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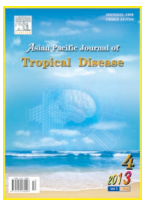

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In-vivo anti-inflammatory and anti-pyretic activities of *Manilkara zapota* leaves in albino Wistar rats

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PEER REVIEW

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Comments

This is a good study in which authors evaluated the anti-inflammatory and anti-pyretic activities of *M. zapota* leaves in carrageenan induced paw edema and yeast-induced pyrexia in rats respectively. The activity was assessed based on percent inhibition of paw edema, reduction of body temperature in experimental animal model. This study is also a novel work and strongly supports the use of *M. zapota* in the treatment of inflammatory disease and pyrexia.
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ABSTRACT

Objective: To screen ethanolic extracts of *Manilkara zapota* leaves (Family: Sapotaceae) and its different solvent soluble fractions for possible anti-inflammatory, anti-pyretic activities in experimental albino Wistar rats.

Methods: Anti-inflammatory activity was evaluated by carrageenan induced paw edema method; anti-pyretic potential was determined by yeast-induced pyrexia method in albino Wistar rats.

Results: In evaluation of anti-inflammatory activity the crude ethanolic (300 mg/kg) and ethyl acetate extract (300 mg/kg) showed significant inhibition of paw edema by 91.98% and 92.41% ($P<0.001$) respectively at 4th h compared to standard diclofenac (86.08% inhibition). In anti-pyretic study by yeast-induced pyrexia in albino Wistar rats, the ethanol extract (300 mg/kg) reduced temperature from 37.90 °C to 37.41 °C ($P<0.01$) and 37.07 °C ($P<0.001$) in 3rd and 4th h respectively. Similarly, both petroleum ether and ethyl acetate fractions exhibited significant anti-pyretic property ($P<0.001$). The maximum body temperature lowering effect (36.86 °C) was noticed by petroleum ether fraction.

Conclusions: The findings of the studies demonstrated both anti-inflammatory and anti-pyretic activities of the leaves of *Manilkara zapota* which could be the therapeutic option against inflammatory disease and pyrexia.

KEYWORDS

Manilkara zapota, Carrageenan, Paw edema, Yeast-induced pyresis

1. Introduction

Inflammation or phlogosis is a pathophysiological response of living tissue to injuries that leads to the local accumulation of plasmatic fluid and blood cells[1]. It is considered as a primary physiologic defense mechanism that helps body to protect itself against infection, burn, toxic chemicals, allergens or other noxious stimuli and usually activated in most disease condition. Although it is a defense mechanism, the complex events and mediators involved in the inflammatory reaction can induce, maintain or aggravate many diseases[1]. The critical role of inappropriate inflammation is becoming accepted in many diseases that affect man, including cardiovascular diseases, inflammatory and autoimmune disorders, neurodegenerative conditions, infection and cancer[2,3]. So, an uncontrolled and persistent

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inflammation may act as an etiologic factor for many of these chronic illnesses[4]. On the other hand pyrexia is a common medical sign characterized by an elevation of temperature above the normal range of 36.5-37.5 °C due to an increase in the body temperature regulatory set-point. This increase in set-point triggers increased muscle tone and shivering. Drugs that are currently used for the management of inflammation and pyrexia are non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids. All these drugs carry potential toxic effects. One study suggests that risk of gastrointestinal bleeding was significantly associated with acute use of NSAIDs like regular-dose aspirin, diclofenac, ketorolac, naproxen or nimesulide. Piroxicam increased the risk of bleeding in both acute and chronic therapy. On the contrary many medicines of plant origin had been used since ages without any adverse effects. It is therefore essential that sedulous efforts should be made to introduce new medicinal plants to develop more effective and cheaper drugs.

