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ANALGESIC AND ANTI-INFLAMMATORY ACTIVITIES OF AQUA FORM OF *CAESALPINIA BONDUCELLA* SEED IN RATS

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ABSTRACT

The objective of this study is to evaluate the analgesic and anti-inflammatory activities of the *Caesalpinia bonducella* in animals. The analgesic action was evaluated in rats using the acetic acid-induced writhing test and the hot-plate test. The anti-inflammatory activity was estimated using carrageenan-induced paw edema in rats. *Caesalpinia bonducella* strikingly and dose-dependently suppressed carrageenan-elicited paw edema in rats, inhibited the writhing response in rats, increased reaction time of in the hot-plate test. These findings propose that aqua of *Caesalpinia bonducella* seed has evident non-central analgesic and anti-inflammatory activities.

INTRODUCTION: Non-steroidal anti-inflammatory drugs (NSAIDs), such as diclofenac, are prescribed globally for the management of pain, inflammation and fever, as well as cardiovascular protection. But their uses were limited because of the key fact with gastrointestinal damage^{1, 2}, regardless of NSAIDs have provided effective management of pain and inflammation highly. In addition kidney damage, increase in blood pressure and some other cardiovascular problems have been found with NSAIDs³. Consequently, during the past decades many researchers have focused on medicinal plants with fewer side-effects for patients to develop anti-inflammatory and analgesic drugs.

The usage of plants, plant extracts or plant-derived pure chemicals to manage disease become a therapeutic modality, which has stood the test of time. As said by the World Health Organization (WHO), about three-quarters of the world population depends upon traditional remedies (mainly herbs) for the health care of its people. The traditional medicines also for a time called as, herbal or natural medicine existed in one way or another in different cultures/civilizations,

such as Egyptians, Western, Chinese, Kampo (Japan) and Greco-Arab or Unani/Tibb (South Asia)^{4, 5}.

Plant is claimed to have multiple therapeutic properties like, antidiuretic, anthelmintic and antibacterial⁶, anti-anaphylactic and antiviral⁷, antiasthmatic⁸, antiamebic and anti-estrogenic⁹. Blood sugar lowering activity of *C. bonducella* has been primarily evaluated with significant result in rabbit¹⁰ and rat models¹¹. In the present study, we examined the analgesic and anti-inflammatory activities of aqua form of *C. bonducella* seed.

Caesalpinia bonducella (*C. bonducella*; Karanjwa) is an important medicinal plant extensively distributed throughout the coastal region of India, Burma, Sri Lanka, and in other tropical and subtropical regions of the world¹²⁻¹⁴.



It is an irregular thorny shrub with large bipinnate leaves. Its flowers are yellow and fruits are inflated pods having 1–2 seeds¹⁵.

Inflammation is a pathophysiological response of living tissue to injuries that leads to the local accumulation of plasma fluid and blood cells and typically characterized by redness, swelling, pain, and heat. The complex events and mediators involved in the inflammatory reaction may induce, maintain or aggravate many diseases. Though, studies have been continuing on inflammatory diseases and the side effects of the currently available anti-inflammatory drugs pose a major problem during their clinical uses. Thus, development of newer and more substantial anti-inflammatory drugs with lesser side effects is necessary¹¹.

The leaves of this plant are traditionally used for the treatment of tumor, inflammation and liver disorders. It has also been recognized for such multiple therapeutic properties that include antipyretic, antidiuretic, anthelmintic, antibacterial, anti-convulsant, antianaphylactic, antidiarrheal, antiviral, antiasthmatic, anti-inflammatory, antiamebic, antiestrogenic, nematocidal, antihyperglycemic and abortifacient activities⁶⁻⁹.

In the present study, we examined the analgesic and anti-inflammatory activities of aqua form of *C. bonducella* seed.

MATERIALS AND METHODS:

Collection and authentication of plant: *C. bonducella* leaves were collected from Jamia Hamdard Botanical Garden, New Delhi- 110062, India. The authenticity and identity of the drugs was confirmed on the basis of classical description in Unani literature at Department of Ilmul Advia, Faculty of Medicine (Unani), Jamia Hamdard, New Delhi and modern botanical information was established by Department of botany, Faculty of science Jamia Hamdard. Voucher specimen of the test drugs have been retained and deposited in the Department of Ilmul Advia, Faculty of Medicine (Unani), Jamia Hamdard, New Delhi.

