

***In vivo* anti-inflammatory, analgesic and antipyretic activities of a medicinal plant, *Caesalpinia bonducella* F.**

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Abstract: This research examined antipyretic, anti-inflammatory, and analgesic activities of ethanolic extract of *C. bonducella* whole seeds in experimental albino rats. Three doses, 100, 200 and 400 mg/kg of the whole seed ethanolic extract prepared as a suspension in 2 ml of 2% gum acacia were used. Acute inflammatory and antipyretic activities were evaluated in experimental animals by carrageenan induced paw edema and brewer's yeast-induced pyrexia models, respectively. A significant ($p < 0.05$) reduction in paw volumes, and pyrexia was noted in experimental animals when compared with control animals. The ethanol seed extract (400 mg/kg) displayed *in vivo* anti-inflammatory, antipyretic and analgesic in terms of reduction in paw edema, % writhes inhibition and rectal temperature by (0.24±0.03), (31.38%) and (36.2±0.1), respectively. Overall the whole ethanolic seed extract at all tested concentrations produced significant ($p < 0.05$) anti-inflammatory, antipyretic and analgesic activities. The results obtained in this study clearly indicated the ethno-medicinal potential of *C. bonducella* in curing pain and inflammation related disorders, supporting its efficacy as a natural analgesic, antipyretic and anti-inflammatory agent.

Keywords: *Caesalpinia bonducella*, whole seed extract, anti-inflammatory effect, anti-pyretic effect, analgesic potential.

INTRODUCTION

As a prime defense mechanism, inflammation has been known to work in body system as a protective strategy against noxious stimuli, toxic chemicals, allergens, burns and infections. Moreover, un-controlled and persistent inflammation also works as an etiologic factor against number of chronic diseases (Kumar *et al.*, 2004). Severe consequences of inflammation may also result in the development of various chronological diseases symptoms (Sosa *et al.*, 2002).

Fever or pyrexia is a consequence of various secondary factors such as damage of tissues, impact of infections, graft rejection, and malignancy of other chronic disease symptoms. Pyrexia is considered as a natural defense mechanism against the causative agents of infection that may result in the non-survival of infected tissues (Hajare *et al.*, 2000). In general, increased production of Il-1 β , Il- α , Il- β , and TNF- α as proinflammatory cytokines is occurred specifically in inflamed or infected tissues. Increased production of proinflammatory cytokines results in the synthesis of prostaglandin (PgE2) eventually triggering hypothalamus leading to increased body temperature (Space and Breder, 1933).

Commercial and synthetic drugs used for the treatment of inflammation have shown their ability to exert severe adverse effects. Hence, plant-based natural anti-inflammatory and antipyretic agents are gaining increasing popularity due to their being less or non-toxic in nature. The development of pharmacological agents has

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been a major subject of pain and inflammatory-related research in view of their multitude of biomedical potential. Hence, the search for alternative sources on potent pharmacological agents including plants has become intensively important to develop novel types of natural ethnomedicinal drugs. In this regard, we attempted to claim ethnomedicinal potential of whole seeds of *C. bonducella* and their role in the prevention of inflammation and pain-related disorders in experimental animals.

Caesalpinia bonducella F. (Leguminosae) has been used in Asian traditional medicinal system historically as a folk medicine (Kirtikar and Basu, 1975). The seeds of *C. bonducella* are reported to have multiple therapeutic properties including adaptogenic, anti-filarial, anti-oxidative, anti-diabetic, anti-inflammatory, immunomodulatory, antimicrobial, and anti-estrogenic effect (Gaur *et al.*, 2008; Shukla *et al.*, 2009; Shukla *et al.*, 2010).

Although fewer reports proved anti-inflammatory potential of *C. bonducella* seed kernel, current research was focused to determine antipyretic, analgesic and anti-inflammatory activities of whole seed ethanolic extract of *C. bonducella* (WSECB) in experimental Swiss albino rats.

MATERIALS AND METHODS

Plant material

Caesalpinia bonducella seeds were collected from Jeevan Herbs Agro Farms, Sagar, MP, India, and were identified

by herbarium in-charge and the specimen (Bot/H/2692) was preserved in the Laboratory of Microbiology and Botany, Dr. H.S. Gour University, Sagar, MP, India. The seeds were kept in airtight bottles for further studies.

