

Evaluation of antiulcer activity of *Manilkara zapota* (Linn) leaves extract in Wistar rats

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Abstract

Aim of the Study: The aim of the study was to evaluate the gastroprotective activity of *Manilkara zapota* leaf extract in Wistar rats. **Materials and Methods:** The leaf of *M. zapota* was air dried in the shade, powdered, and subjected to successive extraction in Soxhlet apparatus. The gastroprotective activity of the ethanol extract of *M. zapota* was evaluated using aspirin plus pylorus ligation and HCl plus ethanol-induced ulcer models. **Results:** Phytochemical analysis of various extracts of *M. zapota* revealed the presence of glycoside, flavonoid, tannins, and phenolic compounds. Ethanolic extract of *M. zapota* (EEMZ) extract treatment shows potent and effective gastroprotection against aspirin plus pylorus ligation and HCl plus ethanol-induced ulcer models. In pylorus-ligated rats, the extract decreases the secretion of pepsin, gastric acidity, volume, and increases gastric pH, glycoprotein, protein, superoxide dismutase, catalase, and non-protein sulfhydryl levels. In the HCl plus ethanol-induced rats, the EEMZ treatment significantly increased the prostaglandin secretion and protects the gastric mucosa compared to the control group rats. **Conclusion:** The results of the study showed that EEMZ possesses a preventive effect against gastric ulcer model in the experimental animals.

Key words: Antiulcer, antioxidant, *Manilkara zapota*, non-protein sulfhydryl, pylorus ligation

INTRODUCTION

Medicinal plants constitute a source of raw materials for both traditional (e.g., Ayurvedic, Chinese, Unani, Homeopathy, and Siddha) and the modern system of medicine. Nowadays, natural products are employed throughout the industrialized and developing world as home remedies. Thus, herbal medicine has led to the discovery of a number of new drugs.^[1] *Manilkara zapota*, commonly known as sapodilla, is long-lived, evergreen forest tree more than 30 m in height and 1.5 m diameter depending on locations.^[2] *M. zapota* (Linn) is well-known ornamental plant and traditionally used for a wide range of biological activities such as anti-inflammatory, antiulcer, anticancer antibacterial, anti-arthritis, and antibacterial.^[3]

Peptic ulcer disease refers to a disruption of the mucosal integrity of the stomach, duodenum, or both, caused by local inflammation, which leads to a well-defined mucosal defect.^[4] This results from an imbalance between the aggressive and defensive factors^[5] for the treatment of peptic ulcer. The number of medicinal agents is

available in the market which is adequately cure the ulcer, act through either decrease the secretion of acid or by producing the cytoprotection,^[6] but none of these are devoid of side effects. Hence, this study was designed to find a drug which produces effective protection, less side effects, and decrease the chances of relapses.

MATERIALS AND METHODS

Plant Material

Fresh leaves of *M. zapota* (Linn) were collected from Gorakhpur district (Uttar Pradesh [U.P]), India. The leaves

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were first washed with running water than shade-dried for 10–15 days. After drying leaves were powdered into moderately coarse powder and extracted in Soxhlet assembly with petroleum ether, chloroform, and afterward with ethanol. Excess of the ethanol was removed under reduced pressure at 40°C. Dried extracts of different solvents were stored in airtight containers in the refrigerator for phytochemical analysis and antiulcer activity.

The preliminary phytochemical investigation was carried out for the presence of constituents such as alkaloids, glycosides, saponin, tannin, and steroids according to the standard method given by Kokate.^[7]

Experimental Animals

Wistar rats of either sex, weighing 180–200 g were used in the study. Animals were procured from IVRI Izatnagar Bareilly, U.P. India. The institutional Animal Ethical Committee approved the experimental protocol; all animals were housed in polypropylene cage under standard conditions of temperature (24 ± 2°C) and relative humidity (30–70%) with a 12:12 h light: dark cycle and had free access to standard pellet food and water *ad libitum*.