Drugs and chemicals:

Preparation of extract:

Aqua of *C. bonducella* seeds: The dried *Caesalpinia bonducella* seeds were distilled three times by distillation process with distilled water (8:2) for 8 hrs and the evaporation is collected in the form of aqua (arq).

Experimental animals: The present study was approved by the Institutional Animal Ethics Committee (IAEC) of Jamia Hamdard, New Delhi (Number; JH/173/CPCSEA). Both the international guidelines for the welfare of the animals and the compatible local regulations for experimenting with laboratory animals were fully considered during the study. Wistar rats (8–15 weeks old, 150–250 g body weight) were issued from Central Animal House Facility, Jamia Hamdard, New Delhi and housed in standard polypropylene cages (6 in each cage) and maintained under controlled room temperature (20–25±2°C) and relative humidity (50 ± 15%) with 12 h light/12 h darkness (day/night) cycle. All the rats were fed with commercially available normal pellet diet (NPD; Amrut Rat Feed, manufactured by Nav Maharashtra Chakan Oil Mills Ltd., Delhi, India) and water *ad libitum*.

Analgesic activity:

Acetic acid-induced Writhing Test: Analgesic activity was assessed by abdominal writhing test using acetic acid¹⁶. Albino rats of either sex weighing 150-250 g were fasted overnight with *ad libitum* access to water. The animals were divided into six groups (n=6 each) viz.: group I- acetic acid control (normal saline, 10 ml/kg, p.o.); group II- indomethacin solution (20 mg/kg, p.o.); group III- *C. bonducella* -I (7 mg/kg, p.o.); group IV- *C. bonducella* -II (10.5 mg/kg, p.o.) and group V- *C. bonducella* -III (14 mg/kg, p.o.).

In the writhing test, 0.2 ml of 0.6 % acetic acid solution was injected intraperitoneally and the number of writhes were counted starting 5 min after injection for a period of 20 minutes. Indomethacin (20 mg/kg, p.o.), was used as standard drug, and the test drug *C. bonducella* in seed aqua form were administered by the intragastric route 1 hour before acetic acid injection.

Hot Plate Test: Analgesic activity was further assessed by hot plate latency assay¹⁶. Albino rats of either sex weighing 150-250 g were fasted overnight with *ad libitum* access to water. The animals were divided into

six groups (n=6 each). The animals were divided into six groups (n=6 each) viz.;

1. Group I: control (normal saline 10 ml/kg, p.o.)
2. Group II: indomethacin (20 mg/kg, p.o.)
3. Group III: *C. bonducella*-I (7 mg/kg, p.o.)
4. Group IV: *C. bonducella*-II (10.5 mg/kg, p.o.)
5. Group V: *C. bonducella*-III (14 mg/kg, p.o.)

In this method, rats in groups I and II were given doses of normal saline (10 ml/kg, p.o.) and indomethacin (20 mg/kg, p.o.) respectively. The rats in Group III-V were given the aqua form of seed of *C. bonducella*. Rats from each group were placed on the hot plate after the administration of treatment drug and the reaction time for the animal to lick the paw or jump from the hot plate was taken as the latency (s). This was also repeated at 60 and 90 minutes from the exact time given. The average of the latency was determined from the six rats in each group. The temperature of the hot plate was maintained at $55 \pm 1^\circ\text{C}$. The cut off time was kept at 20 seconds.

Anti-inflammatory activity:

Carrageenan-induced paw oedema test: Inflammation was produced by administering 0.1 ml of (1%) carrageenan into sub-plantar surface of rat hind paw¹⁷. Albino rats of either sex weighing 150-250 g were fasted overnight with *ad libitum* access to water. The animals were divided in to six groups (n=6 each) viz.;

1. Group I: carrageenan control (normal saline 10 ml/kg, p.o.)
2. Group II: indomethacin (20 mg/kg, p.o.)
3. Group III: *C. bonducella*-I (7 mg/kg, p.o.)
4. Group IV: *C. bonducella*-II (10.5 mg/kg, p.o.)
5. Group V: *C. bonducella*-III (14 mg/kg, p.o.)

In this experiment, all drugs were given orally. One hour later all animals were injected with 0.1ml of 1% Carrageenan solution in the sub-plantar aponeurosis of left hind paw and the paw volume was measured plethysmometrically at 1 hr, 3 hr and 5 hr. Indomethacin (20 mg/kg, p.o.) was used as standard drug and the test drug *C. bonducella* in seed aqua form administered by the intragastric route 1 hr before carrageenan.