Manilkara zapota (Family: Sapotaceae) (*M. zapota*) is an important medicinal plant having various ethno pharmacological uses. It is commonly known as Sofeda or Sobeda in Bengali, Sapota or Chikku in Hindi, Simaiyiluppai in Tamil, Sapotasima in Telugu, Sapotille or Sapodilla in French and American bully in English. It is cultivated throughout Bangladesh and India, though it is native to Mexico and Central America[5]. The major constituents isolated from leaves of *M. zapota* are lupeol acetate, oleanolic acid, apigenin-7-O-α-L-rhamnoside, myricetin-3-O-α-L-rhamnoside and caffeic acid[6]. In traditional system of remedies the leaves of the plant are used to treat cough, cold and diarrhea[7]. The leaves of the plant also posses antioxidant activity[8,9]. The leaves also have antimicrobial property[10,11], analgesic potential, antihyperglycemic and hypocholesterolemic activity[6,12]. Bark is used as tonic and the decoction is given in diarrhea, dysentery and peludism[5,13]. The bark of the *M. zapota* is also traditionally used for the treatment of gastrointestinal disorder, fever, pain and also inflammatory condition[5].

Although *M. zapota* has various ethno pharmacological uses the plant yet have not been undergone any extensive chemical or pharmacological study. Recently anti-inflammatory activity of the bark of *M. zapota* has been reported by Hossain *et al*[5]. To the best of our knowledge, the anti-inflammatory and anti-pyretic activities of the leaf part of the plant have not been reported so far and no literature is currently available to substantiate these above properties. Therefore, the present study was designed to investigate the anti-inflammatory and anti-pyretic potential of the crude ethanolic extract and its fractions of the leaves of *M. zapota* in albino Wistar rats for the first time.

2. Materials and methods

2.1. Plant material

Fresh leaves of *M. zapota* used in this study were collected from Curzon Hall, Dhaka University campus, Bangladesh during the month of February 2012 at the flowering stage. The plant samples were identified and authenticated by experts in the Bangladesh National Herbarium Mirpur, Dhaka. A voucher specimen (accession No: DACB 37661) was deposited there for future reference. The leaves of *M. zapota* were freed from any of the foreign materials. The plant parts, after cutting into small pieces, were sun dried for several days. The plant materials were then oven dried for 24 h for better grinding. The dried cutting pieces were pulverized by a mechanical grinder and stored into an air-tight container.

2.2. Extraction of the plant material and sample preparation

About 1.0 kg of the powdered sample was taken in a clean, round bottomed flask (5 L) and soaked in 4 L of 95% ethanol. The container with its content was sealed by cotton plug and aluminum foil and kept for a period of 15 d accompanying occasional shaking and stirring. The whole mixture was then filtered through cotton followed by Whatman No.1 filter paper and the filtrate thus obtained was concentrated at 39 °C with a Heidolph rotary evaporator. The concentrated extract was then air dried to solid residue. The weight of the crude extract obtained from the *M. zapota* was 50 g. The crude ethanolic extract was partitioned successfully by three solvents of different polarity such as petroleum ether, carbon tetra chloride and ethyl acetate respectively by the modified kupchan partition method[14]. The extracts and standard drug diclofenac and paracetamol were suspended in normal saline using 1.0% Tween-80.

2.3. Chemicals and reagents

Diclofenac and paracetamol were collected from ACI pharmaceuticals and Beximco Pharmaceuticals Ltd. Dhaka, Bangladesh respectively. Carrageenan was purchased from Sigma-Aldrich, Germany. Yeast was obtained from Gonoshastho Pharmaceuticals Ltd. Dhaka, Bangladesh.

2.4. Experimental animal

Albino Wistar rats (*Rattus norvigicus*) of either sex weighing 120-150 g were used for the present study. They were purchased from the animal house of Jahangirnagar University, Bangladesh. They were maintained in the animal house of North South University, Bangladesh for

experimental purpose. The animals were maintained under controlled conditions of temperature (23±2)°C, humidity (50±5) % and 12 h light-dark cycles. All the animals were acclimatized for seven days before the study. The animals were randomized into experimental and control groups and housed individually in sanitized polypropylene cages containing sterile paddy husk as bedding. They had free access to standard pellets as basal diet and water *ad libitum*. Animals were habituated to laboratory conditions for 48 h prior to experimental protocol to minimize if any of non-specific stress. All experimental protocols were in compliance with Dhaka University Ethics Committee on Research in Animals as well as internationally accepted principles for laboratory animal use and care.

2.5. Phytochemical screening

The freshly prepared crude ethanolic extracts of leaves were qualitatively tested for the presence of alkaloids, phenols, tannins, reducing sugar, flavonoids, steroids, terpenoids and saponins by using standard phytochemical procedures[15,16].