Aspirin Plus Pylorus Ligation Induced Gastric Mucosal Ulcers

Healthy wistar rats were divided into five groups Groups I and II served as normal control and ulcer control group and received 1% carboxymethyl cellulose (CMC). Group III served as standard drug-treated group received ranitidine 50 mg/kg, orally. Group IV and V served as test control group, received ethanolic extract of *M. zapota* (EEMZ 200 and 400 mg/kg. p. o.). All animals received 200 mg/kg of aspirin once daily for 3 days except normal control group. On 4th day 30 min after the treatment, under the light diethyl ether anesthesia, the abdomen of the rats was opened and the pylorus was ligated. Abdomen wall was sutured and animals were allowed to recover in their cages for 4 h and then animals were sacrificed with an excess dose of anesthetic ether. The abdomen was opened and the stomach was isolated. During the isolation, proper care was taken so that the gastric juice does not flow out from the cardiac end of the stomach. A small cut in the pyloric region just above the pyloric sphincters was made and contents of the stomach were collected in measuring cylinder. The volume of gastric juice was measured and transferred into a centrifuge tube and centrifuged at 1000 rpm for 10 min. The pH of gastric juice was recorded by pH meter and then contents were subjected to analysis of various biochemical parameters such as protein, pepsin, total acidity, and free acidity.

Acidity was calculated using the formula:

$$\text{Acidity} = \frac{\text{Volume of NaOH} \times \text{Normality} \times 100}{0.1} \text{ mEq/L/100g}$$

The stomachs were washed with running water to see the ulcer index in the glandular portion of the stomach, and the number of ulcer per stomach was calculated, and the severity of the ulcer was measured under a microscope.^[8]

HCl-ethanol Induced Ulcer

Group I served as normal control and received 1% CMC, Group II served as ulcer control group and received HCl and ethanol, Group III received omeprazole (20 mg/kg, p.o.) and served as standard. Group IV and V served as test control group, received ethanolic extract of *M. zapota* (EEMZ 200 and 400 mg/kg. p.o.). After 1 h of drug administration, all animals were treated with 0.2 mL of HCl-ethanol mixture to induce gastric ulcer. 1 h after administration of HCl-ethanol mixture, animals were sacrificed by cervical dislocation. Stomach was excised out and opened, gently rinsed with ice cold saline and examined in a light microscope for the severity of the lesion.^[9]

Evaluation of Gastric pH

Gastric contents were centrifuged at 3000 rpm for 10 min and the centrifuged samples were transferred into the small beaker, pH of the solution was measured using digital pH meter (Labtronics).^[10]

Gastric Wall Mucin

Mucin contents were determined according to the procedure of Corne *et al.* The glandular segments from stomachs were removed and weighed. Each segment was transferred immediately to 10 mL of 0.1% w/v alcian blue solution (in 0.16 M sucrose solution, buffered with 0.05 M sodium acetate pH 5). After immersion for 2 h, the excess dye was removed by two successive rinses with 10 mL of 0.25 M sucrose solution, first for 15 min and then for 45 min. Dye complexes with the gastric wall mucus were extracted with 10 mL of 0.5 M magnesium chloride by shaking intermittently for 1 min after 30 min intervals for 2 h. 4 ml of blue extract were then shaken vigorously with an equal volume of diethyl ether. The resulting emulsion was centrifuged at 3600 rpm for 10 min and the absorbance of the aqueous layer was measured at 580 nm.^[11,12]

Preparation of Tissue Homogenate

Stomach was rinsed with normal saline and then homogenized into ice cold 20 mM Tris-HCl (pH 7.4) to give 10% w/v homogenate. The homogenate was then centrifuged at 3000 rpm for 10 min at 4°C in a cooling centrifuge. The supernatant was used for the determination of antioxidant activity.

