

How to Cite:

Haider, S. A., Asim, F., Rehman, Z. U., Shakir, L., Mustafa, M. W., Masih, S., Israr, U., Asif, K., Siddique, M., Rafaqat, A., Ramzan, H. M., & Shoukat, A. (2023). Exploring the anti-inflammatory and anti-pyretic potentials of methanolic and aqueous fruit extracts from *ceiba speciosa*. *International Journal of Health Sciences*, 7(S1), 2102–2120.
<https://doi.org/10.53730/ijhs.v7nS1.14462>

Exploring the anti-inflammatory and anti-pyretic potentials of methanolic and aqueous fruit extracts from *ceiba speciosa*

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Abstract---Background and Aim: *Ceiba speciosa* (*C.speciosa*) has been used for treating various ailments as a folklore remedy in particularly as an anti-inflammatory agent. The fruit of this plant, however, has not been extensively investigated for its pharmacological activities. Keeping in view the anti-inflammatory activity of the leaves and flowers, this study was intended to explore the anti-inflammatory potential of the fruit of this plant in rats. Materials and Methods: Carrageenan induced inflammation model was applied to study the effects of Methanol and Aqueous extract of *Ceiba speciosa* fruit in Wister rats of both sexes. Diclofenac sodium (10mg/kg) was used as a standard reference for this model. 9 groups of animals were made prior to the administration of methanol extract of *Ceiba speciosa* fruit. Yeast induced pyrexia model was used to study the anti-pyretic effect of methanol and aqueous extracts of *Ceiba speciosa* fruit in rats. Paracetamol (150mg/kg) was used as a standard reference for investigation of anti-pyretic effect. Results: Data compiled at the end of the study illustrated that methanol extract of *C. speciosa* fruit showed significant ($p < 0.0001$) anti-inflammatory effect at doses of 200mg/kg B.W., 400 mg/kg B.W. and 600mg/kg B.W. when related with disease control group. Aqueous extract at doses of 200 mg/kg, 400 mg/kg and 600mg/kg also showed some effect but did not reach to a statistical significance level. Data showed that methanol extract of *Ceiba speciosa* fruit has shown significant ($p < 0.0001$) anti-pyretic effect at dosages of 200, 400 and 600 mg/kg B.W. Conclusion: The assessed parameters demonstrated that methanol extract of fruit from *Ceiba speciosa* demonstrated anti-inflammatory and anti-pyretic effects in rats. Whereas, aqueous extracts did not exhibit significant but depicted some anti-inflammatory and antipyretic effects. Hence, fruit of *Ceiba speciosa* possesses active principle(s) having anti-inflammatory and antipyretic properties. Future studies may attract the researchers to isolate the active components responsible for above activities and explore their individual effects in inflammatory and pyretic models of animals.

Keywords---ceiba speciosa, anti-inflammatory effect, antipyretic effect, rat models, fruit extract.

Introduction

The word inflammation comes from the Latin word “inflammare” (to burn) (de oliveira). The primary objective of an inflammatory response is to protect the body against microbial infection and damage (Abdulkhaleq et al., 2018)¹⁻². Inflammation is the body's feedback to attack by an infectious agent, antigen - experiment or even just physical, chemical or traumatic injury³⁻⁴. To escape harm of the surrounding tissue, inflammatory response must be well-organized and controlled (Ferenčík & Štvrtinová, 1996). Localized inflammation within tissues outside of the brain is demonstrated by the traditional combination of edema, heat, redness and frequently pain⁵⁻⁶. The attack of circulating immune cells (lymphocytes and macrophages), as well as the initiation or inflammatory mediator's activation including cyclooxygenase products, cytokines and kinins have now been recognized as additional mechanisms of inflammation (Lucas et al., 2006). Chemicals released from migratory cells and tissues initiate inflammation. The leukotrienes (LT5), prostaglandins (PGs), bradykinin, histamine, interleukin-1 and platelet-activating factor (PAF) are the most potentially active molecules (Vane, J., & Botting, 1987). Although the normal physiological inflammatory response only continues for a quite brief time, in difference to pathological situations where inflammation can remain for years or months (Valeyev et al, 2010)⁷⁻⁸.

Symptoms of acute inflammation last from hours to a few days. Celsus is attributed to be the first scientist to define the cardinal signs of acute inflammation that include; (i) pain (dolor), (ii) loss of function (function laesa), (iii) redness (rubor), (iv) heat (calor) and (v) swelling (tumor)⁹⁻¹⁰. An acute inflammatory response is certainly the immediate reaction of the human body to any type of insult or trauma and it constitutes the first line of defense against injury or invading microbes or foreign objects into the human body (Rankin, 2004)¹¹⁻¹². This type of response is non-specific and occurs irrespective of the nature of the intruding foreign body. The acute inflammatory response is very well coordinated and involves the endocrine, nervous as well as the immune system. The main objective is to mobilize inflammatory cells and chemicals that eliminate the threat to the human body, restore the physiological state, and initiate repairing mechanisms. Chronic inflammation lasts longer than acute inflammation and the time period may extend from weeks to months¹³⁻¹⁴. Apart from participation of inflammatory mononuclear cells such as macrophages and lymphocytes, chronic inflammation also involves fibroblasts, collagen fibers and connective tissue. If chronic inflammation does not resolve, it may lead to granuloma formation, which is an attempt by the immune and other associated cells to wall off or border the offending organism or other sources of injury to the body¹⁵. This may sometimes lead to tissue degeneration as the toxic chemicals and other mediators of inflammation released are highly reactive. Examples include reactive oxygen and nitrogen species and protease enzymes. Not only these chemicals act to destroy the offending organism or source of injury, they

also participate in remodeling of normal surrounding tissue (Pahwa et al., 2021)¹⁶⁻¹⁷.