Safety of drugs on Gastric Mucosa:

Assessment of the safety of drugs on Gastric Mucosa of rats: This method was performed to assess the safety of the test drugs on the gastric mucosa of rats. In this method albino rats of either sex weighing 150-250 g were fasted overnight with *ad libitum* access to water. The animals were divided in to three groups (n=6 each) viz.:

1. Group I: indomethacin (20 mg/kg p.o.)
2. Group II: *C. bonducella* (14 mg/kg, p.o.)

In this experiment, higher doses of drugs were given orally. After 5 hours animals were sacrificed by an overdose of ether vapors and the stomachs were removed, opened and sum of length of lesions was evaluated for ulcer index given below:

Erosions	Score
1 mm or less	1
1 mm to 2 mm	2
More than 2 mm	3

The overall score was divided by a factor of 10, which was designated as ulcer index¹⁸.

Aqua seed: The drug was administered orally, the very common route for administration of drug in Tibb-e-Unani. The test drug extract was dissolved in distilled water immediately before the administration. As the dose of *C. bonducella* seed are recommended as 5-7 g each in classical Unani literature for human beings, so both the higher and lower doses were taken for extrapolating the dose for animals in the present study.

The animal dose was calculated by multiplying these doses by appropriate conversion factors. The aqua form of seed of *C. bonducella* were given in three different doses i.e. 7 mg/kg, p.o.; 10.5 mg/kg, p.o. and 14 mg/kg, p.o.

Statistical analysis: All the values are expressed as mean \pm S.E.M. The statistical significance was determined by ANOVA followed by Dunnett's test. Values $p < 0.05$ was considered as significant.

RESULTS:

Analgesic activity:

Effect of aqua of *C. bonducella* seed on acetic acid-induced writhing in rats: There was a significant decrease in the number of writhing in 20 minutes

observed. The mean score for writhing decreased by 20.73%, 43.71% and 62.47% respectively over the score of control group. The decreased score for

writhing is statistically significant at both the doses as shown in **Table 1**.

TABLE 1: EFFECT OF AQUA OF *C. BONDUCELLA* SEED ON ACETIC ACID-INDUCED WRITHING IN RATS

Group	Treatment	Dose	No. of writhes in 20 min	% variation
I	Acetic acid control	10 ml/kg	10.66 ± 0.33	--
II	Indomethacin	20 mg/kg	3.80 ± 0.16**	64.35%
III	<i>C. bonducella</i> -I	7 mg/kg	8.45 ± 0.34*	20.73%
IV	<i>C. bonducella</i> -II	10.5 mg/kg	6.00 ± 0.42*	43.71%
V	<i>C. bonducella</i> -III	14 mg/kg	4.00 ± 0.21**	62.47%

Values are expressed as mean ± S.E.M. (n= 6), **p < 0.01, compared with acetic acid control, ANOVA followed by Dunnett's test.

Effect of aqua of *C. bonducella* seed on hot plate reaction time in rats: A significant increase in the reaction time was observed for 30 min, 60 min, and 90 min. In comparison to control group, aqua of the test drug *C. bonducella* in the doses of 7 mg/kg and 10.5 mg/kg and 14 mg/kg showed a significant increase in

the reaction time at 30 min, 60 min and 90 min. The standard drug, indomethacin 20 mg/kg showed a significant increase in the reaction time at 30 min, 60 min, and 90 min (p < 0.01). The increased reaction time for hot plate is statistically significant at both the doses as shown in **Table 2**.

TABLE 2: EFFECT OF AQUA *C. BONDUCELLA* SEED ON HOT PLATE TEST IN RATS

Group	Treatment	Dose	Reaction time (s)		
			30 min	60 min	90 min
I	Control	10 ml/kg	2.38 ± 0.47	4.16 ± 0.47	3.83 ± 0.30
II	Indomethacin	20 mg/kg	9.30 ± 0.49** (290.75%)	9.23 ± 0.80** (121.87%)	10.00 ± 0.30** (161.09%)
III	<i>C. bonducella</i> -I	7 mg/kg	3.00 ± 0.42* (26.05%)	6.24 ± 0.33** (50.00%)	7.30 ± 0.42** (90.60%)
IV	<i>C. bonducella</i> -II	10.5 mg/kg	4.00 ± 0.22* (68.06%)	7.50 ± 0.36 (80.28%)	7.50 ± 0.40** (95.82%)
V	<i>C. bonducella</i> -III	14 mg/kg	5.3 ± 0.22* (122.68%)	8.00 ± 0.47* (92.30%)	8.00 ± 0.55** (108.87%)

Values are expressed as mean ± S.E.M. (n= 6), *p < 0.05, **p < 0.01, compared with control, ANOVA followed by Dunnett's test.

