Original Research Article

An Assessment of Analgesic and anti-inflammatory Activity of *Manilkara zapota* on Rat Model

Abstract:

The word "herbal medicine" refers to the practice of using medicinal plants for the purpose of either preventing or treating sickness. This definition encompasses a broad range of practises, from the common and widespread usage of traditional medicines in every culture to the standardised and tritated extracts of herbs. In this study, an extract of *Manilkara zapota* was administered to rats in order to investigate the analgesic and anti-inflammatory properties of this plant. The carrageenan-induced acute inflammation technique was used to test the anti-inflammatory activity, and the acetic acid writhing and tail flick method was used to evaluate the analgesic efficacy. When it came to the anti-inflammatory action, the 1000 mg/kg dosage extract was the only one that exhibited a highly significant ($p< 0.05$) result, whereas the other doses didn't show any statistically significant findings at all. None of the groups showed statistically significant results in this test using the acetic acid writhing technique. The tail flick technique showed that a dose of 1000 mg/kg was statistically significant ($p <0.05$) at both the 3 and 4 h time periods. This was determined using the tail flick test. It took just 4 hours after the 750 mg/kg dosage for the results to reach statistical significance ($p < 0.05$).

According to these findings, *M. zapota* exhibits both analgesic and anti-inflammatory efficacy when tested on rat models.

Keywords: *Manilkara zapota*, Analgesic, Anti-inflammaoty, Aspirin, Herbal medicine, Phytotherapy.

Introduction

Currently, the International Association for the Study of Pain (IASP) defines pain as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage." [1]. Pain is an unpleasant sensation that is associated with sickness in humans. Acute and chronic pain, peripheral and central pain, nociceptive pain, and neuropathic pain all have inflammation and the inflammatory response as their root causes. White blood cells and the chemicals they release during inflammation work together to rid the body of invading germs and viruses. When there are no outside intruders present, the body's immune system may nonetheless trigger inflammation, as shown in conditions like arthritis [2]. Analgesics and anti-inflammatory pharmaceuticals include common over-the-counter medications like acetaminophen, diclofenac, ketorolac, opioids, etc. Aspirin, codeine, and morphine are the three mainstays of a standard analgesic regimen [3]. Common anti-inflammatory drugs include aspirin, ibuprofen, naproxen, and indomethacin. Inhibiting the enzyme cyclooxygenase (COX) that produces prostaglandins (PGs) is how NSAIDs work to alleviate pain and inflammation [4]. Unfortunately, these drugs also come with a host of potentially life-threatening adverse effects [5-7], including those affecting the digestive tract, the heart, the kidneys, the brain, and the neurological system. At least 100,000 people per year die from these poisons, and adverse reactions to synthetic medications account for 8% of all hospital admissions in the United States [8]. These synthetic drugs have serious adverse effects and may be costly, so the patient may have trouble paying for the whole course of therapy. Therefore, there is a need for highly effective analgesics and NSAIDs with minimal adverse effects.

The use of plants for therapeutic purposes is as ancient as humanity. Documents, historical structures, and even the original plant remedies all attest to the long history of humankind's pursuit of medicinal substances in the natural world [9]. Phytotherapy refers to the scientific study of plants with medicinal characteristics, whereas herbalism refers to their practical use. For thousands of years, people have turned to plants as a source of medicine due to the wide variety of substances found in them. A wide variety of chemically active compounds, including phenols, glycosides, alkaloids, saponins, terpenoids, tannins, polysaccharides, flavonoids, plant lipids, resins, and essential oils, may be found in plants [10, 11]. Increasing or decreasing the concentration of the plant's chemical components by genetic modification may again give the

desired medicinal effect. The manufacture of secondary metabolites like alkaloids may be boosted by using reverse genetics [12]. Traditional remedies for pain and inflammation include several herbs, including *Nigella sativa, Eucalyptus oil, Persea americana Mill,* and *Portulaca oleracea* L [13–16].

