STUDY OF ANTI-INFLAMMATORY ACTIVITY OF *CASSIA AURICULATA* LINN. LEAVES IN WISTAR RATS

ABSTRACT

The present study was undertaken to evaluate the anti-inflammatory activity of aqueous, methanolic, ethyl acetate and hydroalcoholic extracts of *Cassia auriculata* leaves. The study was carried out using the pharmacological model of carrageenan induced rat paw edema. Among all extracts methanolic extract showed maximum anti-inflammatory potential. Indomethacin (10mg/kg) was used as reference compound in the present study. The anti-inflammatory activity of methanolic extract may be due to presence alkaloids, flavonoids, tannins and steroids.

Keywords: *Cassia auriculata*, Carrageenan.

INTRODUCTION

Inflammation is clinically defined as a pathophysiological process characterised by redness, edema, fever, pain and loss of function. Inflammation involves localised increase in number of leucocytes and a variety of complex mediators[1]. It is a protective attempt by the organism to remove the injurious stimuli as well as initiate the healing process of tissue. It is necessary in healing of wounds. If inflammation remains unchecked, it can lead to onset of diseases like vasomotor rhinnorhoea, rheumatoid arthritis and atherosclerosis[2, 3]. The main symptoms of body against inflammatory stimuli are increased body temperature and pain. However the severity of symptoms depends on the type of inflammation i.e. whether it is acute inflammation or chronic inflammation. In acute inflammation symptoms observed are edema, erythraemia, pain, heat and above all loss of function[2].

The presently available drugs for inflammation provide only symptomatic relief and are not free from side effects. The target should be to discover newer drugs from plant kingdom which may provide therapeutic cure and would be free from undesirable effects as well as be economical, which would be accepted by the developing nations like India[4].

Cassia auriculata Linn. is an annual or biennial shrub found throughout India in open forests. It is mainly found in dry zones of southern, western and central India extending up to Rajasthan in north. It is a tall, branched, bushy shrub growing wild throughout forests, along roadsides and in wastelands[5]. Traditionally the plant has been used in ayurvedic medicine as 'Avarai Panchaga Chooram' and as constituent of 'Kalpa' herbal tea[6]. The leaves are bitter, astringent, acrid, constipating and expectorant. Tribals use the poultice of *C. auriculata* leaves for the treatment of pain and inflammatory diseases[7]. Although the leaves of *C.* auriculata have been used for long time in the treatment of inflammation and pain, the anti-inflammatory activity of leaves is not supported by any scientific base. So, the present study was designed to evaluate the anti-inflammatory activity of *Cassia auriculata* leaves.

MATERIALS AND METHODS

Plant Material

The leaves of *C. auriculata* were obtained from their natural habitat in the Pune region of Maharashtra. The plant was identified and authenticated at Botanical Survey of India, Pune). Voucher specimen (no. CAAAAM5) has been deposited in the Botanical Survey of India, Pune, India. The leaves were shade dried and were pulverized in grinder and stored in air tight container for further use.

Preparation of Extracts

The powdered leaves were subjected to extraction by maceration method using water, methanol, hydroalcoholic mixture and ethyl acetate as solvent, separately for 72 h at room temperature.

The extracts were filtered and concentrated under reduced pressure at 50˚C. The % yield of *C. auriculata* leaves was found to be 11.2% for methanolic, 12.5% for aqueous, 7.3% for ethyl acetate and 9.8% for hydroalcoholic extracts.

Phytochemical Analysis

The preliminary phytochemical analysis of all the plant extracts was performed as per the standard qualitative methods. A series of chemical tests were carried out for presence of alkaloids, flavonoids, carbohydrates, glycosides, steroids and tannins[8].

Experimental Animals

Wistar rats (180-200 g) of either sex were used for the study. The animals were procured from the National Institute of Biosciences (NIB), Pune. The animals were kept under standardized animal house conditions with free access to standard pellet diet and water *ad libitum*. The study protocol was approved by institutional animal ethical committee and experimental procedures were conducted in accordance with the regulations of CPCSEA (884/PO/ac/05/CPCSEA).

Acute Toxicity Study

The acute toxicity study of all the extracts was carried out using healthy adult Wistar rats of either sex. They were orally fed with extract in increasing dose levels of 100, 500, 1000, 3000, 5000 and 8000 mg/kg. The mortality, grooming, hyperactivity, sedation, loss of righting reflex and convulsions of the animals were observed periodically for 48 h[9].

Anti-inflammatory Study

Healthy Wistar albino rats (150-180 g) of either sex were used for the study. Rats in groups of six each were treated with vehicle, aqueous extract (250 and 500 mg/kg), methanolic extract (250 and 500 mg/kg), hydroalcoholic extract (250 and 500 mg/kg), ethyl acetate extract (250 and 500 mg/kg) and indomethacin (10 mg/kg) one hour prior to carrageenan injection. 0.1 mL of 1% carrageenan was injected into the sub-plantar region of hind paw of the rats. The test groups of rats were treated with aqueous, hydroalcoholic, methanolic and ethyl acetate extracts of *C. auriculata* leaves, 1 h before the carrageenan injection. At the same time, the control group was administered orally with 1 mL/kg of vehicle and indomethacin 10 mg/kg. The paw volume upto the tibio-tarsal articulation was measured using a plethysmometer (VJ Instruments, Nagpur). The paw volume was determined at 1, 2, 3, 4 and 6 h. The % inhibition in paw volume was calculated by using following formula[10, 11].

$$\% \text{ inhibition in paw volume} = 100 \times (1 - Vt / Vc)$$

Where,
Vt = Mean paw volume in drug treated group.
Vc = Mean paw volume in control group.

