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Pharmacological Investigation of *Manilkara zapota* Seeds Extract for the Anti-ulcer Activity on Rats

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Abstract: The current investigation demonstrated the anti-ulcer activity of the methanolic and ethyl acetate extracts of *Manilkara zapota* wild seeds in an animal model. *Manilkara zapota*, commonly referred to as chicozapote, is an evergreen tree that is indigenous to Belize, Guatemala, and the southern parts of Mexico. Historically, different plant parts have been employed for different medical purposes. An ethanol-induced assay was used to assess the anti-ulcer efficacy. An imbalance between mucosal defense factors (acid and pepsin) and harmful factors (bicarbonate, mucin, prostaglandin, nitric oxide, and other peptides and growth factors) is the cause of gastric ulcer disease. Oral therapy Omeprazole was utilized as the reference standard while testing the anti-ulcer properties of ethyl acetate and methanolic extracts of *Manilkara zapota* at doses of 100 and 200 mg/kg against an ethanol-induced model in rats (Wistar strains). When compared to methanolic extracts, ethanol acetate exhibited a higher percentage of inhibition. A comparison was made between the ulcer control group, the extract-treated group, and the normal control group. The study's animal model, the ethanol-induced model, showed a noteworthy reduction in the formation of ulcers. In comparison to the control group, both doses in the study demonstrated a significant reduction in ulcer activity as measured by the ulcer index, stomach volume, free acidity, and total acidity. The preventive effect of *Manilkara zapota* was demonstrated by the considerable reduction in lesion and bleeding intensity following pretreatment with the extract. The primary components of *Manilkara zapota* wild seeds are flavonoids and tannins, which may be the cause of their ulcer-protective and anti-ulcer properties.

Keywords: Peptic ulcer, *Manilkara zapota*, Wistar rat, Ethanol-induced model, Omeprazole

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Introduction

The plant *Manilkara zapota*, also referred to as sapodilla (Chikoo), possesses a variety of pharmacological characteristics. Its potential in several therapeutic domains, including anti-depressant action, analgesic effects, and wound-healing properties has been demonstrated in earlier investigations (Alsarei *et al*., 2023). More research on the anti-ulcer properties of *Manilkara zapota* seed extract is still desired. Examining the plant's effectiveness in treating ulcers could yield important insights into its medicinal qualities, given its proven pharmacological actions in other fields. The purpose of this study was to assess the anti-ulcer activity of an extract from *Manilkara zapota* seeds in an animal model, with a particular emphasis on how it affects rats' ability to repair ulcers. By building upon the existing knowledge regarding the pharmacological effects of *Manilkara zapota*, this research contribute to the understanding of its therapeutic potential in the context of ulcer treatment. The investigation involved assessing the extract's impact on ulcer healing, potentially offering a natural and effective alternative for managing ulcers (Young *et al*., 2008). As high as 80% of the world population depends on plant-derived medicines for the first line of primary health care, reinforcing the theory that plant extracts can be good sources of new drugs. Ethiopia is a country characterized by a wide range of climatic and ecological conditions possessing enormous diversity of flora and fauna, including a wide range of potentially useful medicinal plants (Rahman *et al*., 2019). In the Indian pharmaceutical industry, anti-ulcer and antacid drugs share 6.2 billion rupees and occupy 4.3% of the market share (Shu *et al*., 2016).

An approximate of 15,000 fatalities are attributed to peptic ulcers annually. Peptic ulcer bleeding and perforation were estimated to occur annually in 19.5–57 and 3.8–14 cases per 100,000 people, respectively. According to Dongo *et al.* (2017) average long-term recurrence of perforation was 12.2%, while the average 7-day recurrence of hemorrhage was 13.9%. Conventional medicines used in PUD therapy today include proton pump inhibitors (PPIs), H2 receptor antagonists, antacids, muscarinic receptor (M1) antagonist pirenzepine, anti-bacterial agents, and its analog, misoprostol (Harman *et al*., 2001)Antiulcer medications are assessed using a variety of models. These models included the indomethacin-induced stomach ulcer model, the pylorus-ligated-induced peptic ulcer model, and the alcohol-induced gastric ulcer model. The pyloric end of the stomach is ligated in pylorus-ligated-induced peptic ulcers, which result in a buildup of gastric acid in the stomach and make it crucial to assess the acidic content of the stomach (Lakshmi *et al*., 2010). Since mucosal prostaglandin production and stomach acid secretion are key components of the underlying pathophysiology, ethanol-induced ulcers, NSAIDs, and are significant when evaluating the potential utility of anti-secretory and cytoprotective medicines (Ulucan *et al*., 2020).