2.6. Acute toxicity test

The acute toxicity of *M. zapota* ethanolic extract and different solvent soluble fractions was determined in rats according to the method of Hilaly[17]. Rats fasted for 16 h were randomly divided into groups of five rats per group. Graded doses of the extract (200, 400, 800, 1 600 and 3 200 mg/kg *p.o.*) were separately administered to the rats in each of the group by means of bulbed steel needle. All rats were then allowed free access to food and water and observed over a period of 48 h for signs of acute toxicity. The number of deaths within this period was recorded.

2.7. Anti-inflammatory activity study

In this experiment, carrageenan-induced rat hind paw edema was used as the animal model of acute inflammation according to the method of Winter[18]. Administration of carrageenan in the sub-plantar region of rat's hind paw leads to the formation of edema *in situ* due to localized inflammation. The animals were weighed and randomly divided into 6 groups of 6 rats in each. Group I (control) received 1% Tween 80 in normal saline (10 mL/kg). Group II (positive control) received 100 mg/kg body weight diclofenac sodium orally. Group III, IV, V and VI received ethanolic crude extract, petroleum ether fraction, ethyl acetate fraction and carbon tetrachloride fraction at the dose of 300 mg/kg body weight. After an hour of oral administration of test materials, 0.1 mL of 1% w/v suspension of carrageenan in normal saline was injected into the sub-plantar surface of the right hind paw of each rat of every group. The paw volume was measured by plethysmometer (Ugo Basile, 7140, Italy) at 1, 2, 3, 4 and 6 h after the carrageenan injection. Mean increase in paw volume were noted for the respective time intervals, thus edema volumes in control [(Ct-Co) control] and in groups treated with test materials [(Ct-Co) treated] were calculated. Percentage inhibition of paw edema was calculated by using the following formula:

% paw edema inhibition=[(Ct-Co) control-(Ct-Co) treated]/(Ct-Co) control ×100

Where, Co=paw volume at zero time (before carragennan injection), Ct=paw volumes at *t* time. (Ct-Co)=paw edema.

2.8. Anti-pyretic activity study

Anti-pyretic activity on albino rats was studied with fever induced by 15% brewer's yeast. Healthy Wistar strain albino rats weighing about 120-150 g were taken. They were fasted overnight with water *ad libitum* before inducing pyrexia and just before inducing pyrexia animals were allowed to quiet in the cage for some time and after that their basal rectal temperature were measured by using a clinical digital thermometer by insertion of thermometer to a depth of one inch into the rectum. After taking the temperature, pyrexia was induced by injecting subcutaneously 15% w/v suspension of brewer's yeast in distilled water at a dose of 10 mL/kg body weight in the back below the nape of the neck. The site of injection was massaged in order to spread the suspension beneath the skin and the rats were returned to their cage and allowed to feed. After 18 h of brewer's yeast injection the rise in rectal temperature was recorded. Only rats which were shown an increase in temperature of at least 0.6 °C were used for further experiment. The animals were divided into 5 groups, each group contains 6 animals. Group I (control) received 1% Tween 80 in normal saline (10 mL/kg). Group II (positive control) received 100 mg/kg body weight paracetamol orally. Group III, IV and V received ethanolic crude extract, petroleum ether fraction and ethyl acetate fraction respectively *p.o.* at the dose of 300 mg/kg body weight. After the drug was administered, the temperature of all the rats in each group was recorded at 1, 2, 3 and 4 h. The mean temperature was calculated for each group and compared with the value of standard drug paracetamol.

2.9. Statistical analysis

All values were expressed as the mean ±standard error of the mean (SEM) and the results were analyzed statistically by one way analysis of variance (ANOVA) followed by Dunnett's

t test by using SPSS Ver.16. $P<0.05$ was considered to be statistically significant.

3. Results

3.1. Phytochemical screening

In preliminary phytochemical screening, the ethanol extract of leaves of *M. zapota* demonstrated the presence of alkaloids, flavonoids, tannins, saponins and glycosides.

3.2. Acute toxicity test

In acute toxicity study, oral administration of graded doses (200, 400, 800, 1 600 and 3 200 mg/kg *p.o.*) of the ethanol extract of *M. zapota* to rats showed no significant changes in behavior, breathing, cutaneous effects, sensory nervous system responses or gastrointestinal effects during the observation period. No mortality or any toxic reaction was recorded in any group at 48 h after administration. *M. zapota* was safe up to a dose level of 3 200 mg/kg body weight.

