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Study On Antiulcer Potentials Of Mangifera Indica Immature Endosperms (Miies)

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ABSTRACT

Mangifera indica immature endosperm (MIIES) is traditionally used for the treatment of gastrointestinal infections. Few studies are available in matured seed kernel biopotential activities, whereas no such studies in immature endosperm, hence in this study antiulcer activity was screened by making use of standard methods. Ethanolic and phenolic extracts were subjected for antiulcer screening. Results revealed that both the extracts produced good antiulcer activity which was evidenced in ulcer score, ulcer index. Extracts also proved antioxidant ability. Among the extracts phenolic extracts proved to be an effective antiulcer agent which may be due to flavonoids.

Keywords: Antiulcer activity, *Mangifera indica*, MIIES, MIIESAE, MIIESPE, immature endosperm.

1. INTRODUCTION

Gastric and duodenal ulcer prevalence is high In India. The point prevalence of active peptic ulcer was 3.4% and the lifetime prevalence was 8.8%. The duodenal-to-gastric ulcer ratio was 12:1. *Helicobacter pylori* was present in 84.6% subjects with this ulcer [1]. Ulcer was more common in elderly and dyspeptic individuals, 1.2% of total mortality is attributed to Peptic Ulcer Disease (PUD). Peptic ulcer is an excoriated area of the gastric or duodenal mucosa, it is a chronic and recurrent disease [2, 3]. It is generally accepted that peptic ulcer is caused by a lack of equilibrium between the gastric aggressive factors and the mucosal defensive factors [4]. Different factor that causes the PUD are bacteria and some synthetic drugs. Ulcer is addressed with proton pump inhibitors and selective H_2 receptor blockers. However, these create side effects and execute their action within a limit. Moreover, the recurrence of ulcer after stopping the medication is very high. Antioxidants may reconcile their upshot by directing reaction with Reactive Oxygen Species (ROS), quenching them and or chelating the catalytic metal ions. Numerous synthetic antioxidants like BHA, BHT are commercially accessible but are perilous and their toxicity is a problem of disquiet. These drawbacks of existing available antioxidant agents necessitate the development of newer generation photogenic drugs. Natural antioxidants are safe and also bioactive. Therefore, in the current years, substantial attention has been directed towards credentials of plants with antioxidant ability that may be utilized for human expenditure [5]. Traditionally peoples of Tamilnadu and Andrapradesh use *Mangifera indica* immature endosperm in a form of pickle for the treatment of intestinal infestations [6]. They also stated the uses of mango matured seed kernel as an antidiarrhoeal, antibacterial and antiulcer agent. Hence, in this study immature endosperm was selected and assessed its antiulcer activity.

2. MATERIALS AND METHODS

Plant Material: *Mangifera indica* Immature Endosperm (MIIES) was collected from Perambalur, Tamilnadu, India during summer months of 2016. The plant material was identified by Dr. John Britto, Professor, Department of Botany, St. Joseph's College, Tiruchirappalli,

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Tamilnadu, India and specimen was deposited at PG and Research Department of Microbiology, M. R. Government Arts College, Mannargudi, Tamilnadu, India.

Preparation of Extracts: The powdered pant material (150gm) was mixed with water and alcohol (1500ml). The MIIES powder was mixed with water for 3 days and filtered with a muslin cloth and condensed in hot air oven at 50°C.Coarsely powdered material was soaked in ethanol for 3 days, filtered and allowed to condense at 50°C. Both extracts were stored in a container and refrigerated for future use.

Antiulcer study

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 model

Ulcer was induced by administering acid alcohol (0.3M hydrochloric acid in 60% ethanol). All the animals were fasted for 36 h before administration of acid alcohol. The animals were divided into nine groups, each consisting of six rats. Rats in group I, served as control, received saline (1 ml) orally. Group II rats were given only acid alcohol, which are considered as a disease control. Rats in group IV, V and VI received MIIESAE at doses of 50, 100 and 200 mg/kg bw, respectively. Similarly rats in groups VII, VIII and IX received MIIESPE at doses of 50, 100 and 200 mg/kg bw, respectively. Gastric ulcers were induced in rats by administrating acid ethanol orally, after 45 min of MIIES and ranitidine treatment. They were kept in specially constructed cages to prevent coprophagia during and after the experiment. The animals were anaesthetized 1 h later with anaesthetic ether and the stomach was incised along the greater curvature and ulceration was scored [7]. Rats in group III were administered with ranitidine (32mg/kg) as a standard reference drug.