Statistical Analysis

The results were statistically analysed using one way ANOVA followed by Dunnet's test of multiple comparison. Value of $P < 0.05$ was considered to be statistically significant.

RESULTS

The qualitative phytochemical analysis of various extracts of *C. auriculata* leaves showed the presence of active constituents such as anthraquinone glycosides, alkaloids, flavonoids, saponins, steroids and tannins. In acute toxicity study, no adverse effect or mortality was observed in rats with oral administration of aqueous, methanolic, hydroalcoholic and ethyl acetate leaves extract of *C. auriculata* leaves upto a high dose of 8000 mg/kg.

Table I shows the anti-inflammatory activity of aqueous, hydroalcoholic, methanolic and ethyl acetate extracts of *C. auriculata* leaves. The aqueous extract of *C. auriculata* 250 & 500 mg/kg shows significant activity at 6 h with percentage inhibition of 31.06 and 30.62% respectively. The ethyl acetate extract of *C. auriculata* at 250 & 500 mg/kg shows significant activity in second phase of inflammation induced by carrageenan with percentage inhibition of 34.16 and 30.79% respectively. The hydroalcoholic extract of *C. auriculata* 250 & 500 mg/kg shows significant activity at 6 h with percentage inhibition of

Table I: Anti-inflammatory activity of *Cassia auriculata* leaves extracts

Treatment	Dose	Paw volume in mL				
		1 h	2 h	3 h	4 h	6 h
Control	1 mL/kg	1.656±0.1	1.959±0.07	2.466±0.2	2.660±0.2	2.962±0.25
Standard	10 mg/kg	1.416±0.09 (14.49)	1.648±0.08 (15.87)	1.482±0.15** (39.90)	1.798±0.17** (32.40)	1.705±0.15** (42.43)
Aqueous extract	250 mg/kg	1.562± 0.03 (5.67)	1.615±0.11 (17.55)	1.790±0.11 (27.41)	1.672±0.12** (37.14)	2.042±0.1** (31.06)
Aqueous extract	500 mg/kg	1.451±0.06 (12.37)	1.669±0.09 (14.80)	1.799±0.14 (27.04)	2.249±0.05 (15.45)	2.055±0.15** (30.62)
Hydroalcoholic extract	250 mg/kg	1.623± 0.1 (1.99)	2.090±0.15	2.088±0.06 (15.32)	2.087±0.07 (21.54)	2.259±0.14* (23.73)
Hydroalcoholic extract	500 mg/kg	1.696±0.08	2.436±0.2	2.104±0.17 (14.67)	2.154±0.14 (19.02)	2.045±0.11** (30.95)
Methanolic extract	250 mg/kg	1.382± 0.01 (16.54)	1.468±0.09** (25.06)	1.799±0.05** (27.04)	2.010±0.06* (24.43)	1.866±0.05** (37)
Methanolic extract	500 mg/kg	1.577±0.07 (4.7)	1.590±0.03** (18.83)	1.899±0.06** (22.99)	2.040±0.08* (23.30)	2.025±0.15** (31.63)
Ethyl acetate extract	250 mg/kg	1.35± 0.03 (18.47)	1.53± 0.1* (21.89)	1.716±0.1** (30.41)	2.269± 0.2 (14.69)	1.95±0.06** (34.16)
Ethyl acetate extract	500 mg/kg	1.63±0.08 (1.5)	2.019±0.08	2.08±0.08 (15.65)	2.312±0.18 (13.08)	2.05±0.1** (30.79)

N=6; Value expressed as mean ± S.E.M., (P<0.05)

23.73 and 30.95% respectively. Similarly, methanolic extract showed inhibition of paw edema in second phase with percentage inhibition of 37% at 250 mg/kg & 31.63% at 500 mg/kg. The standard indomethacin shows significant activity from 3 h onwards, maximum at 6 h with inhibition of 42.56%.

DISCUSSION

The anti-inflammatory activity of various extracts of *C. auriculata* leaves was carried out using carrageenan induced rat paw edema. Carrageenan-induced inflammation represents a classical model of edema formation and hyperalgesia, which has been extensively used for evaluation of anti-edemal effect of drugs[12]. The sub-planter administration of carrageenan in rat is responsible for the typical biphasic edema[13] in which the first phase observed around 0-2 hours is attributed to the release of histamine and serotonin. The second phase of swelling which last for 2-6 hours is due to release of prostaglandin- like substances[14, 15].