Ceiba speciosa is a tree longing about 10-20 meters in length, before known as *Chorisia speciosa* worldwide. It cultivates straight when it is young and becomes bottle designed as it becomes old. It belongs to Malvaceae family (Kausar et al., 2020). Its leaves are lobe shaped with pale green color, flowers are pink colored and fruit is pear-formed capsule with light green color and full with pea-size seeds present in white silk¹⁸. This silk is also applied as water proofing stuffing in pillows, cushions and mattresses. Wood of this tree is used for manufacturing the match sticks. This tree is found frequently in forests of South America. It is also recognized by several other names including “paloborracho” (in Spanish), “toborochi” in Bolivia, meaning “tree of refuge” or “sheltering tree”) (Rosselli et al., 2020) and paineira (in Brazilian Portuguese). It is mainly cultured for ornamental resolutions and silk fiber from the ripened fruits, due to this reason it is also known as silk floss tree¹⁹. This family has been predictably used for giving many health-related illnesses including diarrhea, rheumatism, peptic ulcer and parasitic infections. *Chorisia* species is supposed to have many bioactivities, plus anti-inflammatory, hepato-defensive, cytotoxic, antioxidant, and hypoglycemic activity (Nasr et al., 2018)²⁰

This study aims to determine the Antipyretic and Anti-Inflammatory potential from the fruit of *Chorisia speciosa* in rats by using the Carrageenan induced inflammation model and Yeast induced pyrexia model. To achieve this aim, different parameters like paw size and temperature were investigated in carrageenan induced inflammatory rat model and yeast induced pyrexia model, respectively.

Materials & Methods

Chemicals and drugs

Following standard chemicals and experimental drugs were purchased from the different manufacturers belonging to various countries as mentioned against their names.

- Absolute ethanol, methanol, formalin (Merck, GaA-64721 Darmstadt Germany)
- Normal saline (Otsuka Pakistan Ltd.),
- Carrageenan (Sigma chemical company St. Louis, MO, USA),
- Instant Yeast (Rosepair foods, Lahore Pakistan),
- Diclofenac sodium (Sigma Aldrich)
- Paracetamol (Sigma Aldrich)
- Normal Saline (Otsuka Pakistan Ltd)

Instruments

Different instruments used to measure different parameters in this experimental research work, were of scientific and research grade. These include:

- Analytical balance (AB54-S by mettler Toledo, Switzerland),
- Vernier calipers (Bulgaria manufacturer, 0.05mm-150mm),
- Digital thermometer (Citizen Systems Japan Co. Ltd. Duteck Taiwan factory Shontek, China).
- Electronic heating water bath,
- Rat weighing balance (SF-400A, Smith Process Instrumentation, South Africa),
- Rotary evaporator (Heidolph Lab 4002 Sigma Aldrich, Germany),
- Vortex mixer (MyLab SLV-6 Seoul in Bioscience, Korea).

Experimental animals

Fruit from *Chorisia speciosa* was utilized to test the anti-inflammatory and antipyretic effects on Wistar albino rats of both genders (150-250 g). The animals were kept in the Punjab University College of Pharmacy's animal house at the University of the Punjab in Lahore, Pakistan, under ideal circumstances of humidity (50–60 %), temperature (20–25° C) and a 12-hour cycle of light and darkness. The animals were kept in air-conditioned spaces in wide-mesh, raised-floor stainless steel cages with free admittance to water and food. Before starting the trial, they were kept on a 24-hour fast and only given water. The research methodology was accepted by the "Bioethical Committee of the Department of Zoology, University of the Punjab, Lahore" and the care and handling of the rats were done in agreement with the generally acknowledged standards for the use of experimental animals.

Plant collection and drying

The fruit of *Chorisia speciosa* was acquired from the Lawrence Garden, Lahore Pakistan. The fruit was washed with water, sliced into small pieces and shade dried for 15 days. Plant Identification was carried out at The Botany Department of Government College University, Lahore, Pakistan. The voucher number of the specimen (GC.Herb.Bot.3711) was assigned and the fruit of the plant was saved in the herbarium of the same. Grinding and extraction of the plant was done by slicing the fruit followed by grinding into a fine powder with the help of a blender. The powder was then soaked in methanol with continuous stirring every day for one week.

Filtration and drying of extract

After one week, the extract was filtered using Whatman filter paper with a help of a funnel and removal of the excess solvent was done under decreased pressure by using rotary machine (Heidolph Lab 4002 Sigma Aldrich, Germany). It was then dried in a hot air oven at 40°C to obtain a dark brownish extract which was then refrigerated at 2°C to 8°C for further experimental work.

Acute toxicity study

The study to prove acute toxicity of *Chorisia speciosa* fruit was accomplished as per the guidelines of "Organization for Economic Cooperation and Development" (OECD). Three animals of same sexes were used for evaluation of each toxic dose.

The animals were given 500 mg/kg B.W., 1000 mg/kg B.W. and 1500 mg/kg B.W. of the extract per oral route. No acute or delayed death response, in a study dated of fourteen days was detected. Even at the extreme dose of 1500 mg/kg B.W., the rats displayed no signs of toxicity with regular breathing frequency and nonappearance of fits etc. From these explanations, it was elucidated that LD50 of *Chorisia speciosa* fruit extract is more than 1500 mg/kg B.W.

Drug administration

In the following experiments two different methods were used for the drug administration including oral and subcutaneous. The solid drugs were dissolved in distilled water before administration and given orally by using oral gavage tubes. Disposable syringes of 5cc and 1cc were attached to oral gavage tubes for administration of drugs to rats and mice, respectively. After filling the drug in syringe, oral tube was inserted into the esophagus and the drug was poured directly into the stomach by pushing the plunger of the syringe. In mice, drug was injected intraperitoneal in the space by inserting the tip of the needle of syringe into the inferior right quadrant of the abdomen. Subcutaneous administration of the drug was carried as animals were positioned on lid of the cage and then the loose skin was lifted up from in between the shoulder blades. The 18 gauge needle of the syringe was injected into subcutaneous tissue. Intradermal injection was given in the sub-planter surface of the rat paw. From the ventral side of the rat paw skin from the center was uplifted and carrageenan was injected into the skin.

