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

Human Journals
Research Article
March 2022 Vol.:23, Issue:4

Pharmacological and Phytochemical Investigation on the Seeds of *Caesalpinia crista* for Antipyretic Action in Experimental Animals

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ISSN 2349-7203
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Submitted: 23 February 2022
Accepted: 28 February 2022
Published: 30 March 2022

Keywords: *Caesalpinia crista*, Plant extracts, Phytochemicals, Antipyretic, Brewer's yeast.

ABSTRACT

To evaluate the antipyretic activity of aqueous and ethanol extracts of *Caesalpinia crista* seed extracts. **Methods:** Brewer's yeast-induced pyrexia in rats was used to test the antipyretic efficacy of *Caesalpinia crista* (Fabaceae). At 200 mg/kg body weight, both the aqueous and ethanolic extracts considerably ($p<0.05$) decreased the elevated rectal temperature. **Results:** After 3 hours of treatment, the extracts began to reduce the increased rectal temperature in a dose-dependent manner. After 3 hours of therapy, the aqueous and ethanol extracts lowered 75 percent and 81 percent of the raised rectal temperature, respectively, when compared to the reference medication paracetamol (93 percent). **Conclusion:** As a result, both extracts of *Caesalpinia crista* were shown to have antipyretic action, with the ethanol extract being somewhat more powerful than the water extract.

INTRODUCTION:

Fever is defined as a rise in body temperature above the usual range caused by an increase in hypothalamic set-point temperature [1]. It is a typical medical indication that indicates a rise in body temperature over the normal range of 36.5–37.5 °C [2, 3]. Increased prostaglandin E2 (PGE2) generation in the hypothalamic preoptic area affects neuron firing rate, inducing fever. [4]

Antipyretic medicines block COX-2 expression, resulting in a decrease in PGE2 production, which is a significant fever mediator [5]. Antipyretic medicines suppress prostaglandin formation, however, they do affect body temperature if it is raised due to an increase in ambient body temperature or physical activity [6]. Steroids and opioids are among the other medicinal drugs used in the treatment of pyrexia. Antipyretics such as diclofenac, aspirin, and paracetamol are used to treat fever [7]. Removal of the patients' garments to allow them to dissipate heat to the environment is another non-traditional strategy used in fever control. The patient may also be massaged with a sponge that has been dipped in warm water [8]. This aids in the conductivity of heat.

Caesalpinia crista (Caesalpiniaceae) is a big thorny shrub that may be found in India, Sri Lanka, and the West Indies. It's prevalent in southern India and is frequently used as a hedge plant. Caesalpinia is a pantropical genus with 120-130 species, although its taxonomic history is complicated. Skin illnesses, malaria, cancer, infections, erectile dysfunction, pain, and wounds have all been treated using Caesalpinia species plants in traditional medicine [9]. In Chinese traditional medicine, 14 species, including *C. decapetala* and *C. sappan*, have long been used to cure rheumatism and inflammatory illnesses [10]. *C. pulcherrima* leaves, barks, and roots have been used to treat fungal infections and fever [11].

MATERIALS AND METHODS:

Plant samples collection, preparation, and extraction: After an extensive literature review on the medicinal uses of these plants and with bio-conservation aspect considerations fresh seeds of *C. crista* were collected from Dohra Village, Rohilkhand Region, Uttar Pradesh, India. The samples were identified and authenticated by Dr. Alok Shrivastava, Associate Professor, Department of Plant Science, M. J. P. Rohilkhand University, Bareilly. The plant voucher specimen number was MJP/PS/2019/01 which were deposited at the Plant Sciences departmental Herbarium of MJP Rohilkhand University for future reference. The

ethnobotanical knowledge provided by local herbalists in the region was used to collect the plants pictured. The samples were washed and wrapped in Khaki bags to eliminate dirt and other impurities. For two weeks, the seeds of plant samples were air-dried at the Pharmacy Department at a temperature of 25 °C. They were then pulverised into a fine powder with an electric grinder and kept at room temperature (25 °C) in sealed khaki sheets with suitable labels until extraction.

Chemicals: Paracetamol used was obtained from Yarrow Chem Pvt Ltd.; Tween-80 were of Merck Chemicals Ltd., Germany, while sterile normal saline (0.9% NaCl, Beximco Infusions Ltd., Bangladesh) was used for the study. All solvents and chemicals used were of an analytical grade standard. All solutions were prepared on the same day of the experiments.

Extraction

1. Ethanolic Extract: The seed components of *C. crista* were dried for 2-4 weeks before being pulverized into a coarse powder of 300g. The extract was first defatted with petroleum ether, then ethanol was employed as the solvent in the Soxhlet apparatus for 72 hours. The ethanolic extract was extracted from the Soxhlet after this treatment. This extract was dried at a low temperature (40-60 °C) under decreased pressure. In a Petri dish, the dried ethanolic extract was collected, and the % yield of the ethanolic extract was determined.

