	110 ±3.23**
High dose group CAHE 500 mg/kg	21.83 ±0.87**	81.83 ±3.27**
Standard drug group (Ibuprofen) 40 mg/kg	16.17 ±1.42**	36.66 ±2.63**

*P<0.05, ** P<0.01 as compared to Control group

Table 5. Effect of *Cassia alata* leaves hydro-alcoholic extract on carrageenan induced hind paw oedema in rats

Group	Volume of Paw oedema in ml (Mean ± SEM)	Percentage reduction
Control group	0.54 ± 0.04	----
Low dose group CAHE 100 mg/kg	0.46 ±0.01	15.85
Average dose group CAHE 250mg/kg	0.38 ±0.03*	30.18
High dose group CAHE 500 mg/kg	0.15 ± 0.01**	66.67
Standard drug group (Ibuprofen) 40 mg/kg	0.09 ±0.01**	82.93

*P<0.05, ** P<0.01 as compared to Control group

CONCLUSION

Hydro-alcoholic extract of *Cassia alata* Roxb. leaves was found to be safe up to 2000 mg/kg body weight in Wistar rats. The extract found to possess very significant analgesic activity when screened through acetic acid induced writhing and formalin induced paw licking in mice and anti-inflammatory in carrageenan induced hind paw oedema method in rats. The analgesic activity exhibited by the test extract was less significant in radiant heat method and

hot plate method. The test extract was found to possess more peripheral analgesic activity than central mediated analgesia.

ACKNOWLEDGEMENT

Authors are thankful to Professor Vaidya Dhiman K.S., Director General, CCRAS, New Delhi for providing necessary facilities and encouragement. The technical assistance received from Sri. Sudheesh P.S., Technical Assistant, Quality Control Laboratory, NARIP, Cheruthuruthy is highly solicited.

REFERENCES

1. Singh B, Nadkarni JR, Vishwakarma RA, Bharate SB, Nivsarkar M, Anandjiwala S The hydroalcoholic extract of Cassia alata (Linn.) leaves and its major compound rhein exhibits antiallergic activity via mast cell stabilization and lipoxygenase inhibition. Journal of.Ethnopharmacology. 2012; 41:469-473.
2. Lifongo LL, Simoben CV, Ntie-Kang F, Babiaka SB, Judson PN (2014). A bioactivity versusethno botanical survey of medicinal plants from Nigeria, West Africa. Natural Products Bioprospect. 2014; 4 (1):1-19.
3. Subramanian D P and Venugopal S. Phytochemical investigation of Cassia alata Linn. flowers through various in vitro antioxidant assays. International Journal of Pharmacy& Technology. 2001; 3(4): 3521-3534
4. Ouédraogo, M, Da F.L, Fabré A, Konaté K, Dibala C.I, Carreyre H, S. Thibaudeau S,J, Coustard J.M, Vandebrouck C, Bescond J and Belemtougri R.G.Evaluation of the Bronchorelaxant, Genotoxic, and Antigenotoxic Effects of Cassia alata L.Evidence-Based Complementary and Alternative Medicine. 2013. Online Article ID:162651.
5. Meenupriya, J,SahayaVinisha A and P Priya P Phytochemical screening and bioassay of cassia alata leaf extract to study its skin hyperpigmentation activity. World Journal of Pharmaceutical Sciences. 2014; 2 (12) : 1723-1727.
6. AyurvedicPharmacoepia of India, Part 1 Vol. 8., New Delhi. Ministry of AYUSH. 2011
7. OECD guideline 423 OECD guidelines for testing of chemicals section 4 Health effects OECD France.2001.
8. Gujral, M.C. and Khanna B.K. Comparative evaluation of some narcotic analgesics. Journal of scientific and Industrial research. 1956; 168: 11-13
9. Eddy NB, Leimbach D. Systematic analgesics II.Diethyl butenyl and diethienylbutyl amines. Journal of Pharmacology and experimental therapeutics.. 1953;107:387–93.
10. Witkin LB, Herbner CF, Gaddi F, O'Keefe E, Spitaletta P and Plumer AJ. Pharmacology of 2-aminoindane hydrochloride (SU- 8629).A potent non- narcotic analgesic. J Pharmacology and Experimental Therapeutics. 1961;133:400–8.
11. Hunskaar S and Hole K. The formalin test in mice-dissociation between inflammatory and non-inflammatory pain. Pain. 1997;30:103–4
12. Winter, C.A, Risley, E.A. and Nuss, G.W. Carrageenan induced oedema in hind paw of the rat as an assay of anti-inflammatory drugs. Proceedings of Society of Experimental. Biology and Medicine. 1962;III:544-547.
13. Viana G, Bandeira M and Matos F. Analgesic and anti-inflammatory effects on chalcones isolated from Myeacrodruon Urundeuva Allemao. Phytomedicine, 2003;10(2) : 189-195.
14. Moriyama, H., Iizuka, T., Nagai, M., MiyatakH., and Satoh, T. "Anti-inflammatory activity of heat-treated Cassia alata leaf extract and its flavonoids glycoside." YakugakuZasshi. 2003;123(8): 607-11.