Evaluation of Anti-inflammatory activity

Wister Albino Rats of both genders (150-200g) were used for the anti-inflammatory activity for carrageenan-induced paw edema. Nine groups of five animals each were formed by randomly grouping the animals. Groups 1 and 2 received only normal saline treatment, whereas Group 3 received only an intradermal injection of 0.05 ml of carrageenan (made as a 1% suspension in normal saline) into the hind paws of the rats. Fruit extracts in Methanol were administered to Groups 4, 5 and 6 at doses of 200 mg/kg, 400 mg/kg, and 600 mg/kg, respectively. Aqueous fruit extracts were administered to Groups 7, 8 and 9 at doses of 200 mg/kg, 400 mg/kg, and 600 mg/kg, respectively. All of the rats' normal paw sizes were first determined using a vernier caliper, and then carrageenan (1%, 0.05ml) was subcutaneously injected into the sub-planter tissue of each rat's right hind paw. Paw size was once more assessed 30 minutes after carrageenan administration, and the aforementioned doses were then given orally. Digital vernier calipers were used to measure the paw's inflammation at 0, 1,2,3, and 5 hours intervals.

Evaluation of anti-pyretic activity

Wister albino rats (150–200g) of both genders were chosen to test the anti-pyretic activity. The animals were divided into nine groups, each of which had five rats. By utilizing a digital thermometer to measure each rat's normal rectal temperature, 20% (10ml/kg suspended in normal saline) suspension of Brewer's yeast was then used to produce pyrexia in the rats. After 24 hours, the rectal temperatures of each rat were recorded. The induction of fever was confirmed by a

rise of more than 0.5 degree centigrade. All the groups were fasted for the whole night but given access to drinking water. As a negative control, group 1 was just given normal saline (10ml/kg), while group 2 received the conventional medication paracetamol (150mg/kg). Group 3 received no medication to control the disease, whereas Groups 4, 5, and 6 received 200 mg/kg, 400 mg/kg, and 600 mg/kg of methanol extracts, respectively. Aqueous extracts at doses of 200 mg/kg, 400 mg/kg, and 600 mg/kg were administered to Groups 7, 8, and 9. Rectal temperature was once more recorded periodically at 0, 1, 2, 3, and 5 hours intervals after medication delivery. The reduction in pyrexia was calculated using only temperature variations.

Statistical analysis

The mean and SEM were used to express the data. Utilizing Graph Pad Prism 5, one-way analysis of variance (ANOVA) and Tukey's test were used to establish the statistical significance. $P < 0.05$ was regarded as significant.

Results

Carrageenan-induced rat paw edema (acute inflammatory rat model)

The result of anti-inflammatory effect of, Diclofenac-sodium (10mg/kg), methanol (200mg/kg), methanol (400mg/kg), methanol (600mg/kg), aqueous (200mg/kg), aqueous(400mg/kg) and aqueous (600mg/kg) in carrageenan induced paw edema in rats is shown in table 1. Group 1 was considered as a control group and was treated with normal saline (10ml/kg), at different time intervals (0hr, 1hr, 2hr, 3hr, 4hr, 5hr) rat paw was measured with vernier caliper there weren't any signs of inflammation in control group. Group 2 was administered with carrageenan 1% and rat paws were measured with vernier caliper at different time intervals, paw size increased after half an hour as it was disease control group likewise other groups (group 3 to group 9) were also treated with carrageenan 1% (0.05ml) and same results of inflammation were observed. Paw size increased after half an hour after administration, after inducing inflammation various doses of extracts were administered. Group 3 was administered with diclofenac sodium (10mg/kg) and inflammation was measured at different time intervals (0hr, 1hr, 2hr, 3hr, 4hr, 5hr), paw size decreased from 5.56mm to 3.71mm in 5 hours. Group 4 was treated with methanolic extract of dose 200mg/kg and paw size was measured at different time intervals paw size decreased from 5.60 to 4.78 in 5 hours. Anti-inflammatory response of methanolic extract at a dose of 200mg was evident but it was less than diclofenac sodium (10mg/kg). Group 5 was treated with methanolic extract at a dose of 400mg/kg and response was measured, paw size decreased from 5.28mm to 4.42mm. Group 6 was treated with methanolic extract at a dose of 600mg/kg after induction of inflammation and paw size was measured at different time intervals, paw size decreased from 5.23mm to 4mm. Group 7 was treated with aqueous extract at a dose of 200mg/kg after inflammation and paw size was measured, it decreased from 5.28mm to 4.87mm. Group 8 was treated with aqueous extract at a dose of 400 mg/kg and paw size was measured, it decreased from 5.22mm to 4.79mm. Group 9 was treated with aqueous extract at a dose of 600mg/kg and paw size was measured, it decreased

from 5.23mm to 4.80mm. Group 5 with a dose of 400mg/kg showed maximum response in decreasing the paw size.