2. Aqueous Extract: *C. crista* dry seed powder was put in a round bottom flask with a stopper. Water was employed as a solvent, and it was let to stand for 4 days with constant agitation until the soluble materials disintegrated. The *C. crista* combination was strained (through sieves/net), the mare was passed, the combined liquid was clarified, and the mixture was dried under decreased pressure. Petridis provided the dried plant medicine. [12]

Phytochemical Screening: Using reported methods, the extracts obtained from successive solvent extractions were subjected to various qualitative chemical tests to determine the presence of various phytoconstituents such as alkaloids, glycosides, carbohydrates, phenolics and tannins, proteins and amino acids, saponins, phytosterols, proteins, and amino acids, saponins, and phytosterols. [13]

Thin Layer Chromatographic (TLC) Technique: When it comes to establishing repeatable chromatography with distortion-free zones, the solvent used to apply the sample might be crucial. In general, the application solvent should be suitable for the sample, being as volatile and non-polar as feasible. Thin-layer chromatography (Silica gel coated, Merck) was used to

characterize the aqueous and ethanol seed extracts, with the mobile phase being hexane: ethyl acetate: a few drops of formic acid in the ratio of 7:3:formic acid few drops for flavonoids detection. The stationary phase was chosen because silica gel is an effective adsorbent for TLC separation of most plant extracts and plant medicine extracts. [14]

Experimental Animals: For this investigation, Wistar rats of either sex weighing 130-200g were used. The experiment was carried out in the Department of Pharmacy, M.J.P. Rohilkhand University, Bareilly, and the proper nutritional and environmental conditions were maintained throughout. Prior to the trial, they were kept in polypropylene cages with paddy house bedding under regular laboratory conditions for a 7-day acclimatization period. The institutional ethics committee for animal studies accepted this study (1884/GO/Re/S/16/CPCSEA). The animals had unlimited access to laboratory food and water [15].

Experimental Design: The LD_{50} of *C. crista* is 2000mg/kg. No toxic effects or mortality were observed with doses ranging from 50mg/kg to 2000mg/kg for four weeks. Hence the antipyretic activity of the aqueous and ethanolic seed extracts of *C. crista* was studied as per the following.

Experimental design: Animals were recorded, and they were randomly divided into 4 groups of 6 animals each as follows:

Group I : Brewer's yeast + saline (i.p.-10 ml/kg)

Group II: Brewer's yeast + ethanolic extract of *C. crista* (200 mg/kg, p.o)

Group III: Brewer's yeast + Aqueous extract of *C. crista* (200 mg/kg, p.o)

Group IV: Brewer's yeast + standard drug paracetamol (200 mg/kg, p.o)

Development of Brewer's Yeast-induced pyrexia Model: In this investigation, Wister rats (130-200) were separated into six groups, each with six animals. Subcutaneous injection of a 20 percent w/v brewer yeast solution of dried yeast in 0.9 percent saline at a dosage of 10 ml/kg produced pyrexia in rats. The injection was given to the rat in the back, just below the neck. The animals were fasted for 16 hours before the experiment but were given unrestricted access to water. All of the medications were administered as a fresh aqueous solution in 0.9 percent saline.

The test compounds and the reference medication were given to the rats by oral administration. The starting rectal temperature of rats was measured with an electric thermometer, and the rectal temperature was recorded when it reached its peak. All of the rats in this experiment had a rectal temperature increase of more than 1.2 degrees Celsius. After administration of varied extract C. crista doses and standard medication, the rectal temperature of animals was monitored at 1-hour intervals [16, 17].

Statistical Significance: The statistical analysis was done by ANOVA followed by Dunnet's test for multiple comparisons. $P < 0.01$ was considered significant in the experiment.

RESULTS AND DISCUSSION:

Phytochemical Analysis: The crude aqueous and ethanol extract was screened the present alkaloids, flavonoids, glycoside, carbohydrate, protein, starch, amino acid, steroid, tannins, and saponins using the simple chemical test as reported in a standard reference book.

Table No. 1: Pre-phytochemical Screening of Aqueous and ethanol extract

Chemical Constituents	Chemical test	Aqueous	Ethanol
Carbohydrate	Molisch Fehling Benedict	Negative	Negative
Flavonoid	Shinoda test	Positive	Positive
Alkaloids	Wagners Dragendroff test Mayer Hagers	Positive	Positive
Protein and amino acid	Millions test Ninhydrin	Positive	Positive
Glycoside	Killer Killiani Legal test	Positive	Positive
Saponin	Foam	Negative	Negative
Terpenoid and steroid	Salkowski	Negative	Positive

Thin Layer Chromatography: Hexane: ethyl acetate: few drops of formic acid in the ratio of 7:3:formic acid few drops for ethanolic extract was the optimum solvent system for TLC of C. crista Linn. Using the iodine chamber detecting reagent, TLC of *C. crista* Linn. indicates the presence of 5 compounds with various Rf values in different colors, indicating the presence of 5 compounds in the extract.