Anti-inflammatory activity:

Effect of aqua of *C. bonducella* seed on carrageenan-induced hind paw edema in rats: A significant reduction in the paw volume was observed at 1st, 3rd and 5th hr. In comparison to control group, aqua of the test drug (*C. bonducella*) in the doses of 7 mg/kg and 10.5 mg/kg and 14 mg/kg showed a significant

reduction (p < 0.01) in the paw volume at 1st, 3rd and 5th hr. The standard drug, indomethacin 20 mg/kg showed a significant decrease in paw volume at 1st hr (p < 0.01), 3rd hr (p < 0.01) and 5th hr (p < 0.01). The reduction in the paw volume is statistically significant at both middle and higher doses as shown in **Table 3**.

TABLE 3: EFFECT OF AQUA OF *C. BONDUCELLA* SEED ON CARRAGEENAN-INDUCED HIND PAW EDEMA IN RATS

Group	Treatment	Dose	Increase in paw volume (ml.) after Carrageenan administration (Mean ± S.E.M)			
			0 hr	1st hr	3rd hr	5th hr
I	Control	10 ml/kg	1.03 ± 0.05	1.8 ± 0.03	1.86 ± 0.02	1.71 ± 0.03
II	Indomethacin	20 mg/kg	1.01 ± 0.05	1.40±0.05** (22.22%)	1.00±0.05** (46.23%)	0.9±0.03** (47.36%)
III	<i>C. bonducella</i> -I	7 mg/kg	1.01 ± 0.04	1.70±.03** (5.55%)	1.61±.04** (13.44%)	1.26±0.04** (26.31%)
IV	<i>C. bonducella</i> -II	10.5 mg/kg	1 ± 0.02	1.61± 0.04** (10.55%)	1.43±0.03** (23.11%)	1.2±0.02** (29.82%)
V	<i>C. bonducella</i> -III	14 mg/kg	1 ± 0.02	1.38±0.05** (23.33%)	1.23± 0.03** (33.87%)	1.08 ± 0.0** (36.84%)

Values are expressed as mean ± S.E.M. (n= 6), *p < 0.05, **p < 0.01, compared with carrageenan control, ANOVA followed by Dunnett's test.

Assessment of the safety of test drugs on gastric mucosa of rats: This method was employed to assess the safety of test drugs on gastric mucosa of rats. In this method only the higher dose of the test drugs was given.

In this method, the indomethacin (20 mg/kg) group produced ulcers on the gastric mucosa but the test drugs (*C. bonducella*) caused no ulcers at all as shown in **Table 4**.

TABLE 4: ASSESSMENT OF THE SAFETY OF TEST DRUGS ON GASTRIC MUCOSA OF RATS

Group	Treatment	Dose	Ulcer index
I	Indomethacin	20 mg/kg	5.4
II	<i>C. bonducella</i>	14 mg/kg	0

n= 6

DISCUSSION: The Unani herbal drug *Caesalpinia bonducella* (Karanjwa) are used individually for various anti-inflammatory disorders. *Caesalpinia bonducella* has been used for centuries in Unani system of medicine as Muhallil (Anti-inflammatory) and Musakkin (analgesic) ²⁰⁻²².

Although inflammation is one of the oldest known diseases of mankind and affects a large population of the world, no substantial progress has been made in achieving a permanent cure. The search of screening and development of drugs for anti-inflammatory activity is an unending problem. There is much hope of finding anti-inflammatory drugs from indigenous plants, as these are still used in therapeutics despite the progress made in conventional chemistry and pharmacology for producing effective drugs. Literature survey reveals that the species of 96 genera belonging to 56 families contain anti-inflammatory agents ¹⁹.

The Unani herbal drug *Caesalpinia bonducella* (Karanjwa) are used individually for various anti-inflammatory disorders. *Caesalpinia bonducella* has been used for centuries in Unani system of medicine as Muhallil (Anti-inflammatory) and Musakkin (analgesic) ²⁰⁻²².

The analgesic properties were also studied using sensitive models that could provide different grades of noxious stimuli (in thermal stimulus and chemically induced tissue damage).

In the present study analgesic activity was assessed by abdominal writhing test using acetic acid and hot plate test ¹⁶ applied to rats of both sexes.