Estimation of Gastric Pepsin

Pepsin was assayed as per the method described by kore *et al.*, the pepsin content was expressed as μM of tyrosine liberated/mL.^[13,14]

Estimation of Protein

Protein content was estimated as per the method of Lowry *et al.* 1951.^[15]

Estimation of Antioxidant Enzymes

Centrifuged tissue homogenate was used for estimation of non-protein sulphhydryl (NP-SH)^[16,17] and antioxidant enzyme,

i.e., lipid peroxidation (LPO) catalase (CAT) superoxide dismutase (SOD) assay.^[18-20]

RESULTS

Phytochemical screening of the different extracts of *M. zapota* showed the presence of flavonoid, tannin, phenolic compounds, anthraquinone, and saponin glycosides [Table 1]. Administration of EEMZ to the dose of 2000 mg/kg did not show any type of lethal symptoms and mortality up to 14 days from 1st day.

The results of EEMZ (200 and 400 mg/kg) are shown in Table 2 and pretreatment of EEMZ extract showed a significant ($P < 0.05$) reduction in ulcer index when compared

Table 1: Screening of phytoconstituents in different extracts of *Manilkara zapota*

Phytoconstituents	Ethanolic extract	Chloroform extract	Petroleum ether extract
Tests for cardiac glycosides			
Legal's test	Negative	Negative	Negative
Keller-kiliani test	Positive	Negative	Negative
Tests for anthraquinone glycosides			
Borntrager's test	Positive	Negative	Negative
Modified Borntrager's test for C-glycosides	Positive	Positive	Negative
Tests for saponin glycosides			
Foam test	Positive	Positive	Negative
Tests for steroid			
Salkowski reaction	Negative	Negative	Positive
Liebermann-Burchard reaction	Positive	Positive	Positive
Liebermann's reaction	Positive	Negative	Positive
Tests for flavonoids			
Shinoda test	Positive	Negative	Negative
Residue+Lead acetate solution	Positive	Positive	Negative
Sod. Hydroxide+Residue	Positive	Positive	Negative
Tests for alkaloids			
Dragendorff's test	Negative	Positive	Negative
Mayer's test	Negative	Negative	Negative
Wagner's test	Negative	Negative	Negative
Tests for tannins and phenolic compounds			
5% FeCl ₃ solution	Positive	Positive	Negative
Lead acetate solution	Positive	Positive	Negative
Gelatin solution	Positive	Positive	Positive
Acetic acid solution	Positive	Negative	Positive
Bromine water	Positive	Negative	Negative
Potassium dichromate	Positive	Positive	Negative
Dilute iodine solution	Positive	Positive	Negative
NH ₄ OH+10% AgNO ₃ solution	Negative	Negative	Negative
Dil. Pot. permanganate solution	Positive	Positive	Positive
Dilute HNO ₃	Positive	Negative	Positive

with the ulcer control group in HCl and ethanol-induced ulcer model. Omeprazole treated rats showed a significant reduction ($P < 0.001$) in ulcer index as compared to the disease control group. The percentage protection exhibited by EEMZ (200 and 400 mg/kg) and omeprazole was 50.81, 63.58, and 93.32% against the ulcer control group.

Administration of aspirin (200 mg/kg) and pylorus ligation for 4 h increases the excessive secretion of gastric acid in the stomach. Administration of aspirin (200 mg/kg) and pylorus ligation for 4 h increases the excessive secretion of gastric acid in the stomach. The excessive and continued accumulation of gastric acid in the stomach may leads to

Table 2: Effect of EEMZ on ulcer index in HCl plus ethanol and aspirin plus pylorus ligated rats

Group	Aspirin plus pylorus ligation		HCl plus ethanol	
	Ulcer index	Percentage protection	Ulcer index	Percentage protection
Normal control	0.17±0.10	-	0.25±16	-
Ulcer control	18.63±0.22	-	22.63±1.18	-
Ranitidine (50 mg/kg)	1.17±0.12	93.71***	-	-
Omeprazole (20 mg/kg)	-	-	2.10±0.45	90.72***
EEMZ (200 mg/kg)	9.86±0.25	47.10*	11.13±0.67	50.81*
EEMZ (400 mg/kg)	5.92±0.14	68.28**	8.24±0.53	63.58**

Values are expressed in mean±SEM ($n=6$), statistically analyzed by one-way ANOVA followed by Dunnett t -test * $P<0.05$, ** $P<0.01$ and $P<0.001$ when compared with the control group. EEMZ: Ethanolic extract of *Manilkara zapota*, SEM: Standard error of the mean