Extraction

The air-dried whole seeds of *C. bonducella* (50 g) were crushed and subjected to extraction procedure using ethanol (500 ml) assisted with a Soxhlet assembly. The obtained material was subjected to extraction, filtration, and evaporation steps using a rotary evaporator system. Extract was suspended in dissolved in 2 m of 2% gum acacia in order to maintain various dosages such as 100, 200 and 400 mg/kg body weight.

Animals and acute toxicity assay

Rats (Swiss albino) of either sex which weighed in the range of 100 to 125 g were used for this research. Rats were fed with diet of standard pellet along with tap water and observed under optimal growth condition such as temperature of 25°C, and repetitive light and dark cycle of 12 h.

The protocol followed for animal experiments was approved by the university committee (Animal Ethical Committee IE/98/Reg No 379/01/ab/CPCSEA) and care of the animals was done using international standard parameters (CCAC, 1993). Assay for acute toxicity was done in experimental animals by dividing these animals into different groups, and each group consisted 6 animals. Following overnight fasting, animals were tested by orally administering different graded doses (100, 500 and 1,000 mg/kg) of ethanolic extract, whereas animals belonged to control groups received normal saline (10 ml/kg). Animals belonging to each group were observed continuously for toxic symptoms at least for the first 2 h, and mortality was observed up to 24 h (Litchfield and Wilcoxon, 1949).

Anti-inflammatory effect in carrageenan-induced paw edema

A 10 ml of carrageenan (1%) was used to produce the acute inflammation into sole of the bottom of rat hind paw (Winter *et al.*, 1962). For the induction of acute inflammation, 0.1 ml of 1% carrageenan was injected into sole of the bottom hind paw of the rats (Winter *et al.*, 1962). Test samples of WSECB (100, 200 and 400 mg/kg), and reference drug phenylbutazone (100 mg/kg) were administered orally prior 60 min of carrageenan injection. A thread was used to measure the paw volume at 0, 1, 2, 3 and 4 h in order to calculate diameter size for edema formation. Diameter differences noted between left-hind paw and right-hind paw were used as edema measures.

Analgesic activity by acetic acid-induced writhing test

In this assay, solution of acetic acid (15 mg/ml) at the dose of 300 mg/kg body weight was given

intraperitoneally (i.p.) to measure the analgesic activity of test samples. The writhing numbers during 30 min time were measured (Turner, 1965)]. A significant loss in writhing numbers of test sample of WSECB at 100, 200 and 400 mg/kg was considered as a positive analgesic response in comparison with vehicle treated animals when administered orally. Finally, the % inhibitory effect of WSECB on writhing numbers was calculated. Standard compound aspirin (100 mg/kg) administered i.p. was considered as a reference drug.

Antipyretic activity by induction of brewer's yeast-induced pyrexia test

In this test, in order to measure the antipyretic activity of WSECB, the animals were administered with 20% suspension (20 ml/kg) of brewer's yeast subcutaneously followed by measurement of initial rectal temperature (Smith Hambourger, 1935). The selected animals which showed an increase of rectal temperature from 0.3 to 0.5°C after 18 h of treatment were assessed for antipyretic activity of WSECB. In brief, oral administration of WSECB (100, 200 and 400 mg/kg) was given to consecutive groups whereas control group was administered with normal saline (0.3m) only. Paracetamol at the dose of 100 mg/kg given orally was referred as a reference drug. A thermal probe Ellab themistor thermometer was used to determine the rectal temperature at 1, 2, 3 and 4 h, following the administration of test sample or reference drug.

STATISTICAL ANALYSIS

The data obtained in this study were evaluated using the one-way analysis of variance (ANOVA) test between two mean groups; control and test groups, followed by Student's t-test. Significant levels were considered at $p < 0.05$.

RESULTS

Acute toxicity

Oral administration of the extract of *C. bonducella* whole seeds did not cause any acute toxicity in experimental rats at all the tested dosages, confirming that it has potential safety for consumption. As shown in table 1, gross behavior changes were not observed even at all the doses (100, 500 and 1,000 mg/kg). All rats survived at 100, 500 and 1,000 mg/kg.