Sapodilla sapote, chicozapote, chicoo, chicle, naseberry, and nispero are just a few of the names for the *Manilkara zapota* tree, a long-lived evergreen that is native to the southwestern United States, southern Mexico, Central America, and the Caribbean. It is a member of the Sapotaceae family. The plant has been shown to have anti-inflammatory, anti-cancer, anti-tumour, and anti-arthritic properties [17–23]. Analysis of the phytochemicals in the leaves of M. zapota from India showed that they had alkaloids, flavonoids, tannins, phlobatannins, triterpenes, saponins, and cardiac glycosides. Methanolic extracts of leaves, in particular, were shown to have a high total phenolic content (194.06 1.21 mg/g) and total flavonoid content (35.55 0.21 mg/g) [24, 25]. Flavonoids have been shown to have an antinociceptive effect [26, 27], with much of the research focusing on how they inhibit prostaglandin synthesis. Flavonoids, tannins, saponins, and phenolic components were found in the crude ethanol extract of M. zapota leaves, which may account for the plant's demonstrated peripheral antinociceptive effect. A high concentration of flavonoids (169.37 mg quercetin equivalent per g of dry extract) suggests that the methanolic extract of M. zapota has anti-inflammatory properties. Bioflavonoids, also known as flavonoids, are a class of naturally occurring chemicals found in many types of plants. Both in vitro and in vivo studies have shown that these substances have anti-inflammatory effects [28].

Therefore, considering the exploratory character of the prior work, it is worthwhile to further investigate the analgesic and anti-inflammatory activities of M. zapota leaves. This research set out to test the efficacy of *M. zapota* leaf chloroform and metahnolic extracts as analgesics and anti-inflammatory treatments in animal models.

Materials and Methods

Drugs, Chemicals and Instruments

Sigma Aldrich (Germany) supplied the alloxan, carrageenan, acetic acid, and ethanol. Healthcare Pharmaceutical Limited (UK) provided the ibuprofen and aspirin as free samples. The anti-

inflammatory and analgesic effects were measured using a plethysmometer and an analgesia metre, respectively.

Plant Collection and Extract Preparation.

The medicinal plant garden at the University of Dhaka's Faculty of Pharmacy provided the source for the Manilkara zapota leaf, which was subsequently authenticated and taxonomically identified. The plant specimens were stored in accordance with the regulations of the Bangladesh National Herbarium. For future reference, the 7–10-dday shade-dried and then roughly pulverised leaf was given the accession number 47380 by the herbarium authorities on 11-2-2019. Shaken violently for the whole of the 96 hours, the powdered leaves were soaked in 70% ethanol. When the extract was done soaking, it was filtered, and the resulting liquid was saved. The concentrated extract was then filtered using a rotary evaporator. At last, the concentrated extract was dried and put away for later use.

Experimental Animal Handling

Male Wistar rats weighing between 125 and 200 g were obtained from the Jahangir nogor University Zoology Department in Bangladesh and housed at the University of Dhaka's Institute of Nutrition and Food Science on a 12:12 light:dark cycle with a constant temperature of 25 degrees Celsius. Before beginning the experiment, the rats were maintained there to acclimatise; therefore, standard pellet food and fresh water were supplied on a daily basis. In accordance with the recommendations of the Institutional Animal Ethics Committee (IEAC), all rat experiments were conducted. The Swiss Academy of Medical Sciences (SAMS) and the Swiss Academy of Sciences (SCNAT) provided rules for how animals should be cared for and used in scientific research.

Experimental Guidelines: All studies were conducted in conformity with the 2013 Declaration of Helsinki's ethical guidelines [29]. **Experimental Design**: Individual rats were weighed to determine their body weight, and then the animals were split into groups (Table 1) with an equal distribution of rodents according to their body weight and five rats in each group.

Evaluation of Anti-Inflammatory Activity:

Carrageenan was used to induce inflammation in the rats to examine the anti-inflammatory activity of reference drug and the extract of *Manilkara zapota.*

Table 1: Group specification for anti-inflammatory activity

Group Number	Group Specification	Treatment species	Dose Treatment species (mg/kg)	Abbreviation of Groups
1	Carrageenan Control	N/A	N/A	Car
2	Carrageenan + Ibuprofen	Ibuprofen	10	$Car+Ib_{10}$
3	Carrageenan + *Manilkara zapota*	*Manilkara zapota*	500	$Car+MZ_{500}$
4	Carrageenan + *Manilkara zapota*	*Manilkara zapota*	750	$Car+MZ_{750}$
5	Carrageenan + *Manilkara zapota*	*Manilkara zapota*	1000	$Car+MZ_{1000}$

Carrageenan-Induced Acute Inflammatory Model:

Carrageenan-induced rodent paw edoema testing is the standard method for determining an anti-inflammatory agent's efficacy. The anti-inflammatory test was conducted using a plethysmometer, a specialised kind of device. The next step was to measure the size of each rodent's paw. Subplanar tissue of the left rear paw rat was injected with 1% of the newly manufactured carrageenan solution at a dosage of 0.1 mL per 100 g body weight to stimulate edoema. After that, an hour was allotted. Then, the test medication and extracts were administered to rats in a variety of dosages. The volume of the paw was measured using a plethysmometer between 0 and 6 hours following Carrageenan infusion. The subsequent formula [30, 31] was then used to calculate the rate of edoema obstruction.

$$\text{Percentage Inhibition} = \frac{V_{Pc} - V_t}{V_{pc}} \times 100$$

Here,

VPC = volume of animals' paw in Positive Control rat

V_0=volume of animals' paw in Treatment Group

Evaluation of analgesics activity:

The rodent is stimulated with pain through the acetic acid-induced writhing test and tail-flick method.

