

ANALGESIC AND ANTI-INFLAMMATORY PROPERTIES OF *CAESALPINIA* (BONDUC) SEEDS

Manoj Kumar Sagar^{1*}, Praveen Kumar Ashok¹, Himansu Chopra¹, Manmohan Singh², Kumud Upadhyaya³

Affiliated to: 1. Faculty of Pharmacy, GRD (PG)IMT, Dehradun.
2. Faculty of Pharmacy, Azad institute of Pharmacy, Lucknow.
3. Faculty of Pharmacy, Kumaun University, Bhimtal, Nainital.

ABSTRACT

Caesalpinia bonduc was evaluated for anti-inflammatory action by carrageenin-induced rat paw edema. The analgesic activity was tested by acetic-induced writhing response in albino mice and hot plate method in albino rats. The ethanolic extract of *Caesalpinia bonduc* in doses of 100, 200 and 500 mg/ml showed 70.3, 71.2 and 73.0% inhibition of paw edema respectively at the end of three hour and the percentage of protection from writhing was 45.5, 51.2 and 65.5 respectively. In the hot plate model, the ethanolic extract of *Caesalpinia* in the above doses increased the pain threshold significantly after 30, 60, and 90 min. of administration *Caesalpinia* showed dose dependent action in all experimental models. The plant had Saponins, flavonoids, glycosides, oils, phenols and tannins and significantly increased the reaction time of hot plate. The results of the present study confirm that *Caesalpinia bonduc* has potent analgesic and anti-inflammatory activities.

Keywords: Analgesic, Anti-inflammatory, Carrageenin, *Caesalpinia bonduc*.

1. INTRODUCTION

Caesalpinia bonduc, family Fabaceae commonly called Nata Karanj (Hindi) seeds are a shrub plant. It has twigs and young plant and yellow leaves, fruits inflated pods covered with wiry prickles and yellow flower. The stem is thin woody and the plant produced in India, Myanmar, Vietnam, Sri Lanka, and the Malay Peninsula and distributed domestically in China in Yunnan, Guizhou, Sichuan, Guangdong, Guangxi, Fujian and Taiwan. Many Pharmacological activities of *Caesalpinia bonduc* have been reported treatment of tumors, inflammation

antiviral, antiasthmatic, antiamebic, and antiestrogenic (Negi NC, 1958, Adesina and liver disorders (Kirtikar KR, 1975), antipyretic, antidiuretic, anthelmintic, antibacterial, anticonvulsant, antiviral, antiasthmatic, antiamebic, and antiestrogenic (Negi NC, 1958, Adesina SK, 1982, Dhar ML, *et. al.*, 1968, Gayaraja S, *et. al.*, 1978, Raghunathan K, *et. al.*, 1982), and other activities. The present study was therefore undertaken to investigate some of the folkloric claims especially the use of the plant as a treatment of inflammation (Kirtikar KR, 1975).

2. Materials and Methods :-

2.1. Animals: -

Male albino rats weighing 190 – 230 gm were used for this study. The animals were bred and housed in the central animal house of the Faculty of Pharmacy, GRD(PG)IMT,

* Corresponding Author
Department of Pharmacy, GRD (PG) IMT, 214,
Rajpur Road Dehradun INDIA
Tel.: +91-9412469157
E-mail: manojksagar313@gmail.com

Dehradun, (U.K.). The animals were housed in groups of 6 – 10 under environmentally controlled condition with free access to water and standard food. Food was withheld overnight prior to experiments while water was still provided *ad libitum*. The research was conducted in accordance by Ethical Committee, Faculty of Pharmacy, GRD(PG)IMT, Dehradun (U.K.).

2.2. Drugs and Chemicals: -

The following drugs and chemicals were used: Carrageenan (Sigma - Aldrich), Acetic acid (Ranbaxy Laboratories Ltd., Punjab), Aspirin (Vikash Pharma, Mumbai), Pethidine (Bengal Immunity, Kolkata).