Anti-inflammatory effect

In the present study, the results of carrageenan-induced paw edema method given in Table 2. A slow increased in the volume of edema paw was observed in carrageenan-treated control group. In contrary, WSECB exerted remarkable decrease in edema paw volume experimental animals of treated group. As illustrated in Table 2, WSECB displayed considerable amount of inhibitory

effect on hind paws edema between 2 and 4 h and was found in dose-dependent manner. In addition, the reference standard drug phenylbutazone administered orally at 100 mg/kg displayed significant inhibitory effect on hind paws edema when compared with WSECB.

Table 1: Effect of ethanolic whole seed extract of *C. bonducella* in acute toxicity on rats

Treatment	Dose (mg/kg)	Gross behavior effect	No. of animal died (n=6)
WSECB ^a	100	No change	0
	500	No change	0
	1000	No change	0
Control (Normal saline)	10 ml/kg	No change	0

^a Ethanolic whole seed extract of *C. bonducella*.

Antipyretic activity

The effect of WSECB on normal body temperature of albino rats is given in Table 3. The results confirmed that administration of WSECB (100 mg/kg) resulted in the lowering of body temperature starting at 4 h and simultaneously increased at the increased doses (200 and 400 mg/kg) of test sample, eventually causing a drastic decrease in body temperature up to 4 h. Further administration of the suspension of yeast significantly

decreased the temperature of rectum following 18 h of its administration. Moreover, WSECB (100 mg/kg) had significant effect on reducing pyrexia level at 3 and 4 h (table 3). In case of standard antipyretic drug, paracetamol (100mg/kg) exhibited its effect on lowering the body temperature level started at 2 h (table 3). However increased dose of WSECB (200 and 400 mg/kg) had markedly a significant effect on decreasing the pyrexia level started at 2 h (table 3). Treatment with WSECB (100, 200 and 400 mg/kg) significantly lowered the temperature of rectum in experimental animals which was found in a dose-dependent pattern (table 3). Interestingly, upon administration, WSECB exerted its antipyretic effect at 1 h of administration and maintained for next 4 h. Both WSECB and paracetamol were able to reduce yeast-elevated rectal temperature in animals significantly when compared with control group.

Analgesic activity

The WSECB exerted significant inhibitor effect on acetic acid-induced writhing responses when compared with aspirin. The results presented in table 4 showed that WSECB at the higher dose (400 mg/kg) displayed significantly ($p < 0.05$) higher inhibitory effects (31.38%) on control writhes as compared to aspirin (59.52%) and control groups (table 4). The sensitivity of nociceptive receptors to prostaglandin is associated with abdominal constriction, hence, it might be speculated that WSECB

Table 2: Anti-inflammatory activity of ethanolic whole seed extract of *C. bonducella* on carrageenan induced rat paw edema in the right hind-limb paw of rats.

Treatment	Dose (mg/kg)	Time (h)					Average edema formation
		0	1	2	3	4	
WSECB ^a	100	-	0.40±0.01	0.45±0.02	0.38±0.02	0.30±0.01	0.38±0.11
	200	-	0.38±0.02	0.36±0.04*	0.22±0.00*	0.19±0.02*	0.28±0.01*
	400	-	0.40±0.01	0.24±0.00*	0.19±0.02*	0.16±0.12*	0.24±0.03*
Phenylbutazone	100	-	0.21±0.01	0.22±0.05*	0.22±0.03*	0.17±0.09*	0.21±0.06*
Control (Carrageenan treated)	-	-	0.44 ± 0.11	0.75±0.11	0.88±0.11	0.93±0.10	0.74±0.1

Values are mean ± SEM (n=6), * $p < 0.05$ as compared to control values. ^a Ethanolic whole seed extract of *C. bonducella*.