Table 2: Group specification for analgesic activity by acetic acid writhing method

Group Number	Group Specification	Treatment species	Dose Treatment species (mg/kg)	Abbreviation of Groups
1	Acetic Acid Control	Physiological Saline	10ml/kg	Ace
2	Aspirin +Acetic Acid	Aspirin	100	As_{100}+Acetic Acid
3	*Manilkara zapota*+ *Acetic acid*	*Manilkara zapota*	500	MZ_{500}+Acetic Acid
4	*Manilkara zapota*+ *Acetic acid*	*Manilkara zapota*	750	MZ_{750}+Acetic Acid
5	*Manilkara zapota*+ *Acetic acid*	*Manilkara zapota*	1000	MZ_{1000}+Acetic Acid

Acetic acid-induced writhing test

The acetic acid-induced writhing technique was used to test for peripheral analgesic activity. In the 30 minutes before the intraperitoneal injection of acetic acid, several test samples were given. The rats were given an intraperitoneal injection of 0.9% acetic acid (10 ml/kg) while being exposed to unpleasant stimuli. Over the course of 20 minutes beginning immediately after acetic acid injection, the number of writhes (muscle contraction ions) was counted. The percentage of writhing inhibition was determined by counting the number of times an animal contracted its abdominal muscles, drew its hind limbs towards its abdominal walls, stretched its hind limbs, and periodically arched its back for twenty minutes. Equation [32] was used to figure out what percentage of writhes represents analgesic action.

$$\left\{\frac{A.\ Control\ \ mean - Treatment\ \ mean}{A\ Control\ \ mean}\right\} \times 100$$

Where $T\ Control$ = the mean number of the writhing of each test group

$A\ Control$ = The mean number of the writhing of acetic acid control group

The analgesic activity of the extract is then also assessed via the "Tail Flick Method" on the same experiment rat model after giving a break for seven days. The effect of injected acetic was terminated by this time.

Table 3: Group specification for analgesic activity by tail flick method

Group Number	Group Specification	Treatment species	Dose Treatment species (mg/kg)	Abbreviation of Groups
1	Tail Flick Stress (control)	Physiological Saline	10ml/kg	TFS
2	Aspirin + Tail Flick Stress	Aspirin	100	As_{100}+TFS
5	Manilkara zapota + Tail Flick Stress	Manilkara zapota	500	GP_{500}+TFS
6	Manilkara zapota + Tail Flick Stress	Manilkara zapota	750	GP_{750}+TFS
7	Manilkara zapota + Tail Flick Stress	Manilkara zapota	1000	GP_{1000}+TFS

Tail flick method

The behavioural reaction of animals to painful stimuli is evaluated using a nociceptive test called the tail-flick experiment, which was first outlined by Love and Smith in 1941 [33]. A tail-flick analgesia metre (UGO BASILE®, Germany) programmed with radiant heat was used to determine the lag time between exposure to the stimuli and the onset of the avoidance reaction. A constant current of 4 Amps was supplied through the exposed nichrome to get it up to the proper temperature, and the heat controls helped with this. By applying radiant heat to the rats' tails in the centre, we may make them feel pain. For both untreated and treated rats, the time

required to exhibit a tail-flick reflex was recorded. After administering test compounds to the animals, the experiments were conducted at 0, 15, 30, 45, and 60 minutes.

Statistical analysis:

All of our results (raw data) were recorded and analysed on a spread sheet in MS Excel, and they fall into multiple categories covering a wide range of study factors. Descriptive statistics were applied to the data, and the results are shown as a mean SD. The statistical significance of the observed variation between groups was assessed using the "One Way Anova Test" function in SPSS 1600. So long as the 'p' value is less than 0.05 ($p < 0.5$), we classify the occurrences as statistically significant.

Results:

The data was expressed as time and percent inhibition. The higher dose extract only showed highly significant ($p<0.05$) result at the 4 hours and the other doses didn't show statistically significant results.

Table 4: Anti-inflammatory activity of *M. zapota* extract and Ibuprofen through paw edema test in a rat model (* presents the level of significance of result).