Table 1. Carrageenan-induced rat paw edema (acute inflammatory rat model)

Treatment groups	Paw thickness (mm)					
	0hr	1hr	2hr	3hr	4hr	5hr
Control Normal Saline (10ml/kg)	3.26 ± 0.01	3.24 ± 0.01	3.19 ± 0.01	3.14 ± 0.01	3.12 ± 0.013	3.08 ± 0.01
Carrageenan 1%	5.80 ± 0.02	5.86 ± 0.02	5.90 ± 0.02	5.82 ± 0.02	5.78 ± 0.02	5.74 ± 0.02
Diclofenac Sodium (10mg/kg)	5.56 ± 0.06	5.16 ± 0.07	4.73 ± 0.03	4.10 ± 0.04	3.79 ± 0.05	3.79 ± 0.05
Methanol 200mg/kg	5.60 ± 0.01	5.35 ± 0.04	5.07 ± 0.04	4.97 ± 0.18	4.82 ± 0.18	4.78 ± 0.18
Methanol 400mg/kg	5.28 ± 0.14	4.96 ± 0.13	4.72 ± 0.10	4.56 ± 0.07	4.46 ± 0.05	4.42 ± 0.05
Methanol 600mg/kg	5.23 ± 0.11	4.87 ± 0.09	4.71 ± 0.09	4.60 ± 0.09	4.50 ± 0.08	4.47 ± 0.08
Aqueous 200mg/kg	5.28 ± 0.11	5.15 ± 0.11	5.04 ± 0.10	4.97 ± 0.12	4.89 ± 0.11	4.87 ± 0.11
Aqueous 400mg/kg	5.22 ± 0.12	5.05 ± 0.10	4.94 ± 0.11	4.86 ± 0.11	4.81 ± 0.11	4.79 ± 0.11
Aqueous 600mg/kg	5.23 ± 0.10	5.04 ± 0.11	4.93 ± 0.10	4.85 ± 0.10	4.82 ± 0.01	4.80 ± 0.10

Data of five animals each (n=5) ± SEM

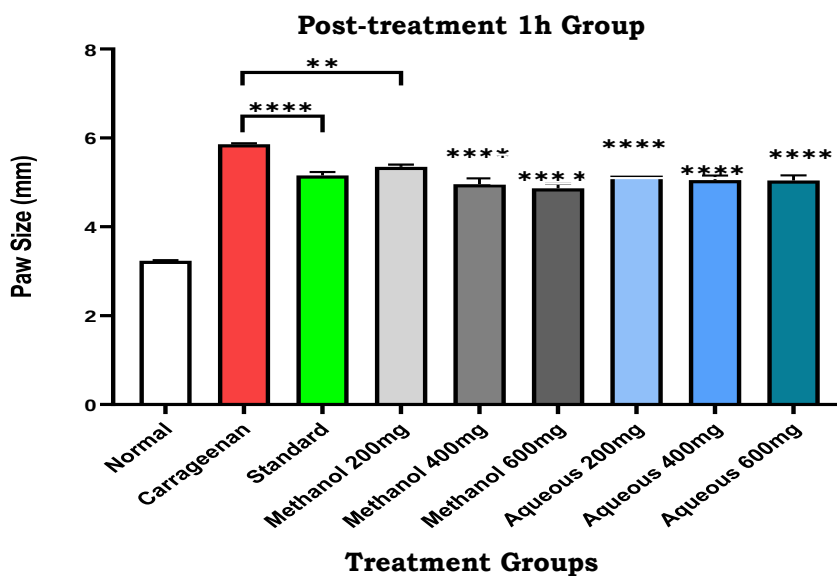


Figure 1. Effect of different treatment groups on carrageenan-induced paw edema in rats at 1 hour

Each bar is the mean of 5 rats while vertical lines on top of each bar indicate SEM.

**** $p < 0.0001$ and ** $p < 0.01$ when compared with Carrageenan group.

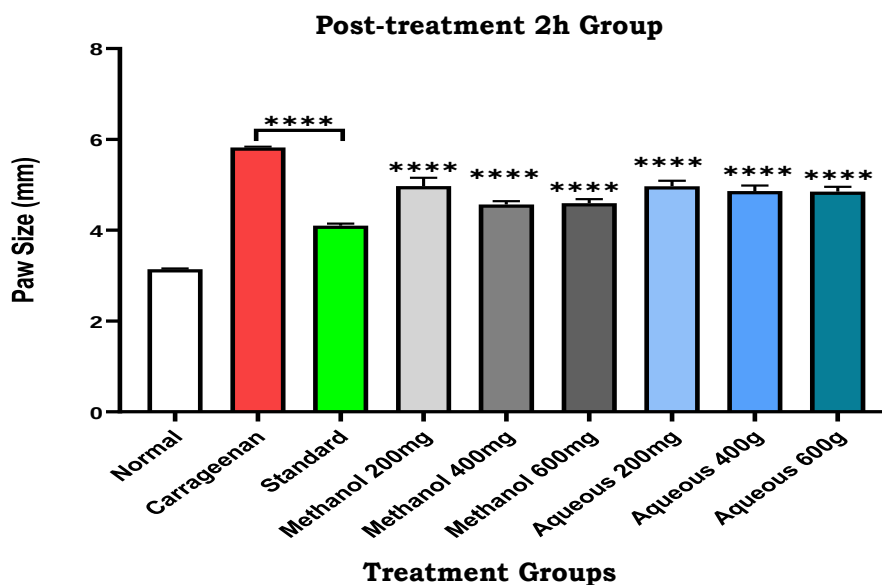


Figure 2. Effect of different treatment groups on carrageenan-induced paw edema in rats at 2 hour

Each bar is the mean of 5 rats while vertical lines on top of each bar indicate SEM.