Table 3: Effect of ethanolic whole seed extract of *C. bonducella* on body temperature in experimental rats

Treatment	Dose (mg/kg)	Rectal temperature (°C) before and after treatment			
		1 h	2 h	3 h	4 h
WSECB ^a	100	38.1±0.3	38.0±0.4	37.5±0.2	37.0±0.1
	200	38.2±0.1	37.5±0.1*	37.0±0.02*	36.6±0.1*
	400	38.0±0.2	37.1±0.1*	36.4±0.4*	36.2±0.1*
Control (Saline treated)	5 ml/kg	38.1±0.3	38.3±0.4	38.6±0.1	39.0±0.1
Paracetamol	100	38.3±0.2	37.0±0.1*	36.2±0.2*	36.0±0.4*

Values are mean ± SEM (n=6), * $p < 0.05$ of the difference between the left and right hind-limb paws. ^a Ethanolic whole seed extract of *C. bonducella*.

exerted its analgesic effect through its inhibitory effect on prostaglandin action or during its synthesis.

Table 4: Effect of ethanolic whole seed extract of *C. bonducella* on writhing induced by acetic acid in experimental rats

Treatment	Dose (mg/kg)	Number of writhes (per 30 min)	Inhibition (%)
Control (Saline treated)	5 ml/kg	38.42±3.98	-
WSECB ^a	100	35.31±2.34	8.09
	200	32.46±3.35	15.51
	400	26.36±2.12	31.38*
Aspirin	100	15.55±1.18	59.52*

Values are mean ± SEM (n=6), **p*<0.05 as compared to control values. ^a Ethanolic whole seed extract of *C. bonducella*.

DISCUSSION

An inflammatory model of rat paw edema induced by carrageenan was used to determine anti-inflammatory potential of various herbal and medicinal drugs (Shenawy *et al.*, 2002). Previous reports have confirmed that various natural compounds have been assessed for their anti-inflammatory potential where use of carrageenan-induced inflammation has served an ideal model for their anti-inflammatory screening (Winter *et al.*, 1962). Sub-plantar administration of carrageenan in rats has led to the development of edema in experimental animals which has been mainly attributed to the production of serotonin, histamine, prostaglandins and kinins (Winter *et al.*, 1962). In our earlier studies, we also reported that oil of *C. bonducella* seeds exhibited anti-inflammatory activity (Shukla *et al.*, 2010). Hereby, it can be hypothesized that antioedematogenic effect of WSECB on carrageenan-induced edema might be correlated due to inhibitory effect on inflammation mediators (Shukla *et al.*, 2010). Confirmatory results on potent anti-inflammatory effects of quercetin-like flavonoid have also been reported (Rajnarayana *et al.*, 2001).

Maintenance of human body temperature needs a proper balancing between loss and production of heat, which can be done by hypothalamus. Suspension of yeast remarkably increased rectal temperature following its subcutaneous injection; the effect of ethanol extract of *C. bonducella* can be measured in terms of reductions in rectal temperatures of body (Shukla *et al.*, 2010). In addition, antipyretic effects of plant-based components

such as flavonoid and tannin have been reported previously (Ahmadiani *et al.*, 2000).

Moreover, analgesic effect of WSECB in experimental animals was evaluated by acetic acid-induced writhing responses which is considered an ideal method for rapid evaluation of analgesic action in peripheral region. This method employs reaction of experimental animals with characteristic stretching behavior known as writhing. It has been known that release of prostaglandins from hypothalamus begins with the process of fever mediation resulting in increased amount of heat whereas decrease in heat eventually led to pyrexia. Results of this study clearly indicated that WSECB exhibited remarkable antipyretic effect on animal body temperature induced by yeast suspension when compared with standard drug paracetamol. Generally, antipyretic action of non-steroid anti-inflammatory drugs (NSAIDs) is correlated to their inhibitory effect on cyclooxygenase enzyme within hypothalamus (Clark and Cumby, 1975). Induction of Cox-2 in non-neuronal cells has been associated with febrile response which is modulated by the inhibitors of Cox-2 (Schwartz *et al.*, 1999). Hence, based on these findings it can be hypothesized that WSECB might exert its antipyretic effect through its inhibitory effect on the synthesis of Cox-2 in hypothalamus (Borikar *et al.*, 2009).

CONCLUSION

This study confirmed the biological significance of the ethanol seed extract of *C. bonducella* in terms of its potent *in vivo* anti-inflammatory, analgesic, and antipyretic activities. These findings conclude that the WSECB may contain bioactive principles with pharmacological potential, and further support the ethnomedicinal value of *C. bonducella* in the treatment of pain, pyrexia and/or inflammatory related disorders. Extension on research are being made on isolation and purification of biologically active molecules from WSECB which were thought to be the mediators of observed effects, in order to confirm their precise mode of action *in vivo*.