Determination of total acidity [8] - Standard methods were adopted to study total acidity of gastric fluid.

Calculation of ulcer score - Ulcer Index - Calculation of Ulcer Index [9]: 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 [10]

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

Evaluation of pH [11]

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.

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Evaluation of gastric acidity

The acid content of the stomach was calculated according to the method of Shay *et al.*,[12] (1954) and expressed as MEq/L. Acidity = Vol. of NaOH × N × 100mEq/L/0.1

Estimation of protein/pepsin

Protein content was estimated by the method of Lowry *et al.*,[13].

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 [14]; SOD [15] and GSH [16].

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.

3. RESULTS

` Acid alcohol was used to induce ulcer in animals to assess the antiulcer activity of MIIES extracts. Aqueous and phenolic extracts at three dosage levels were used for the treatment (Group IV, V, VI, VII, VIII and IX). Group III animals were treated with ranitidine whereas group II was considered as disease control. Lower pH and total and free acidity were noticed in gastric juice of group II animals, which primarily indicated the presence of ulcer induction process and was not neutralized at any means. Similarly increased pH and increased total and free acidity was noted in extract treated and ranitidine treated animal groups which indicated the role of extracts on ulcer prevention process.

Table-1 Effect of MIIES extracts in ulcer prevention

S. No	Group	Treatment	Ph	Total Acidity (m eq/L)	Free Acidity(m eq/L)	Ulcer Score	Ulcer No / animal	Ulcer %	Ulcer Index
1	I	Normal Control	2.95±0.03	05.1±0.5	2.53±0.24	0.00±0.00	0	0	0
2	II	Disease Control	1.11±0.01	18.9±0.4	9.48±0.22	4.66±1.36	8	100	3
3	III	Treatment Control	2.96±0.01	06.3±0.1	3.16±0.05	2.66±0.81	1	12.5	0.5
4	IV	MIIESAE (50mg/kg/bw)	1.82±0.01	16.8±0.5	8.40±0.23	4.16±0.75	6	75	1.5
5	V	MIIESAE (100mg/kg/bw)	2.62±0.18	12.1±0.2	6.07±0.11	4.00±0.63	3	37.5	1
6	VI	MIIESAE (200mg/kg/bw)	2.80±0.01	06.9±0.5	3.45±0.25	2.83±0.75	2	25	0.5
7	VII	MIIESPE (50mg/kg/bw)	2.49±0.01	17.4±0.4	8.74±0.20	4.50±0.83	5	62.5	1
8	VIII	MIIESPE (100mg/kg/bw)	2.78±0.01	12.8±0.3	6.41±0.15	4.16±0.75	3	37.5	1
9	IX	MIIESPE (200mg/kg/bw)	2.87±0.01	08.2±0.2	4.13±0.09	3.66±0.51	1	12.5	0.5

Ulcer score, ulcer number and ulcer index was also very high in group II animals when compared to normal and treated animal groups. One animal in phenolic extract treated group and ranitidine treated group showed low level ulcer with one ulcer number, 12.5 ulcer percentage and 0.5 ulcer index. This indicated the effectiveness of phenolic extract (group IX). Aqueous extract

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at 200mg/kg/b.w also showed good ulcer prevention (Group VI). These group animals also showed low frequency of ulcer formation with 0.5 ulcer index and 2.83±0.75 ulcer score. Phenolic extracts treated animals of Gp IX showed good ulcer control with only 12.5% incidence of ulcer and only one animal showed ulcer with 0.5 ulcer score. MIIESPE effectively controlled ulcer formation. Phenolic extract produced 87.5% protection of ulcer at 200mg/kg/bw concentration.

Ulcer is one of the inclining factors for the reduction of digestion, which leads to malnutrition and other health related issues. Table 2 indicated the levels of pepsin and amylase in normal, ulcer induced and treated animal groups. Higher quantities of pepsin and amylase were detected in normal animals (Group I), where as it was significantly reduced in acid ethanol ulcer induced animal groups. Plant extracts and ranitidine treated animal groups recaptured its enzyme secretion, which are not disturbed in normal digestion and absorption mechanisms. This showed that extracts prevented ulceration and inflammation there by enzyme secretion was not stopped but only acid alcohol disturbed peptic mechanisms which ultimately disturbs enzyme secretion.

