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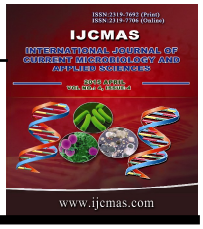

Original Research Article

Assessment of Antiulcer Activity of Ethanolic Extract of *Mangifera indica* Seed Kernel Using Acid Ethanol Induced Ulcer Model

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A B S T R A C T

Mangifera indica (Family: Anacardiaceae) is being used in Ayurvedic and indigenous medical systems for the treatment of various diseases including gastric ulcer. The present work was undertaken to analyze the antiulcer potential of ethanol extracts of *M. indica* seed kernel against acid alcohol induced gastric ulcer. The ethanolic extracts (400mg/kg) of *M. indica* seed kernel significantly reduced the ulcer index, pH, total acidity, LPO and protein levels. It also acted as good antioxidant agent. In conclusion, the present study provide preliminary data on the antiulcer potential of *M.indica* seed kernel and support the traditional uses of the plant for the treatment of gastric ulcer.

Keywords

Mangifera indica, Seed kernel, Anti ulcer, Antioxidant

Introduction

Nature has provided a complete storehouse of remedies to cure ailments of mankind (Gangadhar *et al.*, 2012; Rahman *et al.*, 2011). There is a widespread belief that green medicines are healthier, though the recovery of disease by plant medicine is slow, the therapeutic use of medicinal plant is becoming popular because of its inability to cause side effects and effective for antibiotic resistant microorganisms (Perumalsamy *et al.*, 2008). India is well known for its rich traditional system of medicine, besides a vast reservoir of living traditions of ethno medicine.

Mangifera indica L. (Anacardiaceae) is one of the most important tropical plants, part of this plant is used in all system of medicine throughout the globe (Sharma *et al.*, 1997). Seed kernel of this plant contributed about 17-22% of the fruit, it is discarded as a waste and contributes a source of pollution and rich in phenolic compounds and stable fat rich in saturated fatty acids (Agarwal, 2005). Traditionally *M.indica* Seed kernel (MISK) is used to cure chronic diarrhoea, wound healing, to expel tapeworms and other worms (Warrier, 1996). It also possesses antioxidant (Cadenas and Packer,

1996) and antidiarrhoel activity (Yean and Philip, 2004). Peptic Ulcer Disease Deaths in India reached 1.2% of total deaths. The age adjusted Death Rate is 12.37 per 100,000 of population ranks India is 5 in the world. Peptic ulcer is an excoriated area of the gastric or duodenal mucosa, is a chronic and recurrent disease (Guyton and Hall, 2000). It is generally recognized that peptic ulcer is caused by a lack of equilibrium between the gastric aggressive factors and the mucosal defensive factors (Muralidharan and Srikanth, 2009). The predominant causes of peptic ulcer are infection with the bacterium called *Helicobacter pylori (H.pylori*) and the use of Non Steroidal Anti-Inflammatory Drugs (NSAIDs) such as aspirin and ibuprofen (Goroll and Mulley, 2009). Ulcer is treated with proton pump inhibitors and selective H2 receptor blockers can efficiently cure ulcer. But none of these are devoid of side effects and execute their action within a limit. Moreover, the recurrence of ulcer after stopping the medication is very high. The antioxidants may reconcile their upshot by directing reaction with ROS, quenching them and or chelating the catalytic metal ions.

Several synthetic antioxidants like BHA, BHT are commercially accessible but are perilous and their toxicity is a problem of disquiet. These drawbacks of currently available antiulcer medicines necessitate the development of newer generation photogenic drugs. Natural antioxidants are safe and also bioactive. Therefore, in current years, substantial attention has been directed towards credentials of plants with antioxidant ability that may be used for human expenditure (Aman *et al.*, 2011). Hence it is very essential to develop some cost effective herbal drugs without or less side effects to combat the burning problem gastric ulcer.

Materials and Methods

Plant material collection

The fruits of *M. indica* were purchased from the local market of Tiruchirappalli, Tamilnadu, India and seeds were separated from the fruit. The hard seed coat was removed and the seed kernel were dried. These dried seed kernels were coarsely powdered and stored in closed container for further use.

Extraction - Ethanol

200gm of *M. indica* seed kernel powder was soaked in 1200ml of ethyl alcohol and incubated for 72 hrs. Then it was filtered and evaporated to dryness. The extracts (MISKEE) obtained were subjected to pre-clinical screening.

Animal

Albino rats of both sexes weighing 100- 120 g were utilized for the study. They were purchased from the Tamil Nadu Veterinary and Animal Sciences University, Madhavaram Milk Colony, Chennai 600 051, India. They were maintained in well equipped polypropylene cages at room temperature of 24°C and exposed to both light and dark cycle. All the animals were allowed to adapt for two weeks in animal house of Srimad Andavan Arts and Science College, Tiruchirappalli.

The animals were fed with standard pelleted diet and water was allowed *ad libitum*.

The container for the food and water was washed daily as the food and water were renewed every day to ensure hygiene and maximum comfort for the animal. The study protocol was approved by CPCSEA NO 790/03/ac/CPCSEA.