**** $p < 0.0001$ when compared with Carrageenan group.

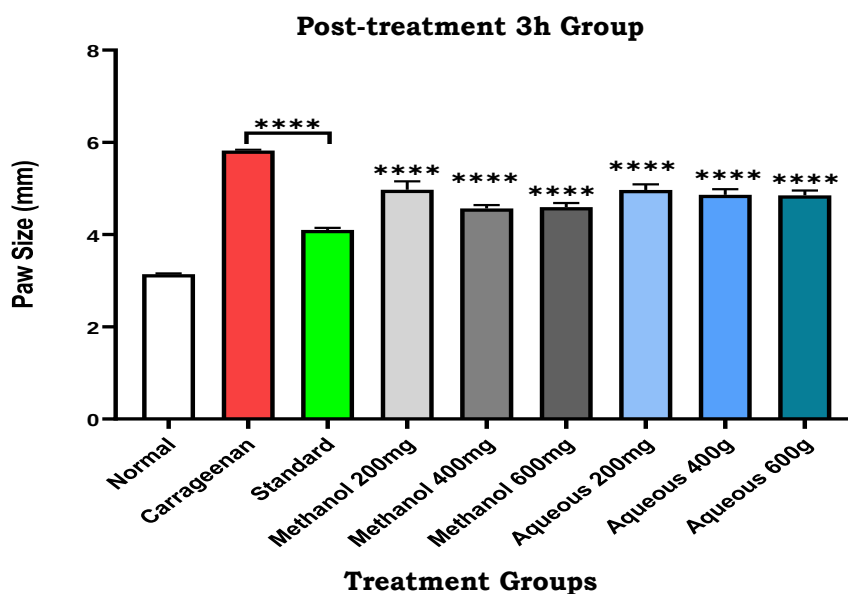


Figure 3. Effect of different treatment groups on carrageenan-induced paw edema in rats at 3 hours

Each bar is the mean of 5 rats while vertical lines on top of each bar indicate SEM.

****p < 0.0001 when compared with Carrageenan group.

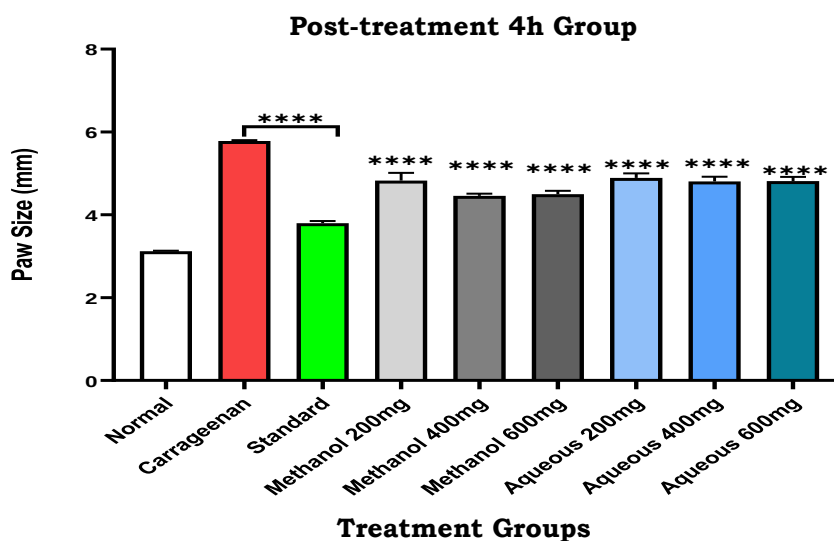


Figure 4. Effect of different treatment groups on carrageenan-induced paw edema in rats at 4 hours.

Each bar is the mean of 5 rats while vertical lines on top of each bar indicate SEM.

****p < 0.0001 when compared with Carrageenan group.

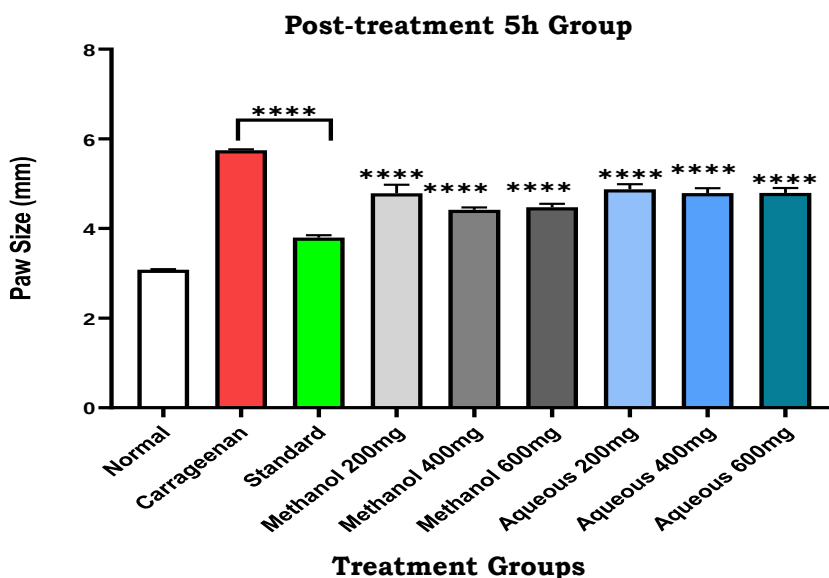


Figure 5. Effect of different treatment groups on carrageenan-induced paw edema in rats at 5 hours

Each bar is the mean of 5 rats while vertical lines on top of each bar indicate SEM.

**** $p < 0.0001$ when compared with Carrageenan group.

Anti-pyretic results

The anti-pyretic results of paracetamol 150mg/kg, methanol 200mg/kg, methanol 400mg/kg, methanol 600mg/kg, aqueous 200mg/kg, aqueous 400mg/kg, aqueous 600 mg/kg in yeast induced pyrexia model in rats are shown in table. Group 1 was considered as a control group and was treated with only normal saline 10ml/kg after rectal temperature is measured at different time intervals (0hr, 1hr, 2hr, 3hr, 4hr, 5hr). Group 2 was administered with yeast only and rats' temperatures were measured at different time intervals of (0hr, 1hr, 2hr, 3hr, 4hr and 5hr) and their temperature remained around 38.92°C at 5th hour. Group 3 was treated with Paracetamol standard 150mg/kg and the temperatures were measured at different hours (1, 2, 3, 4, 5), it decreased from 39.11°C to 37.60°C in 5 hours. Group 4 was treated with methanolic extract of dose 200mg/kg and their temperatures decreased from 39.05°C to 38.55°C. Group 5 was treated with methanolic extract of dose 400mg/kg and their temperatures decreased from 39.06°C to 38.45°C. Group 6 was treated with methanolic extract of dose 600mg/kg and their temperatures decreased from 39.03°C to 38.34°C. Group 7 was treated with aqueous extract of 200mg/kg and their temperatures decreased from 39.04°C to 38.86°C. Group 8 was treated with aqueous extract of 400mg/kg their temperatures decreased from 39.02°C to 38.74°C. Group 9 was treated with aqueous extract of 600mg/kg their temperatures decreased from 39.02°C to 38.79°C. Group 6 dose (600mg/kg) showed maximum response in decreasing the temperature.