Table-2-Effect of MITFES extracts in digestive enzymes secretion in ulcer induced animal models

S. No	Group	Treatment	Pepsin	Amylase
1	I	Normal Control	1540.5±32.8	250.3±8.3
2	II	Disease Control	0249.6±39.5	070.8±5.1
3	III	Treatment Control	1403.2±31.9	211.6±7.7
4	IV	MIIESAE (50mg/kg/bw)	0356.4±33.1	089.3±5.0
5	V	MIIESAE (100mg/kg/bw)	0962.2±45.4	139.9±6.6
6	VI	MIIESAE (200mg/kg/bw)	1351.6±31.5	205.7±7.1
7	VII	MIIESPE (50mg/kg/bw)	0334.1±47.4	083.7±6.1
8	VIII	MIIESPE (100mg/kg/bw)	0906.9±46.2	129.6±7.4
9	IX	MIIESPE (200mg/kg/bw)	1289.3±31.2	200.5±6.2

MIIESs ethanolic and phenolic extracts are considered as good antioxidants which is evidenced in assessing stomach tissues of ulcer induced extract treated animal models (Table 3). Levels of LPO, SOD, GSH and Catalase were normalized in extract and ranitidine treated animal tissues, which could be due to extracts prevented inflammation and other pathological events in stomach. In Group II animals ulcer index is 3 and all the animals of this group showed ulcer and ulcer score is 4.66. This group also showed 410.4±2.6 LPO, which is far high when compared to group I and Group IX animals and also extract treated animal groups. SOD level is very low in group II animals, which is normalized in MIIES extract treated animal groups.

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Table-3-Effectiveness of MITFES extracts on antioxidants of stomach tissues of ulcer induced animals.

S. No	Group	Treatment	LPO	SOD	GSH	Catalase
1	I	Normal Control	162.6±2.8	0.162±0.004	2433.8±63.9	343.3±05.6
2	II	Disease Control	410.4±2.6	0.032±0.009	0569.2±34.2	044.4±13.5
3	III	Treatment Control	169.8±1.9	0.153±0.006	2413.3±36.7	330.3±06.9
4	IV	MIIESAE (50mg/kg/bw)	279.5±3.9	0.066±0.007	0882.7±67.1	081.8±11.1
5	V	MIIESAE (100mg/kg/bw)	218.3±5.2	0.085±0.006	1828.7±78.5	171.8±14.3
6	VI	MIIESAE (200mg/kg/bw)	171.8±3.5	0.140±0.011	2376.6±55.3	317.9±02.1
7	VII	MIIESPE (50mg/kg/bw)	291.2±7.4	0.041±0.008	0796.5±47.4	068.5±15.1
8	VIII	MIIESPE (100mg/kg/bw)	220.3±4.4	0.081±0.004	1760.0±86.4	149.7±16.2
9	IX	MIIESPE (200mg/kg/bw)	174.8±2.7	0.136±0.008	2258.7±68.7	311.6±07.5

The levels of protein concentration in the gastric juice of different groups of animals received varying doses of aqueous and phenolic extracts (MIIEAE and MIIESPE), which was found to decrease significantly proving the efficacy of the treatment **(**Table -4**).** Increase in protein content of gastric juice resulted in a decrease in total carbohydrate and protein ratio in aspirin induced animal stomach leading to the damage of gastric mucosa. Treatment with MITFES extracts decreased the plasma protein leakage from gastric mucosa by strengthening the mucosal barrier. Total hexoses, hexosamine and fucose concentration also increased significantly in MIIES extracts treated animal groups and standard drug treated group. Mucus acts as defensive factor by preventing physical damage to mucosa leading to a lesser degree of ulceration. The individual carbohydrates such as hexoses, fucose and hexosamine concentrations are the index of mucin content in gastric juice.

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Table-4-Effect of MITFES extracts on gastric carbohydrate and Protein

S. No	Group	Treatment	Hexose	Hexosamine	Fucose	Protein
1	I	Normal Control	0.19±0.013	0.10±0.007	0.22±0.007	3.74±0.03
2	II	Disease Control	0.38±0.003	0.21±0.012	0.36±0.017	1.08±0.07
3	III	Treatment Control	0.22±0.003	0.13±0.001	0.24±0.24	4.13±0.32
4	IV	MIIESAE (50mg/kg/bw)	0.37±0.002	0.19±0.005	0.32±0.001	2.17±1.08
5	V	MIIESAE (100mg/kg/bw)	0.31±0.008	0.17±0.004	0.31±0.004	2.73±0.04
6	VI	MIIESAE (200mg/kg/bw)	0.27±0.003	0.15±0.002	0.29±0.002	3.67±0.03
7	VII	MIIESPE (50mg/kg/bw)	0.32±0.003	0.17±0.003	0.31±0.004	2.18±0.26
8	VIII	MIIFESPE (100mg/kg/bw)	0.28±0.005	0.15±0.003	0.29±0.001	3.11±1.13
9	IX	MIIESPE (200mg/kg/bw)	0.23±0.003	0.14±0.002	0.26±0.003	3.83±1.37