Acid alcohol induced ulcer

Wistar strains of female albino rats weighing 100gm- 120gm were used as the experimental models. The rats were divided into six groups comprising of six rats each. Group I rats served as the normal control, Group II rats were administered orally with Hcl and Ethanol (0.3M hydrochloric acid in 60% ethanol - 1:1) and served as the disease control, Group III rats were treated with Ranitidine (32mg/kg bw) for 15 days, Group IV, V, VI Rats were treated with ethanolic extract of *M.indica* (100, 200, 400mg / Kg b.w) for 15 days. After the experimental period, animals were sacrificed by cervical decapitation. Stomach was dissected out and washed in ice- cold saline. Stomach was homogenized in 0.1 M phosphate buffer, pH 7.4 and used for various experiment (Deshpande *et al.*, 2003).

Calculation of ulcer score – Ulcer Index

The stomach was opened along the greater curvature and washed slowly under running tap water. It was put on a glass slide and observed under 10X magnification for ulcers. Mean ulcer score in each group was calculated and was designated as ulcer index and percentage. They were calculated as

Calculation of Ulcer Index (Jyoti *et al.*, 2012): U1 = UN + US + UP x 10-1 U1 = Ulcer Index; UN = Average of number of ulcer per animal; US = Average of severity score; UP = Percentage of animal with ulcer; Mean ulcer score for each animal is expressed as ulcer index

% Protection= (C-T/C) X 100

Where C= ulcer index in control group; T= ulcer index in treated group

Ulcer scoring

Normal stomach - (0); Red coloration - (0.5); Spot ulcer - (1); Hemorrhagic streak..(1.5); Ulcers - (2); Perforation - (3); Mean ulcer score for each animal was expressed as ulcer index.

Evaluation of pH (Nwinyl and Kwanashie, 2013**)**

The stomachs were removed and the contents were drained into a graduated centrifuge tube through a small nick along the greater curvature. The tubes were centrifuged at 3000rpm for 10mins and the centrifuged samples were decanted and analyzed for pH.

Evaluation of gastric acidity

The gastric juice collected from the dissected stomachs of the animals in Hcl & ethanol induced ulcer group was washed using saline and centrifuged at 3000 rpm for 5 min. The clear supernatant was titrated against, 0.01mol/L solution of sodium hydroxide (NaOH) to pH 7.0. The acid content of the stomach was calculated according to the method of Shay *et al.*, (1954) and expressed as MEq/L.

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

Estimation of protein/pepsin

Protein content was estimated by the method of Lowry *et al.*, (1951).

Estimation of antioxidant enzymes

Stomach tissue homogenate was used for assessing antioxidant enzymes like Lipid peroxidase (LPO), Superoxide Dismutase (SOD) and Glutathione Peroxidase (GSH).

Standard methods were used to assess LPO (Ohkawa *et al.*, 1979); SOD (Misra and Fridovich, 1972) and GSH (Lawrence and Burk, 1976).

Statistical analysis

All the results were expressed as mean ±S.E.M. The data were statistically analyzed by one – way analysis of variance (ANOVA) and P values □0.05 were considered significant.

Result and Discussion

Animal model on antiulcer study evidenced the antiulcer potentials of MISK ethanolic extracts. Ulcer index was significantly reduced ranitidine treated groups (2.71 ±0.14) as well as in ethanolic extracts treated animal groups (Group IV, V and VI). Among different doses of extract treated animal groups 400mg/kg bw treated group (group VI) showed significantly reduced ulcer index (3.02±0.15) and produced 70% ulcer protection / healing (Table 1).

Ulcer protection effect of the MISK ethanolic extracts was also evidenced in the level of pH, total acidity and protein (Table 2). Intestinal content of group showed pH (2.80 ± 0.015), which is similar to group I animals and higher than disease control (1.34 ± 0.020). In the same manner group VI animals (treated with 400 mg/kg body weight) showed lower acidity (110 ± 0.956) and lower protein i.e., lower enzymatic activity (7.06 ±0.710), when compared to group II disease control animals.

Extracts and ranitidine treated animals showed similar pattern of results and it is comparatively very close to the normal animals. This indicated that acid ethanol induced ethanolic extracts treated animals regained its normal status.

MISK ethanolic extract treatment significantly reduced LPO activity, increased SOD and GSH activity when compared to the disease control. LPO activity was very high in Group II animals (325 ± 1.856) whereas it was found to be 288.6 ± 4.72 for ranitidine treatment and 160±1.894 for 400mg/kg bw ethanolic extract treated group. Lower SOD was noted in diseases animal (42.50 ± 1.625) whereas higher SOD activity was noted in Group IV animals, which is also higher than ranitidine treated group. Similar kind of result was noted in GSH activity. GSH activity was high in group IV animals (63.08 ±0.925), when compared to disease control (20.56 ± 1.285). These results revealed that ethanolic extract of *Mangifera indica* seed kernel considered as an effective antioxidant agent thereby prevents ulcer (Table 3).