Table 3: Effect of EEMZ on gastric parameters in aspirin plus pylorus ligated rats

Group	Gastric pH	Gastric volume (mL)	Free acidity mEq/L	Total acidity mEq/L
Normal control	4.70±0.27	2.12±0.19	38.34±2.45	58.17±3.52
Ulcer control	1.56±0.20	4.57±0.28	97.33±3.63	126.33±4.82
Ranitidine (50 mg/kg)	5.80±0.28***	2.23±0.18**	42.83±2.75**	65.17±3.75**
EEMZ (200 mg/kg)	3.40±0.24*	3.03±0.23*	52.66±3.26*	86.17±4.07*
EEMZ (400 mg/kg)	4.50±0.25**	2.45±0.17**	45.67±2.35**	72.50±3.82*

Values are expressed in mean±SEM ($n=6$), statistically analyzed by one-way ANOVA followed by Dunnett t -test * $P<0.05$ ** $P<0.01$ and *** $P<0.001$ when compared with control group. EEMZ: Ethanolic extract of *Manilkara zapota*, SEM: Standard error of the mean

Table 4: Effect of ethanolic extract of *Manilkara zapota* on changes in gastric tissue and gastric mucosal secretion in aspirin plus pylorus ligated rats

Group	Protein (µg/ml)	Pepsin (µg/ml)	Mucin (µg/g wet tissue)
Normal control	283.44±16.24	1.46±0.48	239.94±16.84
Ulcer control	177.47±10.29	7.05±0.92	113.39±13.34
Ranitidine (50 mg/kg)	260.13±8.08**	3.35±0.67*	217.10±15.75**
EEMZ (200 mg/kg)	197.18±12.82*	6.08±0.86 ^{NS}	160.34±15.34*
EEMZ (400 mg/kg)	221.60±10.44*	4.84±0.72*	194.77±14.77**

Values are expressed in mean±SEM ($n=6$), statistically analyzed by one-way ANOVA followed by Dunnett t -test * $P<0.05$ ** $P<0.01$ and $P<0.001$ when compared with the control group. EEMZ: Ethanolic extract of *Manilkara zapota*, SEM: Standard error of the mean

Table 5: Effect of EEMZ on gastric mucosal antioxidant (SOD, CAT, LPO, and NP-SH) enzyme levels in aspirin plus pylorus ligated rats

Group	SOD (µg/mg protein)	CAT (µg/mg protein)	NP-SH (µg/mg protein)	LPO (nmol/mg protein)
Normal control	164.01±7.24	58.90±3.74	10.27±1.08	38.57±4.64
Ulcer control	82.29±5.88	25.45±2.41	3.23±0.20	128.79±8.48
Ranitidine (50 mg/kg)	157.65±6.49**	46.55±4.70*	8.28±0.66**	48.80±6.88**
EEMZ (200 mg/kg)	108.29±5.78*	38.25±3.44*	5.33±0.36*	82.96±7.04*
EEMZ (400 mg/kg)	138.32±5.41*	42.55±3.64*	6.98±0.39**	62.33±7.87*

Values are expressed in mean±SEM ($n=6$), statistically analyzed by one-way ANOVA. Followed by Dunnett t -test * $P<0.05$ and ** $P<0.01$ when compared with the control group. EEMZ: Ethanolic extract of *Manilkara zapota*, SEM: Standard error of the mean, SOD: Superoxide dismutase, CAT: Catalase, NP-SH: Non-protein sulfhydryl, LPO: Lipid peroxidation

degradation of gastric mucosa as well as lesions formation. Oral administration of EEMZ (200–400 mg/kg) in aspirin and pyloric ligated rats produce the significant ($P < 0.01$ and $P < 0.05$) dose-dependent reduction in gastric mucosal damage or ulcer index by 47.10 and 68.20% as compared to the disease control group. Similarly, ranitidine (50 mg/kg) showed significant ($P < 0.001$) 91.10% reduction against the negative control group. Pretreatment of experimental animals with EEMZ and ranitidine reduced the volume of gastric juice, free acidity, total acidity, pepsin level and increase protein, mucin, and gastric pH levels significantly ($P < 0.05$) as compared to the disease control group as shown in Tables 3-5.