2.3. Plant materials:-

The plant (*Caesalpinia bonduc*) used for this study was collected from Dehradun and identified by Dr. Kumud Upadhyaya, Principal, College of Pharmacy, GRD(PG)IMT, Dehradun. A voucher specimen has also been deposited in the herbarium of the institute for future references. The air dried seeds of the plant were cleaned and reduced to powdery form with mortar and pestle, after which 150 gm of powdered sample was exhaustively extracted with 2.5 lt of ethanol (analytical grade), for 3 days (by soxhlet apparatus). The plant material was separated by filtration and the ethanolic extract was concentrated (by Rotavapour, Büchi, Switzerland) and lyophilized to preserve it. The residue was obtained 3.2gm and dilutions of the extract were made in 2% gum acacia for the various studies. Preliminary phytochemical screening was carried out on the extract using the standard screening method of Trease and Evans (1983).

2.4. Analgesic activity:-

2.4.1. Acetic acid – induced writhing in mice: -

The writhing acetic acid test was performed in mice as originally described by Ghosh, MN, 1984. The Prescreened animals were divided into groups as shown in Table 1. Aspirin in doses of 50, 100, and 150 mg/kg, suspended in 2% gum acacia was used as the standard drug and administered subcutaneously. Writhing was induced 30 min. later by intraperitoneal injection of 10 ml/kg of 0.6% acetic acid in distilled water. Aspirin is a well known peripheral analgesic drug and it was used as a positive control in the present investigation. The mice were then placed in an observation box, and the number of writhes was counted for 20 min. after acetic acid injection.

2.4.2. Hot plate method: -

The hot plate latency assay was based on the method of Eddy *et. al.*, (1950). The temperature of the hot plate set at $55 \pm 0.5^{\circ}\text{C}$. The plant extract, saline, and indomethacin were given to the animals (each group 6 animals) orally after a 12 hr. fast. All the animals in each group were placed on a hot plate 30 min. after the administration of extract, standard drug and saline. The average of the two readings was obtained as the initial reaction time (T_b). The reaction time (T_a) was recorded when the animals to lick the foot or jump off the hot plate and the mean of the latency for each group at 60 and 90 min. after the administration of extract, saline and indomethacin. The following calculation was:

$$\text{Percentage analgesic activity} = \frac{T_a - T_b \times 100\%}{T_b}$$

2.5. Anti-inflammatory activity:-

2.5.1. Inflammatory paw edema in rats:-

This assay was determined as described by Winter *et. al.*, (1962). Animals were divided into five groups comprising six animals in each group with 2% gum acacia in normal saline. An injection of 0.1 ml of carrageenin

suspension (200µg/paw) was made into the right hind foot of each conscious rats under the subplantar (150-170gm). The control, standard and test groups were treated orally with saline, indomethacin and the extracts 1hr. before carrageenin injection. The paw

volume was measured plethysmometrically (Ugo Basile, Italy) at '0' and '3' hour after the carrageenin injection. The difference between the two readings was taken as the volume of paw edema and percentage inhibition was calculated.

$$\text{Percentage inhibition} = \frac{(C_t - C_0)_{\text{control}} - (C_t - C_0)_{\text{treated}}}{(C_t - C_0)_{\text{control}}} \times 100$$

Where C_t = paw circumference at time t, C_0 = paw circumference before carrageenin injection

2.6 Statistical analysis

In this study, Results are expressed as mean $\pm$ S.E.M. statistical evaluations were made using ANOVA followed by t-test (Prism 3.0) and P values less than 0.05 were considered significant. Data are represented as mean $\pm$ S.E.M.

3. Results: -

In the anti-inflammatory tests, the results show oral treatment of animals with ethanolic extract of *Caesalpinia bonduc* (100 – 500 mg/kg b.w.) and Indomethacin (5 mg/kg). The test and standard drugs produced significant inhibition of paw edema in comparison to the control (Table-1).

Table 1. Effect of the ethanolic extract *Caesalpinia bonduc* on carrageenin-induced paw edema in rats.