The abdominal constriction response induced by acetic acid is a sensitive procedure to establish peripherally acting analgesics. The response is thought to involve local peritoneal receptors. A significant decrease in the number of writhings in 20 minutes was observed. The mean score for writhing was decreased by 20.73%, 43.71% and 62.47%, and 64.35% in standard (indomethacin) lower middle and higher doses of aqua form of *Caesalpinia bonducella* seed respectively over the score of control group. The decreased score for writhing was statistically significant at all the doses ($p < 0.01$).

In hot plate test, nociceptive reaction towards thermal stimuli in rats is a well-validated model for detection of opiate analgesic as well as several types of analgesic drugs from spinal origin ²³. A significant increase in the reaction time at various dose levels of aqua form of *C. bonducella* seed (7, 10.5 & 14 mg/kg) was observed at 30 min, 60 min and 90 min increased the reaction time in a dose dependent manner to the thermal stimulus. The nociception inhibition of thermal stimulus was exhibited at a lower dose of the extract is 90.60%, & at higher dose 108.87% at 90 min which is comparable to indomethacin (161.09%). These findings suggest that the test drug exerts analgesic effect similar to non-steroidal anti-inflammatory drugs.

Thus the anti-nociceptive activity shown by *Caesalpinia bonducella* in aqua form on hot plate and acetic acid-induced writhing test might possess centrally and peripherally mediated anti-nociceptive properties.

Anti-inflammatory agents have widely been incriminated as one of the important causes of gastritis and gastric ulceration (peptic ulcers). The gastric lesions produced are the result of prostaglandin inhibitory effect of anti-inflammatory agents, produced in the cyclo-oxygenase pathway of arachidonic acid metabolism. Prostaglandins generated through cox-1 enzyme pathway have got a gastroprotective role and inhibition of cyclo-oxygenase results in the depletion of both the cox-1 and cox-2 enzymes, hence devolving mucosal barrier from the protective effect of cox-1 mediated prostaglandins.

In view of this, the drug was investigated for the gastric irritation potential also. The results of the study revealed that no gastric irritation sign was observed whereas the Indomethacin produced some ulcers.

Carrageenan-induced paw edema was used for assaying the effect of the test drug extract on acute inflammation. Carrageenan-induced paw inflammation is a test largely used to study both steroidal and non-steroidal anti-inflammatory drugs²⁴.

In the present study, the aqua form of *C. bonducella* seed at various dose levels (7, 10.5 & 14 mg/kg) showed significant anti-inflammatory activity on carrageenan-induced edema in rats in a dose dependent manner. *C. bonducella* seed at 7 mg/kg produced a significant inhibition ($p < 0.01$) of 5.55%, 13.44% and 26.31% at 1st hour, 3rd hour and 5th hour respectively while at the dose of 10.5 mg/kg also produced significant inhibition ($p < 0.01$) of 10.55%, 23.11% and 29.82% at 1st hour, 3rd hour and 5th hour respectively and at higher dose it produced significant inhibition ($p < 0.01$) of 23.33%, 33.87% and 36.84% at 1st hour, 3rd hour and 5th hour respectively.

The standard drug, indomethacin (20 mg/kg) showed an inhibition of 22.22% ($p < 0.01$), 46.23% ($p < 0.01$) and 47.36% ($p < 0.01$) at 1st hour, 3rd hour and 5th hour respectively.

Drug might be having different compounds responsible for the inhibition of different phases of carrageenan-induced inflammation. The higher degree of inhibition in the 5th hour shows that the drug might be interfering with prostaglandins i.e. the drug might be antagonizing the prostaglandin induced rat hind paw edema.

The inhibition at the 1st hour and 3rd hour may be due to interfering of the drug with histamines and serotonin induced vascular changes, mobility of polymorphonuclear leucocytes and the resultant exudation, which are characteristic of acute inflammation.

Thus, the test drug Karanjwa seed (*C. bonducella*) may be considered safer for use as compared to indomethacin, which although having well anti-inflammatory and analgesic activity produces gastric ulcers.

CONCLUSION: In conclusion, our findings demonstrated the favorable non-central analgesic and anti-inflammatory activities of aqua form of *C. bonducella* seed, and affirmed the claim by traditional medicine practitioners that *C. bonducella* could be used to treat pain and inflammation. These effects are possibly involved in adjustment of local synthesis and release of prostaglandins. However this plant extract contains too many compounds and possibly has several action pathways for its activities. Hence, it is necessary to further investigate the chemical components of this plant in order to clearly elucidate its mechanisms of action.

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