Table 2. Anti-pyretic activity in rat model by yeast-induced pyrexia method

Treatment groups	Rectal temperature (°C) of rats after					
	0hr	1hr	2hr	3hr	4hr	5hr
Control Normal saline (10ml/kg)	37.08± 0.01	37.08± 0.01	37.08± 0.01	37.07± 0.01	37.06± 0.01	37.04± 0.01
Brewer yeast (15%)	39.14± 0.03	39.05± 0.05	39.01± 0.05	38.97± 0.04	38.94± 0.04	38.92± 0.04
Paracetamol (150mg/kg)	39.11± 0.04	38.56± 0.06	38.12± 0.06	37.83± 0.03	37.63± 0.04	37.60± 0.04
Methanol 200mg/kg	39.05± 0.02	38.82± 0.05	38.63± 0.02	38.56± 0.02	38.56± 0.02	38.55± 0.02
Methanol 400mg/kg	39.06± 0.04	38.75± 0.03	38.51± 0.02	38.45± 0.02	38.45± 0.01	38.45± 0.02
Methanol 600mg/kg	39.03± 0.02	38.62± 0.02	38.44± 0.02	38.35± 0.02	38.34± 0.02	38.34± 0.02
Aqueous 200mg/kg	39.04± 0.02	38.97± 0.01	38.92± 0.01	38.87± 0.02	38.86± 0.01	38.86± 0.02
Aqueous 400mg/kg	39.02± 0.02	38.89± 0.01	38.82± 0.01	38.76± 0.01	38.75± 0.01	38.74± 0.01
Aqueous 600mg/kg	39.02± 0.02	38.88± 0.01	38.81± 0.01	38.76± 0.01	38.79± 0.01	38.79± 0.01

Data are expressed as mean; n=5 with SEM

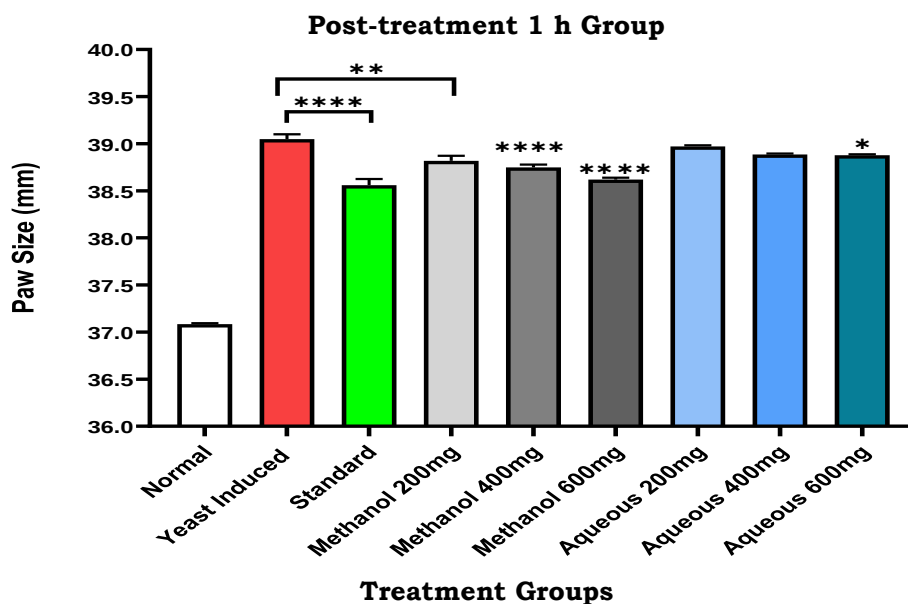


Figure 6. Effect of different treatment groups on Yeast-induced pyrexia in rats at 1 hour.

Each bar is the mean of 5 rats while vertical lines on top of each bar indicate SEM. **** $P < 0.0001$, *** p value is 0.0004, ** $p < 0.001$ and * $p < 0.01$ when compared with Yeast induced group.

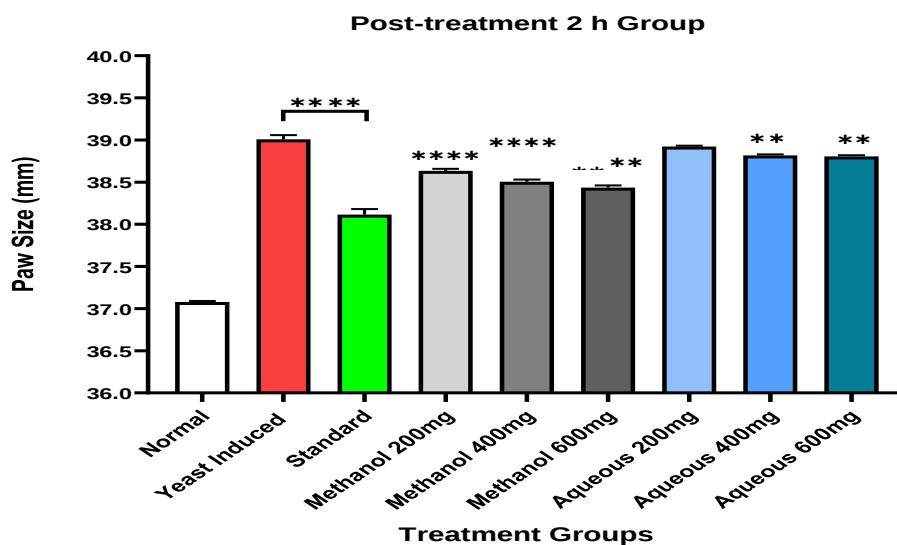


Figure 7. Effect of different treatment groups on Yeast-induced pyrexia in rats at 2 hours

Each bar is the mean of 5 rats while vertical lines on top of each bar indicate SEM. **** $P < 0.0001$ and *** p value are 0.0004, ** $p < 0.001$ and * $p < 0.01$ when compared with Yeast induced group.