3.3. Anti-inflammatory activity study

The effects of ethanolic crude extract and its different fractions of the leaves of *M. zapota* (300 mg/kg) in carrageenan induced paw edema in rats are shown in Table 1 and Figure 1. The crude ethanolic extract prevented the formation of edema induced by carrageenan and thus showed significant anti-inflammatory activity ($P<0.001$) and reduced the edema induced by carrageenan by 47.8%, 62.87%, 70.96%, 91.98% and 95.65% respectively after 1st, 2nd, 3rd, 4th and 6th h injection of noxious agent carrageenan. The rate of anti-inflammatory activity was increased with time and reached the peak level at the 6th hour of the study which is even better than that of standard drug diclofenac sodium at 100 mg/kg (% inhibition: 92.75%) at 6th h (Figure 1).

The petroleum ether fraction (300 mg/kg) of *M. zapota* also showed moderate anti-inflammatory activity having paw edema inhibition of 58.99% ($P<0.01$), 70.04% ($P<0.01$) and 86.96% ($P<0.001$) at 3rd, 4th and 6th h of the study respectively. While the ethyl acetate fraction (300 mg/kg) demonstrated significant anti-inflammatory activity ($P<0.001$) and inhibited edema by 74.73%, 74.75%, 83.41%, 92.41% and 92.27% after 1st, 2nd, 3rd, 4th and 6th h respectively. The interesting finding was that the anti-edematogenic effect of ethyl acetate fraction increased with the time up to 4th h and it showed most significant ($P<0.001$) anti-inflammatory effect which is comparable to that of standard diclofenac sodium. However the carbon-tetrachloride fraction (300 mg/kg) didn't show any paw edema inhibition up to 3rd h but revealed to a lesser extent of reduction in edema after 4th and 6th h compared to standard and other fractions.

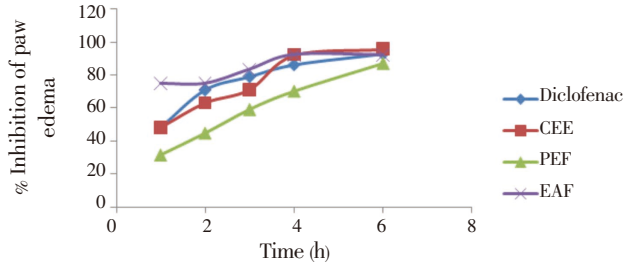

Figure 1. Percent inhibition of paw edema at different time intervals of different groups of rats receiving different extracts of leaves of *M. zapota*. CEE: Crude ethanol extract of leaves; PEF: Petroleum ether fraction; EAF: Ethyl acetate fraction.

3.4. Anti-pyretic activity study

The effect of ethanolic extract and two fractions of *M. zapota* on normal body temperature in rats are presented in Table 2. In this test, the ethanol extracts and its fractions of *M. zapota* at a dose of 300 mg/kg body weight caused significant lowering of the body temperature up to 4 h. The ethanol extract reduced temperature from 37.90 °C to 37.41 °C in 3rd h ($P<0.01$) and 37.07 °C ($P<0.001$) in 4th h. Similarly both the petroleum ether and ethyl acetate fractions exhibited significant anti-pyretic property ($P<0.001$) as

Table 1

Anti-inflammatory activity of different extracts of *M. zapota* leaves on carrageenan-induced edema paw volume in Wistar rats (mean±SEM, $n=6$).

Group	Treatment	Dose (mg/kg body weight)	Paw volume (mL) (mean±SEM)				
			1st h	2nd h	3rd h	4th h	6th h
I	Control	-----	1.12±0.05	1.16±0.05	1.19±0.05	1.23±0.06	1.17±0.06
II	Positive control	100	0.91±0.02**	0.84±0.04***	0.81±0.04***	0.79±0.04***	0.75±0.03***
III	CEE	300	0.91±0.02**	0.87±0.03**	0.85±0.04***	0.76±0.04***	0.74±0.03***
IV	PEF	300	0.99±0.03	0.97±0.06*	0.92±0.08**	0.89±0.06**	0.80±0.03***
V	EAF	300	0.66±0.03***	0.67±0.04***	0.64±0.03***	0.60±0.05***	0.60±0.05***
VI	CTF	300	1.15±0.07	1.33±0.04	1.38±0.04*	1.23±0.07	1.16±0.06

$^{***}P<0.001$, $^{**}P<0.01$, $^{*}P<0.05$ compared to control (one way ANOVA followed by Dunnett's *t* test)

Positive control: Diclofenac, CEE: Crude ethanol extract; PEF: Petroleum ether fraction; EAF: Ethyl acetate fraction; CTF: Carbon-tetra chloride fraction.