The current investigation demonstrated the anti-ulcer activity of the methanolic and ethyl acetate extracts of *Manilkara zapota* wild seeds in an animal model.

Materials and Methods

Plant material: *Manilkara zapota* fresh plant seeds were collected in Krushnoor. Dr. S. S. Bodke, Botany Department at Yashwant Mahavidyalaya in Nanded, Maharashtra, India, performed the authentication. Verified to be a member of the Sapotaceous plant family, the voucher specimen has been placed at the Herbarium under the accession number (H-5 NPC/M.Pharm/ Herbarium/2021/12/21). The established procedure for the collecting, processing, identification, authentication, and storage of plant material has been adhered to.

Preparation of extract: A mechanical grinder was used to reduce the dried material to small particles, or powder. Sieve number 14 was used to pass the powder through. A Soxhlet apparatus was used to extract ethyl acetate and methanol using the continuous hot extraction method after the

250 g of dried powdered seeds of *Manikara zapota* were first defatted with petroleum ether at 60–80 °C. After being standardized, the extracted materials were used to assess their anti-oxidant and anti-ulcer properties *in vitro* and *in vivo*, respectively.

Animal: In the current investigation, 180–230 g Wistar rats of both sexes were employed. The Ethical Committee accepted the use of the animal house (Registration No. 1613/PO/Re/S/12/ CPCSEA) of Nanded Pharmacy College, Nanded, to manage and supervise animal studies (CPCSEA procedure). The animals were kept in this facility under conventional laboratory conditions. The animals were kept in 12 h light/dark cycles at a temperature controlled at 22 ± 2 °C. They were fed commercial pellet food and given access to water. Before the experiment started, every animal was given at least one week to get used to the lab setting. The study's experimental protocol was adhered to in compliance with the Institutional Animal Ethics Committee's guidelines.

Drugs and Chemicals: Omeprazole (OMEZ-20, Dr. Reddy Pharmaceutical India) was used as reference standards for anti-ulcer activity.

Acute oral toxicity test: Both ethyl acetate and methanolic extract were evaluated based on internationally accepted protocol drawn by OECD guideline-425 (OECD TN.425, 2008).

Experimental Protocol: On the day of the experiment, randomly 48 h fasted animals were given study medicines, standard drugs, and vehicles. One approach has been chosen as the anti-ulcer model for the current investigation.

The animals were divided into Seven treatment groups. Each group contained six animals (n = 6).

Group I: Control (0.5 % Dimethyl sulfoxide orally).

Group II: Negative control (ethanol 90% (1 ml/200 g) orally.

Group III: Standard (Omeprazole 20 mg/kg) orally.

Group IV: *Manilkara zapota* Ethyl acetate extract (MZEA) 100 mg/kg orally.

Group V: *Manilkara zapota* Ethyl acetate extract (MZEA) 200 mg/kg orally.

Group VI: *Manilkara zapota* Methanolic extract (MZME) 100 mg/kg orally.

Group VII: *Manilkara zapota* Methanolic extract (MZME) 200 mg/kg orally.

The anti-ulcer activity was carried out using one model, i.e. ethanol induced model.

Ethanol-induced model:

Procedure:

Male Wistar rats weighing 180–230 g were deprived of food for 48 h before the experiment but were allowed free access to water. During this time, they were kept in restraining cages to prevent coprophagy. The rats were administered either the appropriate vehicle or the cytoprotective drug, e.g., a proteinoid, intra-arterially for 30 min before administration of 1 ml of absolute ethanol. Untreated animals were employed as controls. One hour after administration of ethanol, the animals were euthanized with CO_2, the stomachs were excised, cut along the greater curvature, and the genus was rinsed under tap water. A circular, full-thickness about 13 mm in diameter, is cut with a cork borer from each lobe of the fundus just below the ridge dividing the glandular from the non-glandular portion of the stomach. The stomach was stretched on a piece of foam core mat, mucosal site up. The subjective scores of the treated tissues were recorded; the graded response reflects the least (0) to most (3) damage. A Plexiglas template (19 × 14 × 0.3 cm), burnished on one side with emery cloth and with four rows with six holes 13 mm in diameter, is placed on a sheet of clear glass, burnished side up, and bound to the glass with photographic tape along the periphery. The excised pairs of tissue from each stomach were placed into the holes of the template. Pairs of tissues from each stomach were examined to minimize sampling errors. The template is

positioned on a rectangular central opening of an Aristo Model T-16 cold cathode transilluminator (38 × 38 cm) containing a W-45 blue-white lamp. A camera is mounted on a copy stand directly above the template. Photographs were taken, the film was processed in a standard manner, and a contact sheet was made from the negatives (Ousman Ahmed *et al*., 2022).