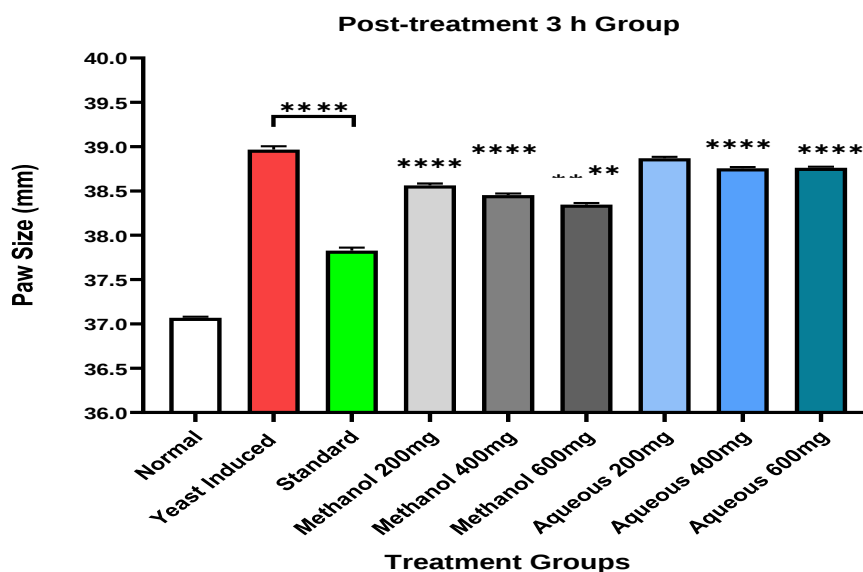


Figure 8. Effect of different treatment groups on Yeast-induced pyrexia in rats at 3 hours

Each bar is the mean of 5 rats while vertical lines on top of each bar indicate SEM. **** $P < 0.0001$ and p value are 0.0004, $p < 0.001$ and $p < 0.01$ when compared with Yeast induced group.

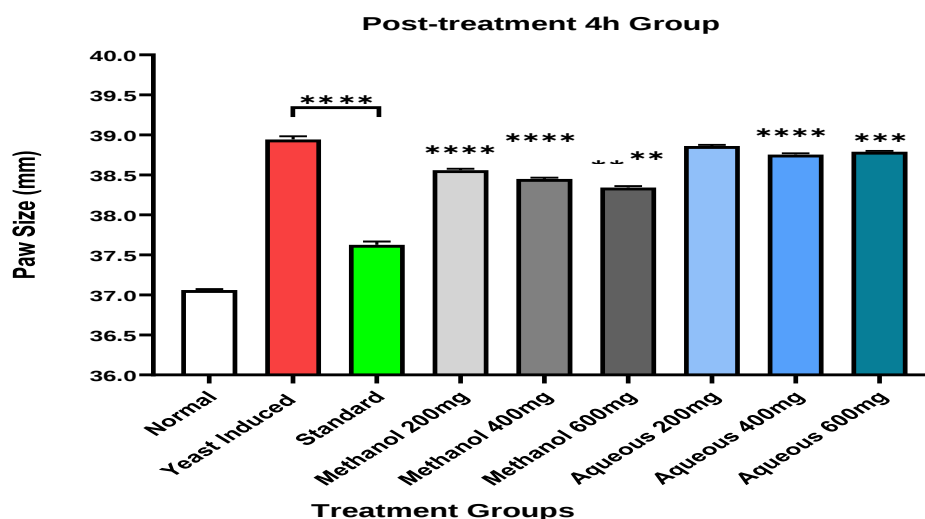


Figure 9. Effect of different treatment groups on Yeast-induced pyrexia in rats at 4 hours

Each bar is the mean of 5 rats while vertical lines on top of each bar indicate SEM.

**** $P < 0.0001$ and p value are 0.0004, $p < 0.001$ and $p < 0.01$ when compared with Yeast induced group

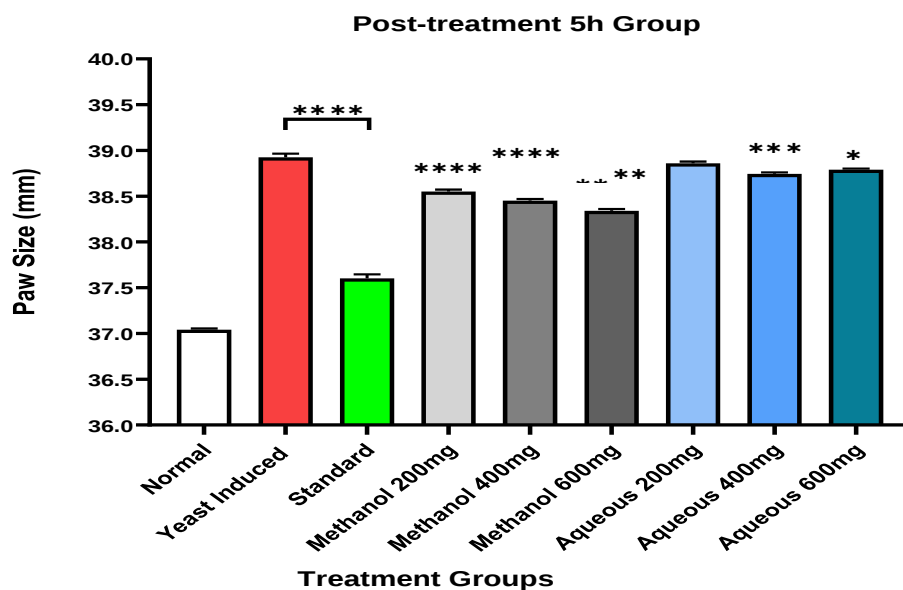


Figure 10. Effect of different treatment groups on Yeast-induced pyrexia in rats at 5 hours

Each bar is the mean of 5 rats while vertical lines on top of each bar indicate SEM.