S.No.	Groups	Dose orally (mg/kg. p.o.)	Initial paw size	Paw edema		Inhibition	
				3 hr	4 hr	3 hr	4 hr
1	Control	---	2.0 $\pm$ 0.3	7.4 $\pm$ 0.5	8.4 $\pm$ 0.5	----	----
2	Caesalpinia	100	1.9 $\pm$ 0.5	2.2 $\pm$ 0.2	1.0 $\pm$ 0.2**	70.3	88.1
3	Caesalpinia	200	2.0 $\pm$ 0.2	2.1 $\pm$ 0.5	0.8 $\pm$ 0.2**	71.2	90.2
4	Caesalpinia	500	1.9 $\pm$ 0.2	2.0 $\pm$ 0.7**	0.6 $\pm$ 0.3**	73.0	92.9
5	Indomethacin	5	1.9 $\pm$ 0.4	2.8 $\pm$ 0.6*	0.2 $\pm$ 0.3**	62.0	97.6

n = 6 in each group, Each values is the mean $\pm$ S.E.M.

*P < 0.05 compared to control

**P < 0.001 compared to control

In the analgesic studies, the ethanolic extract of *Caesalpinia bonduc* (100 – 500 mg/kg b.w., s.c.) suppressed the acetic acid –

induced writhing significantly in a dose – dependent manner. The standard drug (Aspirin) in increasing doses produced

increased inhibition of writhing movements. The results were found to be highly significant ($P < 0.001$) in comparison to the control (Table-2).

Table 2. Effect of the ethanolic extract of *Caesalpinia bonduc* on acetic acid induced writhing response in mice.

S.No.	Group	Dose (mg/ kg, s.c.)	No. of writhing movements	% of protection
1	Control	-	83.30 ± 0.95	-
2	Caesalpinia	100	41.57 ± 4.22	45.5
3	Caesalpinia	200	$39.33 \pm 6.47^{**}$	51.2
4	Caesalpinia	500	$27.66 \pm 3.46^{*}$	65.5
5	Aspirin	50	$30.33 \pm 2.47^{**}$	62.3
6.	Aspirin	100	$20.33 \pm 6.71^{**}$	69.7
7.	Aspirin	150	$16.00 \pm 5.24^{**}$	80.2

n= 6 in each group, Each value is the mean $\pm$ S.E.M.

* $P < 0.1$ compared to control

** $P < 0.001$ compared to control

The results from hot plate test show that at 30 min the oral doses of ethanolic extract of *Caesalpinia bonduc* and indomethacin

increased the reaction time were significantly increased in comparison to the control (Table- 3).

Table 3. Effect of the ethanolic extract of *Caesalpinia bonduc* on hot plate test method in rats.

S.No.	Groups	Dose (mg/kg, p.o.)	Reaction time ^a (s)		
			30	60	90
1	Control	-	5.8 ± 0.8	3.8 ± 0.6	2.8 ± 0.3
2	Caesalpinia	100	6.8 ± 0.5	$9.1 \pm 0.2^{**}$	$10.2 \pm 0.3^{**}$
3	Caesalpinia	200	7.4 ± 0.8	$9.2 \pm 0.9^{**}$	$10.4 \pm 1.2^{**}$
4	Caesalpinia	500	7.8 ± 0.5	$9.4 \pm 0.8^{**}$	$10.2 \pm 0.4^{**}$
5	Indomethacin	5	$8.4 \pm 0.7^{*}$	$9.0 \pm 0.8^{**}$	$9.4 \pm 0.8^{**}$

n= 6 in each group, Each value is the mean $\pm$ S.E.M.

* $P < 0.05$ compared to control

** $P < 0.001$ compared to control

4. Discussion: -

Two different analgesic laboratory models were employed in the current investigation with the objective of identifying possible peripheral and central effect of the test substances, using both acetic acid – induced writhes and hot plate thermal stimulation. It

was observed that the ethanolic extract of *Caesalpinia bonduc* possessed analgesic effects against both models. This observation indicates that *Caesalpinia bonduc* have both peripheral (writhe reduction) and central (thermal reaction time prolongation) effects. The abdominal constriction response induced by acetic acid

is a sensitive procedure to establish peripherally acting analgesics. The number of writhing movements during 30 min. observation in the control group was 83.30 ± 0.95 which corresponds with the findings of other workers (Hajare SW. *et. al.*, 2000, Effraim KD, *et. al.*, 1998). In hot plate method, the latency of the animals were highly increased compared to the control, likewise the licking time was significantly reduced by administration of the extract showing analgesic activities. This observation can provide useful information if a choice is desired to be made regarding the species of *Caesalpinia* as a source of analgesic drugs.