Table No. 2: TLC of ethanolic extract of seeds of *C. crista*

Extract	Solvent System	No. of Spots	Rf value
Ethanolic Extract	hexane: ethyl acetate: few drops of formic acid (7:3:formic acid few drop)	5	0.32 0.43 0.64 0.68 0.72

Figure No. 1: TLC of ethanolic extract of *C. crista*

ANTIPYRETIC ACTIVITY:

Day 1:

1. Standard vs. Test 1, Test 2, and Control: Each value represents mean ± SEM (n=6), compared to standard group by Thermometer during the time session of 21 hours. Test T1 and T2 group consist of six animals, each group treated with *Caesalpinia crista* (L.) of dose 200mg/kg (aqueous extract) and 200mg/kg (ethanolic extract) respectively for 21 hours and

control group consisted of six animals, in each group treated with normal saline of dose 1ml/kg for 21 hours. After treatment, I was tested by Thermometer to evaluate its antipyretic effects during the time session of 21 hours. The temperature of the animal was recorded by the thermometer and noted down it.

Table No. 3: Standard Vs Test 1st, Test 2nd, and control group

Treatment	Initial temp. before Br's yeast injection	Temperature after 18 hrs (0 Hr.)	1 Hr.	2 Hr.	3 Hr.
Standard group	36.59±0.13	38.40±0.15	37.42±0.12	37.15±0.07	36.40±0.07
Test 1 group	36.50±0.11	38.41±0.89	37.91±0.04	37.33±0.14	36.77±0.06
Test 2 group	36.62±0.05	38.28±0.10	37.68±0.08	37.43±0.10	36.58±0.13
Control group	36.57±0.19	38.14±0.03	38.24±0.04	38.30±0.03	38.51±0.09

DAY 2

1. Standard vs. Test 1, Test 2, and Control: Each value represents mean + SEM (n=6), compared to the standard group by Thermometer during the time session of 21 hours. Test T1 and T2 group consist of six animals, each group treated with *Caesalpinia crista* (L.) of dose 200mg/kg (aqueous extract) and 200mg/kg (ethanolic extract) respectively for 21 hours and control group consisted of six animals, in each group treated with normal saline of dose 1ml/kg for 21 hours. After treatment, I was tested by Thermometer to evaluate its antipyretic effects during the time session of 21 hours. The temperature of the animal was recorded by the thermometer and noted down.

Table No. 4: Standard Vs Test 1st, Test 2nd, and control group

Treatment	Initial temp. before Br's yeast injection	Temperature after 18 hrs (0 Hr.)	1 Hr.	2 Hr.	3 Hr.
Standard group	36.45±0.09	38.28±0.09	37.75±0.08	37.31±0.05	36.43±0.04
Test 1 group	36.78±0.10	38.40±0.06	37.93±0.03	37.68±0.03	36.53±0.01
Test 2 group	36.81±0.09	38.32±0.04	37.82±0.04	37.29±0.09	36.53±0.14
Control group	36.56±0.11	38.31±0.05	38.32±0.07	38.47±0.05	38.54±0.04

Yeast-induced fever, which mimics pathogenic fever, is a cost-effective and reliable way to test novel antipyretics. In this technique, the presence of proteins in yeast is connected to fever via an inflammatory response [18]. Proinflammatory cytokines including interleukin-1 (IL-1) and IL-6, interferon (IFN), and tumor necrosis factor (TNF-) as well as prostaglandins like PGE2 and PGI2 are also responsible for raising body temperature via acting on the brain [19, 20, 21]. Paracetamol (PCM), Nimesulide, Aspirin, and other similar drugs have a harmful impact on the body's numerous organs. Flavonoids, tannins, proteins, alkaloids, carbohydrates, phytosterols, saponins, diterpenoids, and triterpenoids were found in the ethanolic and aqueous extracts of the plant *Caesalpinia crista* (L.). Prostaglandins, which are implicated in the late stages of pyrexia, inflammation, and pain perception, are known to be targeted by flavonoids and tri-terpenoids. By avoiding or reducing the beginning of cell necrosis and enhancing vascularity, flavonoids and triterpenoids diminish lipid percolation.

In general, nonsteroidal anti-inflammatory medications (NSAIDs) work by inhibiting prostaglandin production in the hypothalamus. Nature's gift to humans is medicinal plants, which aid in the pursuit of a disease-free, healthy existence. Plants have been utilized as medicines by humans for thousands of years. *Caesalpinia crista* (L.) may serve as an antipyretic by inhibiting prostaglandin production. *Caesalpinia crista* (L.) is a member of the Caesalpiniaceae family. *Caesalpinia crista* (L.) is a plant with a diverse set of chemical components that have a variety of therapeutic effects. Because of its efficacy and safety, Caesalpinia crista (L.) has considerable potential for the development of innovative medications to treat a variety of human diseases. [22]

CONCLUSION:

The antipyretic properties of the aqueous and ethanol extracts from *C. crista* seeds were found to be substantial. Because this is a preliminary study, more research is needed to confirm the findings, as well as more extensive investigations on chemical identification and isolation, as well as the underlying mechanism for the observed impact, to ensure its therapeutic application.

ACKNOWLEDGEMENT: Nil

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