Antiulcer activity of ethanolic extract of *Mangifera indica* seed kernel was evaluated using acid alcohol induced ulceration model. Ulcers are thought to be due to an imbalance between offensive factors such as acid and pepsin and defensive factors such as mucin secretion, cell proliferation, prostaglandins, etc (Rao *et al.*, 2001). Acid induces histamin, which is a potent stimulator of acid secretion. Ethanol causes disturbances in gastric secretion, damage to the mucosa, alterations in the permeability, gastric mucous depletion and free radical production (Sakat and Juvekar, 2009). This is attributed to the release of superoxide anion and hydroperoxy free radicals cause acute and chronic ulceration in the gastric mucosa (Jude, E. O., Paul, A. 2009). Ethanol also induced gastric lesion formation, which contributes to the development of the haemorrhage and necrotic tissue injury (Soll, 1990; Surendra, 1999). MISK ethanolic extract significantly reduces mean ulcer

count. It may be due to antisecretory effect of MISK, which significantly reduces the formation of ulcers. It also increases the pH, reduce the acidity of stomach and also it reduces the protein content in ulcer affected animals. Phytochemical constituents of the MISK ethanolic extracts were highly responsible for reducing ill effects of acid alcohol by preventing mast cell activity and normalize the H_2 secretion in the stomach. Rajan *et al.*, (2001a), Rajan *et al.*, (2011b), Rajan *et al.*,(2012) and Rajan and Thirunalasundari, (2012) revealed the presence of flavonoids, phenolic compounds, tannins, alkaloids in ethanolic extracts of MISK. One of our previous study also indicated similar type of phytochemicals (Prabhu and Rajan, 2014). Tannins, flavonoids were responsible for antiulcer activity. Flavonoids prevents ulcer by free radicals scavenging mechanisms.

In vivo antioxidant assay (LPO, SOD and GSH) revealed that MISK could be considered as an effective antioxidant compound and prevents ulcer and its related complications. Tannins have been reported to possess antioxidant, wound healing, antimicrobial and antiulcer activity. MISK ethanolic extract could be considered as a good antiulcer agent may be due to the green chemicals found on the plant materials. Polyphenolic substance of this plant also may responsible for antioxidant and antiulcer activity (Wu *et al.*, 2015). The results of the present study suggest that the ethanolic extract of *Mangifera indica* seed kernel may be beneficial in the treatment of gastric lesions. Further studies to identify the active compounds and elucidation of the mechanism of action are recommended.

Table.1 Comparison of the ulcer index in treatment groups with control groups

Groups	**Ulcer index**	**% of healing**
Disease control(Hcl:ethanol)	10.0 ±0.15	----
Standard Drug(Ranitidine)	2.71 ±0.14	73%
Dose I(100 mg/kg body weight)	6.12 ±0.14	39%
Dose II(200 mg/kg body weight)	3.2±0.19	68%
Dose III (400 mg/kg body weight)	3.0±0.15	70%

Table.2 Effect of *Mangifera indica* seed kernel extract on various parameters in Hcl & Ethanol induced ulcer

Groups	**Treatment**	**pH**	**Total acidity MEq/L**	**Protein g/dl**
I	Normal control	2.85 ±0.025	105 ± 1.781	6.98 ±0.565
II	Disease control	1.34 ± 0.020	275 ± 2.125	10.45 ±0.835
III	Standard drug (Ranitidine)	1.74 ±0.081	216.52 ±3.391	9.205 ± 0.039
IV	Ethanolic extract of MISK(100 mg/kg b.w)	2.19 ± 0.047	174.33 ± 4.22	8.35 ± 0.041
V	Ethanolic extract of MISK(200 mg/kg b.w)	2.71±0.028	115.16 ± 3.56	7.15 ±0.04
VI	Ethanolic extract of MISK(400 mg/kg b.w)	2.80 ± 0.015	110 ± 0.956	7.06 ±0.710

Values are expressed as mean ± SEM (n=6) $P<0.05$ statistically significant when compared Group IV, V & VI with Group III

Table.3 Effect of *Mangifera indica* seed kernel extract on various parameters in Hcl & Ethanol induced ulcer

Groups	Treatment	LPO µm of MDA/ g tissue	SOD U/mg protein	GSH µg/g tissue
I	Normal control	155 ± 1.725	87.06 ± 1.478	65.35 ±1.045
II	Disease control	325 ± 1.856	42.50 ± 1.625	20.56 ± 1.285
III	Standard drug (Ranitidine)	288.6 ± 4.72	54.26 ± 1.262	29.02 ± 0.515
IV	Ethanolic extract of MISK(100 mg/kg bw)	215 ± 2.61	72.88 ± 1.862	45.81 ± 0.353
V	Ethanolic extract of MISK(200 mg/kg bw)	168.5 ± 4.461	84.12 ± 0.759	61.61 ± 0.431
VI	Ethanolic extract of MISK(400 mg/kg bw)	160 ± 1.894	86.50 ± 1.176	63.08 ±0.925

Values are expressed as mean ± SEM (n=6) P<0.05 statistically significant when compared Group IV, V & VI with Group III

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