$$\% \text{ Ulcer index} = (\text{Ulceration area} / \text{Total stomach area}) \times 100$$

The Inhibition of ulcer was calculated by using the following formula:

$$\% \text{ Inhibition} = \frac{\text{Ulcer Index control} - \text{Ulcer Index test}}{\text{Ulcer Index control}} \times 100$$

Determination of total Acidity:

The entire stomach contents of the treated animal were extracted and placed in a centrifuge tube to measure the gastric output. For 5 min, the stomach contents were centrifuged at 1000 rpm. Using phenolphthalein as an indicator, titration was performed against pre-standardized sodium hydroxide (0.1N) using 1 ml of supernatant that had been diluted with 9 ml of distilled water. A 50-ml conical flask was filled with an aliquot of 1 ml of gastric juice that had been diluted with 1 ml of distilled water. Two drops of phenolphthalein indicator were added, and the mixture was titrated with 0.1N NaOH until a persistent pink hue was seen. The 0.1N NaOH volume was raised until a persistent pink hue was noticed. It was observed how much NaOH was consumed. The total acidity is expressed as mEq/L by the following formula:

$$\text{Acidity} = \frac{\text{Vol. of NaOH} \times \text{N} \times 100 \text{ mEq/L}}{0.1}$$

Statistical Analysis: Each piece of data is represented as mean± S.E.M. A student t-test and a one-way analysis of variance (ANOVA) were used to evaluate the results. Additional multiple comparisons were performed using Tukey's test as the post hoc test whenever the ANOVA was significant. The statistical analysis was carried out using GraphPad InStat version 5. $P>0.05$ was deemed non-significant (ns) when compared to the control group, while $p<0.05$ to $p<0.001$ was the range of statistical significance. Non-significant (ns) was declared when $p > 0.05$ was compared to the control group.

Results and Discussion

The higher dose of ethyl acetate shows the most similar activity to the standard (Table 1). Methanolic and ethyl acetate extract are giving more antidepressant activity as compared to the control.

Determination of volume and pH: The Volume of gastric juice of each rat was measured after centrifugation with 1000 rpm for 10 min and an aliquot of 1 ml of gastric juice was diluted with 1 ml of distilled water and pH of the solution was measured using a pH meter.

The ethyl acetate and methanol extracts of *Manilkara zapota* seeds exhibit a minor variation in gastric volume in comparison to the standard (Table 2). Calculating the "p" of gastric juice, which measures the acidity of the stomach, showed that, although standard and test extracts demonstrated a drop in the pH of gastric secretion, control Group D may have contributed to an increase in stomach acidity.

Ethanol induced ulcer: Gross microscopic study of effect of *Manilkara zapota* seeds extract on Gastric mucosal ulcer has been depicted in Figure 1.

The safety status of medicinal plants needs to be investigated because, despite common belief, they may be poisonous. A stomach, duodenum, or esophageal ulcer is known as a peptic ulcer. Millions of individuals throughout the world suffer from peptic ulcers. Anti-inflammatory drug use, cigarette smoking, and the stomach bacterium *Helicobacter pylori* are all linked to the development of ulcers. There may not be a relationship between the intensity or existence of ulceration and pain from peptic ulcer. Peptic ulcer complications can result in bleeding, perforation, and stomach blockage (gastric obstruction). There are drawbacks to the majority of synthetic

Table 1: Effect of *Manilkara Zapota seeds* extract in ethanol induced gastric ulcer

Group	Ulcer index	Ulcer protective%
Positive Control	32.42±0.66	0
Negative Control	34.62±0.78	0
Standard	7.1±0.53	92.90**
MZEA100	8.2±0.68**	83.38**
MZEA200	8.1±0.36**#	86.98**#
MZME100	32.53±0.98**	64.98**
MZME200	27.98±0.89**	69.66**

Values are expressed as mean ±SEM (n=6), **p<0.001, *p<0.05 v/s Vehicle (One-way ANOVA followed by Tukey's test), #p>0.05 non-significant difference when compared with standard.

Table 2: Impact of MZEA and MZMA on pH and total acidity

Group	pH	Total acidity
Positive control	4.2±0.30	75.5±0.20
Negative Control	2.7±0.25	105±0.50
Standard	5.8±0.04**	28.45±0.40**
MZEA100	4.5±0.08**	41.45±0.48**
MZEA200	5.3±0.11**#	28.55±0.1**#
MZMA100	4.8±0.16	34.12±**
MZMA200	5.0±0.25	44.78±0.46**

Values are expressed as mean ±SEM (n=6), **p<0.001, *p<0.05 v/s Vehicle (One-way ANOVA followed by Tukey's test), #p>0.05 non-significant difference when compared with standard. # shows the most similar activity to standard. ** Ethyl acetate and Methanolic and extract gives more activity as compared to control.