4. DISCUSSIONS

Acid alcohol induced ulcer model was used to assess antiulcer activity of ethanolic and phenolic extract of *Mangifera indica* immature endosperm. Ethanol induces imbalance of acidity, mucous and hormones and induces ulcer formation [17, 18]. *M. indica* immature endosperm phenolic extract significantly reduces mean ulcer count. It may be due to anti secretory effect of *M. indica* immature endosperm, which significantly reduces the formation of ulcers (Table-1). It clearly indicates the 200 µg of extracts act as antiulcer medicine. It also increases the pH, reduce the acidity of stomach and also it reduces the protein content in ulcer affected animals (Table-1 & 4). Phytochemical constituents of the *M. indica* endosperm phenolic 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.*, [19] revealed the presence of flavonoids, phenolic compounds, tannins; alkaloids in phenolic extracts of *M. indica* endosperm. Phenols, Tannins, flavonoids were responsible for antiulcer activity. Flavonoid prevents ulcer by free radicals scavenging mechanisms. In Vivo antioxidant assay (LPO, SOD and GSH) revealed that *M .indica* endosperm could be considered as an effective antioxidant compound and prevents ulcer and its related complications (Table 3). Tannins have been reported to possess antioxidant, wound healing, antimicrobial and antiulcer activity. *M. indica* endosperm phenolic extract could be considered as a good antiulcer agent may be due to the green chemicals found on the plant materials. Polyphenolics substance of this plant also may responsible for antioxidant and antiulcer activity [20].

The present study proved the potentials of MIIESPE significantly reduced gastriculceration as indicated by the reduction in ulcer index in the acid alcohol induced animal models. These results suggested that both extracts possesses anti-secretary potency as well as acid neutralizing effect. Furthermore, based on findings of Ubaka *et al.*, [21] the anti-secretory effect is suggested to be one of the mechanism through which the extracts were able to protect the stomach mucosa from acid and alcohol induced damage. Abdulla *et al.*, [22] demonstrated

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that the reduction of neutrophil infiltration into ulcerated gastric tissues helped to prevent gastric ulcers in rats. Wasman *et al.*, [23] showed that the oral administration of plant extract prior to ethanol administration significantly decreased neutrophil infiltration into the gastric mucosa. Ethanol causes extensive damage to the gastric mucosa and leads to increased neutrophil infiltration into this tissue.

Level of catalase, SOD and GSH were high among the tissues treated with herbal extracts and ranitidine. Variable antioxidant enzyme level was noted in Gp II. Other group (Gp III, IV, V, VI and VII) animals regained the nature of antioxidant enzymes as normal. SOD converts superoxide to hydrogen peroxide (H_2O_2), which is converted into water by catalase in the lysosomes or by glutathione peroxidase in the mitochondria [24]. MDA is the final product of lipid peroxidation and is used to determine lipid peroxidation levels [25]. Lipid peroxidation causes a loss of membrane fluidity, impaired ion transport and membrane integrity and ultimately a loss of cellular function. Oxidative stress plays an important role in the pathogenesis of various diseases including gastric ulcer, with antioxidants being reported to play a significant role in the protection of gastric mucosa against various necrotic agents [26]. Antioxidants could help to protect cells from damage caused by oxidative stress while enhancing the body's defense systems against degenerative diseases. Administration of antioxidants inhibits ethanol-induced gastric injury in rat [27]. In addition, phenolic compound are the major contributors to the antioxidant activities of MIIESPE.

5. CONCLUSION

This study revealed that ethanolic and phenolic extracts of MIIES reduces Ulcer score, ulcer number and ulcer index in treated animal groups. MIIESPE effectively controlled ulcer formation. Phenolic extract produced 87.5% protection of ulcer at 200mg/kg/b.w concentration. Hence, it exhibits very good antiulcer activity.

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