Table 2

Anti-pyretic effect of crude ethanolic extract, petroleum ether and ethyl acetate fractions of *M. zapota* leaves in Wistar rats (mean±SEM, n=6).

Group	Treatment	Dose (mg/kg body weight)	Initial rectal temperature before yeast injection (°C)	Rectal temperature after 18 h of yeast injection (°C)				
				0 h	1st h	2nd h	3rd h	4th h
I	Control	–	37.22±0.11	38.11±0.17	38.17±0.15	38.17±0.10	38.16±0.11	38.14±0.11
II	Positive control	100	36.98±0.16	38.09±0.18	37.68±0.07*	37.33±0.11***	37.15±0.03***	36.84±0.05***
III	CEE	300	36.98±0.10	37.90±0.13	37.87±0.15	37.72±0.16	37.41±0.19**	37.07±0.22***
IV	PEF	300	36.97±0.10	37.71±0.05	37.53±0.06**	37.38±0.07**	37.18±0.11***	36.86±0.10***
V	EAF	300	36.93±0.10	37.68±0.09	37.57±0.10**	37.24±0.14***	37.06±0.15***	36.89±0.13***

$^{***}P$<0.001, $^{**}P$<0.01, $^{*}P$< 0.05 compared to control (one way ANOVA followed by Dunnett's t test).
Positive control: Paracetamol; CEE: Crude ethanol extract; PEF: Petroleum ether fraction; EAF: Ethyl acetate fraction.

shown in Table 2. The maximum body temperature lowering effect (36.86 °C) was noticed by petroleum ether fraction. The anti-pyretic properties of the extracts were comparable to that of the standard drug paracetamol. It was evident from the study that the observed anti-pyretic effects of the extract were similar in both magnitude and time course.

4. Discussion

The carrageenan-induced rat paw edema model, frequently used to evaluate the anti-inflammatory activity of natural products is believed to be a biphasic process[19]. Carrageenan is the phlogistic agent of choice for testing anti-inflammatory drugs as it is not known to be antigenic and is devoid of apparent systemic effects. The initial phase, which occurs between 0-2 h after injection of the phlogistic agent, has been attributed to the release of histamine or serotonin (5-HT)[20-22], and the second phase of inflammatory reaction is associated with the production and release of prostaglandin like substances, bradykinin, protease and lysosome[20]. The major components of inflammation are the edema formation, leukocyte infiltration and granuloma formation[23]. Formation of edema in the paw is the result of a synergism between various inflammatory mediators that increase vascular permeability or the mediators that increase blood flow and development of edema induced by carrageenan is commonly correlated with the early exudative stage of inflammation[24,25]. It has been reported that the second phase of edema is sensitive to both clinically useful steroidal and non-steroidal anti-inflammatory agents[19,26]. Since the extract showed inhibition of paw edema at 1-2 h after carrageenan injection, the anti-inflammatory activity observed may be due to an inhibitory effect of the extract on the release of histamine and/or serotonin. The crude extract and different fractions of leaves of *M. zapota* also exhibited prominent inhibition of edema at 3rd to 4th h of the study in comparison to control. In the biphasic process of inflammation the action of the extract was more significant in the second phase and it can be explained by the reduction of prostaglandin synthesis via inhibiting enzymes of cyclooxygenase pathway[27]. This study has shown that the crude ethanolic extract of leaves of *M. zapota* and its petroleum ether and ethyl acetate fractions at a dose of 300 mg/kg body weight possess potential anti-inflammatory activity in carrageenan induced paw edema method. Among the all fractions, the ethyl acetate fraction showed most prominent (P<0.001) effect which is comparable to that of standard diclofenac sodium.