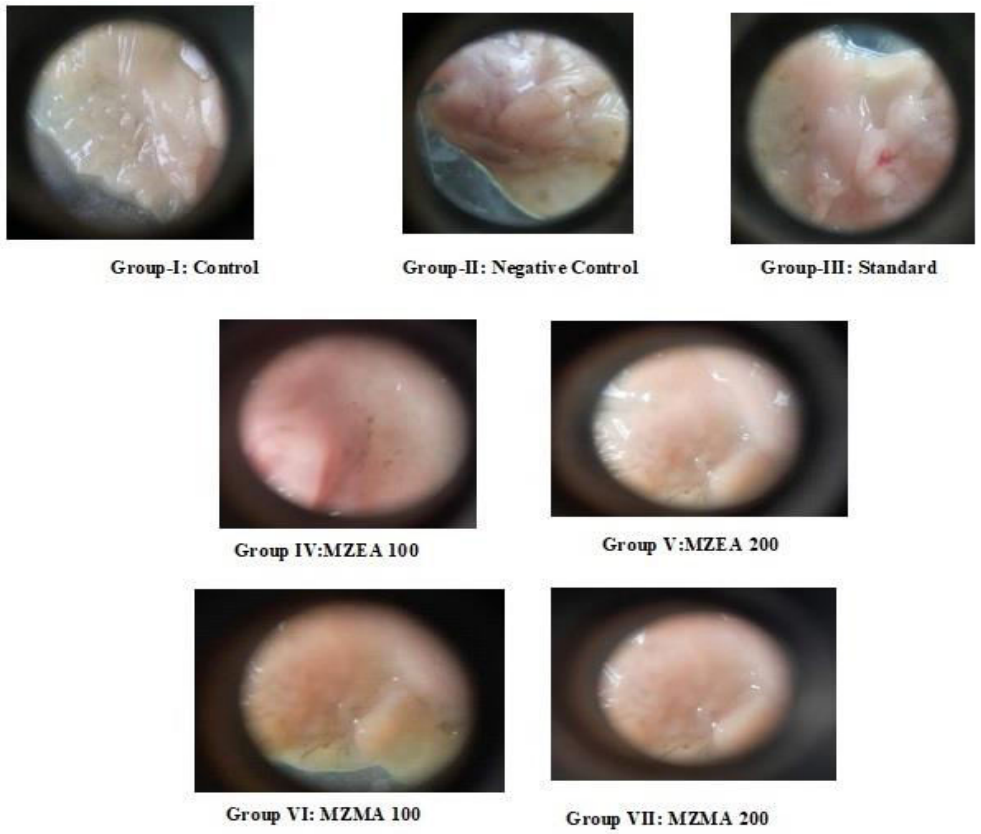

Fig. 1: Effect of *Manilkara zapota* seeds extract on Gastric mucosal ulcer.

medicines used to treat ulcers. As a result, people have been looking for herbal products more often, particularly in the past ten years (Mickelberk *et al*., 1996). Flavonoids, tannins, terpenoids, and saponins were responsible for the gastro-protective effects of plant extracts (Mosaddik and Alam, 2000). Tannins also possess anti-ulcer effects of their stringent property and vasoconstriction effects (Jesus *et al*., 2012). Significant antioxidant activity is shown by MZEA (83.38%), and MZME (64.98%). In contrast to the other extracts, ethyl acetate extract (MZEA) had the highest level of antioxidant activity. Ethyl acetate and methanolic extracts of *Manilkara Zapota* seeds were found to have strong anti-ulcer efficacy in an *in vivo* anti-ulcer research conducted on rats with ulcers produced by ethanol. At 200 mg/kg ethyl acetate extract exhibits 86.98% protection against ulcers; methanolic extract demonstrates 69.66%; and standard, at 20 mg/kg exhibits 92.90% protection. This indicates that the potential of ethyl acetate extract is greater than that of methanolic extract and nearly equal to that of standard (omeprazole 20 mg/kg) at a dose of 200 mg/kg.

Conclusion

Manilkara zapota wild seeds' main ingredients include flavonoids, tannins, and phenolic compounds which may have anti-ulcer and ulcer-protective qualities. Different extracts of *M anilkara zapota* seeds show significant anti-ulcer activity. The ethyl acetate extract shows more significant activity at respective doses compared to the methanol extract. This is baseline work; further investigation is needed at the molecular level.

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