The anti-inflammatory effects of the extract on acute inflammatory process such as carrageenin – induced edema in rats paw was dose dependent (Di Rosa, 1972). At 200 mg/kg, the extract showed at least 50% inhibitory activity throughout the measurement intervals and the efficacy of indomethacin (5 mg/kg) was comparable to 500 mg/kg of the extract.

Preliminary phytochemical screening of the ethanolic extract shows the presence of flavonoids and saponins. Flavonoids act as an anti-inflammatory response in the same way as the non – steroidal anti-inflammatory drugs, i.e. by inhibiting the enzymes that cause the synthesis of prostaglandins (Berknow, 1992). Further studies may reveal the extract mechanisms of action responsible for the analgesic and anti-inflammatory activities of *Caesalpinia bonduc*.

5. Acknowledgements: -

I am very much grateful thanks to the Management and Director of Pharmacy, GRD(PG)IMT, Rajpur Road, Dehradun, Uttarakhand, India for their financial

assistance by providing the reagents and animals without any interruption.

References

1. Kirtikar KR, Basu BD: *Indian Medicinal Plants*. Vol 2, 2nd ed. Dehradun, India: Bishen Singh Mahendra Pal Singh.1975; p. 842–844.
2. Negi NC, Nayak KP: Biological investigation of *Caesalpinia bonduc* F. *Indian J Pharmacol*. 1975; 20: 95–100.
3. Adesina SK: Studies on some plants used as anticonvulsant in Amerindian and African traditional medicine. *Fitoterapia*. 1982 53: 147–162.
4. Dhar ML, Dhar MM, Dhawan BN, Mehrotra BN, Roy C: Screening of Indian plants for biological activity. *Indian J Exp Biol*. 1968; 6: 232–247.
5. Gayaraja S, Shinde S, Agarwal SL: Antiasthmatic properties of *Caesalpinia bonduc* leaves. *Indian J Pharmacol*. 1978; 10: 86–89.
6. Raghunathan K, Mitra R, Karanja: In: Raghunathan K, Mitra R, editors. *Pharmacognosy of indigenous drugs*. Part-1. New Delhi, India: Central Council for Research in Ayurveda and Siddha; 1982; p. 484–510.
7. Trease, G.E., Evans, W.C.: *Pharmacognosy*. Ballière Tindall Press, London; 1983; p. 309, 706.
8. Ghosh MN: Evaluation of analgesic agents, *Fundamentals of experimental pharmacology*. 2nd ed. Calcutta: Scientific Book Agency; 1984.
9. Eddy, N.B., Touchberry, C.F., Lieberman, I.E.: *Synthetic analgesics, a methadone isomers and derivatives*.

- Journal of Pharmacology and Experimental Therapeutics*. 1950; 98, 121–137.
10. Winter, C.A., Risley, E.A., Nuss, G.W.: Carrageenan-induced edema in hind paw of the rat as an assay for anti-inflammatory drugs. *Proceedings for the Society of Experimental Biology and Medicine*. 1962; 111, 544–547.
11. Hajare SW, Chandra S, Tandon SK, Sarma J, Lal J, Telang AG: Analgesic and antipyretic activities of Dalbergia Sissoo leaves. *Indian J Pharmacol*. 2000; 32:357-60.
12. Effraim KD, Osunkwo UA, Onyeyilli P, Ngulde A: Preliminary investigation of possible antinociceptive activity of aqueous leaf extract Ziziphus spina Christi (Linn) desf. *Indian J Pharmacol*. 1998; 30:271-2.
13. DiRosa, M.: Biological properties of carrageenan. *Journal of Pharmacy and Pharmacology*. 1972; 24, 89–102.

Source of support: Nil, Conflict of interest: None Declared