Group	**Time µL**				
	0 Minute(Just before carrageenan injection)	1 hour (just before treatment	2 Hours	3 Hours	4 Hours
Car	118.26±3.94	113.34±5.86	140.22±5.59	146.37±6.10	152.49±5.45
Car+Ib$_{10}$	115.20±4.04	133.21±6.40	127.41±5.40	121.29±4.93	117.46±4.10
Car+MZ$_{500}$	119.29±5.43	135.20±5.99	139.37±6.61 0.61%	135.73±4.19 7.27%	131.97±5.21 13.46%
Car+MZ$_{750}$	114.39±3.44	133.20±4.19	136.96±6.22 2.32%	132.14±5.96 9.72%	128.36±4.44 15.82%

Car+MZ$_{1000}$	116.30±4.16	134.69±5.71	134.08±5.10 4.38%	128.31±5.19 12.33%	121.90±6.14 20.06%

Analgesic Activity of *Manilkara zapota:*

Writhing test: The result of acetic acid writhing test is shown below in table 2. No groups showed statistically significant results in this test.

Table 5: Analgesic effect of different doses of *Manilkara zapota* and Aspirin by acetic acid writhing test (*presents the level of significance of result).

Group specification	Dose	Number of writhing	% Inhibition
Ace		93.71±6.42	
As$_{100}$+Acetic Acid	100	70.42±5.84	
MZ$_{500}$+Acetic Acid	500	92.46±7.14	1.33%
MZ$_{750}$+Acetic Acid	750	92.21±6.19	1.6%
MZ$_{1000}$+Acetic Acid	1000	89.19±5.39	4.82%

Tail flick test (TFS): Table 3 shows the outcomes of the exam. Treatment with MZ improved the pain threshold in a dose-dependent way in both non-diabetic and diabetic rats; however, the impact was less than that of the gold standard medication, aspirin. Only 4 hours after the 750 mg/kg dosage showed statistically significant results ($p< 0.05$). Statistical significance ($p< 0.05$) was seen at both the 3- and 4-hour time points for a dosage of 1000 mg/kg.

Table 6: Analgesic activity of *Manilkara zapota* and Aspirin by the tail-flick test method.

Group	Group	Basal	Reaction time in second

No	Specification	Reaction	After 30 minutes	After 1 Hour	After 2 Hour	After 4 Hour
1	TFS	3.93±0.86	4.11±0.93	4.24±1.01	4.45±0.93	4.76±0.80
2	As_{100}+TFS	3.44±0.77	3.50±0.84	3.73±0.89	3.89±0.69	4.10±0.96
3	MZ_{500}+TFS	3.77±0.81	3.96±0.73	4.14±0.84	4.02±0.91	4.19±0.86
4	MZ_{750}+TFS	3.99±0.94	4.21±0.91	4.49±0.81	4.87±0.95	5.53±1.01
5	MZ_{1000}+TFS	3.92±0.92	4.47±0.87	4.99±0.94	5.86±0.84	6.24±0.91

Discussion:

The healing potential of plants has been known for a long time. Traditional herbal therapy has been used by indigenous peoples all over the globe for hundreds of years to cure a wide variety of illnesses. In this research, we looked at the effectiveness of *Manilkara zopata* leaves as an analgesic and as an anti-inflammatory. At a time interval of 4 hours, the effects of a dosage of 1000mg/kg on anti-inflammatory activity were statistically significant ($p<.0.05$). However, no statistically significant differences were seen with any other dosage. High levels of flavonoids [34, 35] are responsible for their potent anti-inflammatory actions. Two investigations using *Manilkara zapota* reported similar outcomes [19, 36]. The number of writhing rats in the acetic acid writhing technique dropped across all tested dosages, although this trend was not statistically significant relative to the positive control groups. In the tail flick test, 1000 mg/kg was shown to be statistically significant ($p< 0.05$) at both the 3 and 4 h time periods. Statistical significance ($p < 0.05$) was seen only 4 hours after the 750 mg/kg dose. Previous investigations [37, 38] indicate that the high alkaloid and flavonoid content is responsible for the pain-relieving effects. Further research revealed the same thing about *Manilkara zapota* [39, 40].

More research is needed to pinpoint the specific molecule responsible for the analgesic and anti-inflammatory effects.

Conclusion:

This study demonstrated, using a rat model and varying doses of ethanolic extract and reference medications, that the plant had anti-inflammatory and analgesic properties. More study is

required to identify the component of the extract responsible for the intended effect. The next step in the research process is to isolate the active compounds.

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