**** $P < 0.0001$ and p values are 0.0004, $p < 0.001$ and $p < 0.01$ when compared with Yeast induced group.

Discussion

The rat paw edema induced by Carrageenan model was used to determine Anti-inflammatory activity of treatment groups in acute inflammatory disease (Khan et al., 2015). It is a basic and fundamental screening model that is mostly utilized for determining the anti-inflammatory activity²¹. Inflammation is the body's reaction to any foreign infectious agent or antigen. Inflammation is demonstrated by the occurrence of swelling in the tissues which can be both, acute or chronic (Iwakiri, 2007). Acute inflammation is an immediate reaction of the body to any injury, with the aim of mobilizing the inflammatory cells and chemicals including the neutrophils and macrophages (Kumar et al., 2004)²²⁻²³. Chronic inflammation generally lasts longer than acute and may extend from weeks to months. In chronic inflammation, not only the basic inflammatory mediators including the neutrophils and lymphocytes, but also the fibroblasts and collagen fibers are also mobilized. (Pahwa et al., 2021) Both acute and chronic inflammation help the body to fight against the foreign invasion causing edema that occurs in two phases, the initial phase is characterized by infiltration of histamines and serotonins, whereas the delayed phase involves the release of bradykinins and prostaglandins (Aoki & Narumiya, 2012)²⁴⁻²⁵. In the recent past many efforts have been made by the scientists to develop new therapies free of side effects which can be used to treat inflammation. Many therapies currently in use to treat inflammation including NSAIDs and corticosteroids which are limited by their side effects and adverse drug reactions.

Therefore, this study was designed to study the anti-inflammatory possibility of fruit extracts from *Ceiba speciosa* by using the carrageenan model. Carrageenan model had been used previously by many scientists for the purpose of evaluating the anti-inflammatory studies. Acute toxicity studies were also carried out to establish the safety profile of fruit extracts before administering the oral dose of extracts to the experimental animals²⁶. Two different extracts had been used including aqueous extract and methanolic extract. Animals had been divided into nine different groups including normal, disease control, standard and other treatment groups. Carrageenan had been used to induce inflammation in the paws of rats. Later animals were given oral dose of aqueous and methanolic extract. Paw sizes of experimental animals before and after administering the oral doses were noted down at different intervals to study the anti-inflammatory activity and body temperatures of experimental animals were recorded for the purpose of studying the anti-pyretic activity. Animals were divided into nine different groups. Group 1 had been administered the normal saline only which was called normal group, groups 2 was administered with carrageenan 1% called disease control, group 3 with standard that was diclofenac sodium called standard, group 4, 5 and 6 with Methanol extract of *Ceiba speciosa* fruit and group 7, 8 and 9 with Aqueous extract of fruit called treatment groups. Results elaborated that group 5 which was administered the methanolic extract of fruit with dose of 400 mg/kg has shown the highest anti-inflammatory activity with the paw size reduction of 0.86mm whereas the group 6 which was also administered the same extract but with the higher dose of 600 mg/kg has shown the paw size reduction of 0.76mm. When groups 7, 8 and 9 were administered the aqueous extract with doses of 200 mg/kg, 400 mg/kg and 600 mg/kg, comparison of these groups illustrated that group 8 and 9 amongst three groups have shown highest and almost same anti-inflammatory activity with the paw size reduction of 0.43mm²⁷. However, when the results of aqueous extracts were compared with the methanolic extracts it was observed that methanolic extracts possess better anti-inflammatory potential so this can be deduced that methanolic extract has shown better anti-inflammatory activity.

For the evaluation of anti-pyretic activity yeast was used to induce pyrexia in experimental animals (Khan et al., 2015)²⁸. This model has been used previously by many researchers for the assessment of anti-pyretic activity of various drugs. Increased amount of pro-inflammatory mediators are responsible for the increased synthesis of PGE₂ near the preoptic hypothalamus area, so hypothalamus is triggered to elevate the body temperature due to infection or tissue damage (Gao et al., 2013). Paracetamol has been used as a standard in this study. Mechanism of the paracetamol involves the blockade of chemical messengers in the brain (Graham & Scott, 2005)²⁹. For evaluation of this anti-pyretic activity body temperature was recorded at different intervals and later results were compiled to evaluate this activity. Similar to the anti-inflammatory study, animals had been divided into 9 groups. Amongst the groups which had been administered the methanolic extract at different doses of group 4 (200 mg/kg), group 5 (400mg/kg) and group 6 (600 mg/kg), group 6 has shown the highest activity as compared to other groups. Groups 7, 8 and 9 were those groups which had been administered the aqueous extract at different doses of group 7 (200mg/kg), Group 8 (400mg/kg) and group 9 (600mg/kg). However, when the results of aqueous extracts were compared with the methanolic extracts

it was observed that methanolic extracts possess better anti-pyretic potential³⁰⁻³². So it is concluded that the methanolic extracts of the fruit of *Chorisia speciosa* possess better anti-pyretic activity than the aqueous extracts.

Conclusion

The experimental results have clearly shown that the methanol extract of fruit from *Ceiba speciosa* has significant anti-inflammatory and antipyretic effects whereas the aqueous extract of fruit from *Ceiba speciosa* shows minimum anti-inflammatory and anti-pyretic effect.

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