EEMZ treated group also decrease the level of oxidative stress marker (LPO) and increases antioxidant enzyme (SOD, CAT, and NP-SH) levels as compared to the disease control group.

DISCUSSION

The present study for the first time demonstrates that EEMZ possesses cytoprotective activity against aspirin plus pylorus ligation and HCl ethanol-induced ulcer model.

Phytochemical screening of the different extracts of *M. zapota* leaves revealed the presence of anthraquinone and saponin glycosides, flavonoid, tannin, and phenolic compounds. It is well known reported that the phenolic compounds, flavonoid, and other phytoconstituents are responsible for antioxidant and inflammatory mechanism.^[21]

In the present study, the activities of EEMZ leaves were evaluated in HCl plus ethanol and aspirin plus pylorus ligation model. Moreover, pylorus ligation causes continued accumulation of gastric acid and facilitates the autodigestion of the gastric mucosal wall and disruption of the gastric mucosal barrier.^[22-25] However, aspirin produces direct aggravation impact and mucosal injury by interrupting with prostaglandin synthesis.^[26-27] The result of our study shows that EEMZ significantly and dose-dependently decrease the intensity of gastric ulcer in aspirin plus pylorus ligation model. It is assumed that the vagal enactment by incitement of pressure receptor in the antrum of the gastric mucosa is responsible for gastric acid secretion.^[28] The present information clearly demonstrated that EEMZ not only diminished the volume of gastric acid but also decrease the free and total acidity and inhibited the lesion formation in the glandular portion of the stomach.

Moreover, the status of mucus secretion is important to determine the status of the mucosal barrier. Mucus consists of mucin glycoprotein this high molecular weight glycoprotein are mainly responsible for viscous and gel-forming capacity of mucus^[29] and protect the gastric mucosa from injury. It was observed in the current study that EEMZ has caused a significant increment of gastric wall mucus that has been decreased by the hyperacidity in the aspirin and pylorus

ligated model. This further confirms the capability of EEMZ to counteract or improve the impact of damaging agents. This finding demonstrates that EEMZ retains the gastric mucus secretion and reinforce the defensive factor of gastric mucosa in experimental animals. Tissue damage is constantly connected with oxidative stress, and promoting the loss or destruction of protein synthesis and damages to lipids and the thiol-dependent antioxidant defense.^[30] In the case of enzymatic oxidative stress, the level of SOD and CAT was decreased in aspirin plus pylorus ligation induced gastric injury. SOD plays an essential role in providing the gastroprotection by halting the oxidative damage. The result of the current study demonstrated that EEMZ reestablished the activities of SOD and CAT.

Our outcomes of this study indicate a marked reduction in NP-SH level of the gastric mucosa. It is well known reported that the sulfhydryl compounds play an important role in maintains of gastric mucosa especially when free radical or reactive oxygen species are involved in the pathogenesis of tissue damage.^[31] In addition to decreasing the NP-SH level EEMZ also reverse the level of LPO in the stomach, which was increase by aspirin and pylorus ligation in rats. Alcohol consumption is a contributor of gastric ulcer. HCl and ethanol produced ulcers due to the distraction of superficial epithelial cells; both the chemical agents irritate the gastric mucosal cell and decrease the release of bicarbonate and mucus secretion and increase the production of reactive oxygen species.^[32] EEMZ produced the cytoprotective effect against the HCl and ethanol-induced ulcer. Ethanol extract of *M. zapota* exhibited a less ulcer index as compared to the negative control group. The cytoprotective effect of EEMZ could be due to increasing the viscosity and production of mucus which produces a protective barrier against the corrosive agents or decreasing the production of reactive oxygen species by stimulating the antioxidant enzymes.

CONCLUSION

The results of the present study show that the EEMZ have good antiulcer activity.

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