REFERENCES

- Ahmadiani A, Hosseiny J, Semnianian S, Javan M, Saeedi F, Kamalinejad M and Saremi S (2000). Antinociceptive and anti-inflammatory effects of *Elaeagnus angustifolia* fruit extract. *J. Ethnopharmacol.*, **72**: 287-292.
- Borikar VI, Jangde CR, Philip P, Rekhe DS and Atole SK (2009). Study of antiulcer activity of *Bauhinia racemosa* lam in rats. *Veter. World*, **2**: 217-218.
- CCAC (1993). Guide to the care and used of experimental animals, vol. 1. The Canadian Council on Animal Care, <http://www.ccac.ca/>.

- Clark WG and Cumby HR (1975). The antipyretic effect of indomethacin. *J. Physiol.*, **248**: 625-638.
- Gaur RL, Sahoo MK, Dixit S, Fatma N, Rastogi S and Kulshreshtha DK (2008). Antifilarial activity of *Caesalpinia bonducella* against experimental filarial infections. *Indian J. Med. Res.*, **128**: 65-70.
- Hajare SW, Tandan SK, Sharma J, Lal J and Telang AG (2000). Analgesic and antipyretic activities of *dalbergia sissoo* leaves. *Indian J. Pharmacol.*, **32**: 357-360.
- Kumar VA, Abbas K and Fausto N (2004). 7th edition, Elsevier Saunders, Philadelphia, Pennsylvania, USA, pp.47-86.
- Kirtikar KR and Basu BD (1975). *Caesalpinia crista* Linn. In: Indian Medicinal Plants 2, CSIR: New Delhi, India. pp.842-845.
- Litchfield JT and Wilcoxon F (1949). A simplified method of evaluating dose-effect experiments. *J. Pharm. Exp. Ther.*, **96**: 99-135.
- Rajnarayana K, Reddy SM and Chaluvadi MR (2001). Bioflavonoids classification, pharmacological, biochemical effects and therapeutic potential. *Indian J. Pharmacol.*, **33**: 2-16.
- Schwartz JJ, Chan CC and Mukhopadhyay S (1999). Cyclooxygenase-2 inhibition by rofecoxib reverse naturally occurring fever in humans. *Clin. Pharmacol. Ther.*, **65**: 653-660.
- Shenawy SM, Abdel-Salam OM, Baiuomy AR, Batran S and Arbid MS (2002). Studies on the anti-inflammatory and antinociceptive effects of melatonin in the rat. *Pharmacol. Res.*, **46**: 235-243.
- Shukla S, Mehta A, Jinu J, Mehta P, Vyas SP and Shukla S (2009). Immunomodulatory activities of the ethanolic extract of *Caesalpinia bonducella* seeds. *J. Ethnopharmacol.*, **125**: 252-256.
- Shukla S, Mehta A, Mehta P, Vyas SP, Shukla S and Bajpai VK (2010). Studies on anti-inflammatory, antipyretic and analgesic properties of *Caesalpinia bonducella* F. seed oil in experimental animal models. *Food Chem. Toxicol.*, **48**: 61-64.
- Smith PK and Hamburger WE (1935). The ratio of the toxicity of acetanilide to its antipyretic activity in rats. *J. Pharm. Exp. Ther.*, **54**: 346-349.
- Sosa S, Balicet MJ, Arvigo R, Esposito RG, Pizza C and Altinier GA (2002). Screening of the topical anti-inflammatory activity of some Central American plants. *J. Ethnopharmacol.*, **81**: 211-215.
- Spacer CB and Breder CD (1933). The neurologic basis of fever. *New Eng. J. Med.*, **330**: 1880-1886.
- Turner RA (1965). Analgesics: Screening Methods in Pharmacology. Academic Press, New York, USA. p. 100.
- Winter CA, Risley EA and Nuss GW (1962). Carrageenan-induced edema in hind paws of the rat as an assay for anti-inflammatory drugs. *Proc. Soc. Exp. Biol. Med.*, **111**: 544-552.