In the present study, methanolic extract of *C. auriculata* leaves showed potent anti-inflammatory activity compared to aqueous, hydroalcoholic and ethyl acetate extracts. As the anti-inflammatory effect was more significant during later phase of inflammation, it can be concluded that there might

be inhibition of inflammatory mediators such as prostaglandins, leukotrienes, polymorphonuclear cells or bradykinins. In accordance with previous studies steroids, flavonoids, alkaloids, terpenoids and tannins have been shown to possess of anti-inflammatory activity[16]. Thus the anti-inflammatory effect of methanolic extract may be due to presence of of active constituents like alkaloids, flavonoids, tannins and steroids. However, chemical constituents and mechanism responsible for the pharmacological activities remain to be investigated.

ACKNOWLEDGEMENTS

The authors are very grateful to the management, P.E. Society's Modern College of Pharmacy, Nigdi, Pune, India for providing required facilities of this work. Authors are also grateful to P. G. Diwakar (Joint Director, Botanical Survey of India, Pune) for authentication of plant material.

REFERENCES

1. Hyun P.O., Kun H.S., Hyeun W.C., Sam S.K.: Anti-inflammatory plant flavonoids and cellular action mechanisms, **J Pharmacol Sci.** 2004, 96, 229-245.
2. Suralkar A.: *In vivo* animal models for evaluation of anti-inflammatory activity, 2008. Available from: http://www.pharmainfo.net/reviews.
3. Singh A., Malhotra S., Subban R.: Anti-inflammatory and analgesic agents from Indian medicinal plants, **International Journal of Integrative Biology.** 2008, 3, 57-72.
4. Shah B.N., Nayak B.S., Seth A.K., Jalalpure S.S., Patel K.N., Patel M.A., Mishra A.D.: Search for medicinal plants as a source of anti-inflammatory and anti-arthritic agents - A review, **Pharmacognosy Magazine.** 2006, 2, 77-86.
5. Gaikwad S.A., Kale A.A., Jadhav B.G., Deshpande N.R., Salvekar J.P.: Anthelmintic activity of *Cassia auriculata* L. extracts - *in vivo* study, **J. Nat. Prod. Plant. Resour.** 2011, 1, 62-66.
6. Doshi G.M., Aggarwal G.V., Shanbagh P.P., Shastri K.V., Agarwal O.K., Bhalerao A.B., Desai S.K.: Studies on *Cassia auriculata* plant parts by improved *in vitro* antioxidant models, **J. of Pharma and Biomed Sci.** 2011, 10, 1-2.
7. Balakrishna R.B., Jagir R.P., Prabhakaran V.: *Cassia auriculata* – A phytopharmacological review, **J. Adv Drug Research.** 2011, 1, 46-56.
8. Khandelwal KR.: Practical Pharmacognosy: Techniques and Experiments. 10th edition, Nirali Prakashan, Pune. 2006, 149-150.
9. Bandawane D.D., Juvekar A.R., Juvekar M.R.: Preliminary study on antidiabetic activity of *Withania coagulans* (Dunal) fruit extract in normal and streptozotocin induced diabetic rats, **Indian Drugs.** 2010, 47, 72-76.
10. Kumar S., Kumar V., Prakash O.M.: Pharmacognostic study and anti-inflammatory activity of *Callistemon lanceolatus* leaf, **Asian Pacific J. Trop. Med.** 2011, 177-181.
11. Benni J.M., Jayanthi M.K., Suresha R.N.: Evaluation of the anti-inflammatory activity of *Aegle marmelos* (Bilwa) root, **Indian J. Pharmacol.** 2011, 43, 393-397.
12. Guay J., Bateman K., Gordon R., Mancini J., Riendeau D.: Carrageenan-induced paw edema in rat elicits a predominant prostaglandin E_2 (PGE_2) response in the central nervous system associated with the induction of microsomal PGE_2 synthase-1, **J Biol Chem.** 2004, 279, 24866-24872.
13. Fabiana R.N., Tais A.A.B., Angelica M.L., Carlos E.C.O., Ricardo R.D.S., Milena B.P.S., Cristiane F.V.: Anti-inflammatory and antinociceptive activities of *Blechnum occidentale* L. extract, **J. Ethnopharmacol.** 2009, 125, 102-107.
14. Kaushik M.L., Jalalpure S.S.: Evaluation of anti-inflammatory effect of ethanolic and aqueous extracts of *Curcuma zedoaria* rose root, **Int. J. Drug Der. & Res.** 2010, 3, 360-365.
15. Cuman R.K.N., Bersani-Amadio C.A., Fortes Z.B.: Influence of type-2 diabetes on the inflammatory response in rats, **Inflam. Res.** 2001, 50, 460-465.
16. Lilian E.P., Teresita G., Americo O.J., Eduardo G.: Acute and chronic anti-inflammatory effects of plant flavonoids, **Il Farmaco.** 1998, 53, 421-424.

Department of Pharmacology
Progressive Education Society's Modern College of Pharmacy,
Sector no 21, Yamunanagar, Nigdi, Pune-44, Maharashtra.
E-mail : rspatil_pharma@yahoo.co.in

Mali A. A., Hivrale M. G., Bandawane D. D.* and Chaudhari P. D.

*For correspondence

(Received 22 August 2012) (Accepted 09 October 2012)