Yeast-induced pyrexia is called pathogenic fever and its etiology involves production of prostaglandins, which set the thermoregulatory centre at a lower temperature. The production of prostaglandins are mainly the most potent pyretic agent, phenyl glycidyl ether 2 appears to be a final pathway responsible for fever production induced by several pyrogens. The anti-pyretic activity is generally exhibited as one of the properties of non-steroidal anti-inflammatory drugs, resulting from their inhibitory effects on prostaglandin biosynthesis in the central nervous system[28]. A number of plant extracts modulate enzymes of cyclooxygenase pathway, which inhibit leukotriene and prostaglandin synthesis by inhibiting COX-1 and COX-2 pathways[29]. Therefore, in the present study, it is reasonable to assume that the inhibition of prostaglandin biosynthesis by various fractions of the *M. zapota* may be the reason for the anti-pyretic activity.

The presence of phytoconstituents like terpenoids, steroids, flavonoids, tannins, glycosides have been previously found to be responsible for anti-inflammatory and anti-pyretic activities in plant[30,31]. The presence of the above constituents like flavonoids, saponins, tannins, glycosides shown by the phytochemical screening in crude extract of *M. zapota* leaves may be responsible for this observed activity. The observed anti-inflammatory and anti-pyretic activities of the leaves of the plant could be attributed to some active constituents like lupeol acetate, oleanolic acid, apigenin-7-O-α-L-rhamnoside and myricetin-3-O-α-L-rhamnoside isolated from *M. zapota* leaves which were previously reported by Shazly[6].

In conclusion, for the first time we have reported the anti-inflammatory and anti-pyretic properties of the *M. zapota* leaves. The overall results of the present study indicate that ethanolic crude extract and its petroleum ether and ethyl

acetate fractions showed prominent anti-inflammatory activity. Among these, the anti-edematogenic effect of ethyl acetate fraction is most significant ($P<0.001$) at 4th h of the study. In anti-pyretic study, almost all the fractions demonstrated significant anti-pyretic effect in albino Wistar rats. Among these, maximum body temperature lowering effect (36.86 °C) was noticed by petroleum ether fraction at 4th h. The observed results indicate potent anti-inflammatory and anti-pyretic potentials of the leaves of *M. zapota* which deserves further investigation to isolate the bioactive constituents. Again, no mortality was recorded in the acute toxicity test justifying the safe and beneficial uses of the plant. Therefore, our findings provide scientific supports for the use of *M. zapota* in the treatment of inflammatory diseases and pyrexia in ethno medicine.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

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Comments

Background

Inflammation is a reaction to infection, irritation or foreign substance. It is part of the host defense mechanisms. But an uncontrolled and persistent inflammation may act as an etiologic factor for many chronic illnesses. Pyrexia is a common medical sign characterized by an elevation of body temperature above normal range. Drugs presently used against these disorders carry potential side effects. Therefore there is a need of new, potent and safe anti-inflammatory and anti-pyretic agents all over the world.

Research frontiers

The present research work depicts anti-inflammatory activity of ethanolic extract and its fractions of *M. zapota* leaves in carrageenan induced paw edema method and anti-pyretic potential of the same extracts in yeast-induced pyrexia method. Inhibition of paw edema volume and reduction of body temperature are considered as indicators of the above activities.

Related reports

In this study, the anti-inflammatory and anti-pyretic properties of the *M. zapota* leaves have been demonstrated. Similar study was conducted to report the analgesic potential of the plant by Shivhare *et al*. (2011).

Innovations & breakthroughs

M. zapota has been used by many rural people traditionally in the treatment of diseases like gastrointestinal disorder, inflammation, fever and pain. But there is no extensive pharmacological study to validate these uses. In the present study, authors have demonstrated the anti-inflammatory and anti-pyretic activities of *M. zapota* in experimental rat models.

Applications

From the literature survey and acute toxicity study conducted in this research work, it has been found that *M. zapota* is safe for use. It is effective against inflammation and pyresis as well. This scientific study supports and suggests the use of this plant as an alternative to commonly used synthetic drug having various toxic effects.

Peer review

This is a good study in which authors evaluated the anti-inflammatory and anti-pyretic activities of *M. zapota* leaves in carrageenan induced paw edema and yeast-induced pyrexia method in rats respectively. The activity was assessed based on percent inhibition of paw edema, reduction of body temperature in experimental animal model. This study is also a novel work and strongly supports the use of *M. zapota* in the treatment of inflammatory disease and pyrexia.

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