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EVALUATION OF ANTIULCER ACTIVITY OF *MANGIFERA INDICA* KERNEL, VITAMINS AND ZINC SULPHATE ON PYLORUS LIGATION AND ETHANOL INDUCED ULCER MODELS IN RATS

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ABSTRACT

The present study was designed to evaluate the anti-ulcer activity of ethanolic extract of *Mangifera indica* kernel (EEMI) alone and in combination with Menadione, Zinc sulphate and Vitamin C in ethanol and pylorus ligation induced peptic ulcers in rats. The ethanolic extract of *Mangifera indica* kernel was studied in two dose levels (200 and 400mg/kg, oral). The effect of extract was studied by measuring the ulcer index, percentage protection, in vivo antioxidant levels and histological changes in the stomach tissue of treated animals. The volumes of gastric secretion, free and total acidity were also estimated in pylorus ligated rats. In the present study EEMI, Menadione, Zinc sulphate and Vitamin C showed a significant gastroprotective activity and antioxidant property in a dose dependent manner. The ethanolic extract of *Mangifera indica* kernel (EEMI) with a dose of 400mg/kg body weight has shown better gastroprotective activity than 200mg/kg body weight in both ethanol and pylorus ligation induced ulcer models in rats. Treatment in combination of EEMI with Menadione, Zinc sulphate and Vitamin C has shown no significant higher gastroprotective potential than the individual treatment.

Key words: *Mangifera indica*, Anti-ulcer, Ethanol, Pylorus ligation.

INTRODUCTION

Peptic ulcer disease manifests as a result of imbalance between defensive factors and aggressive factors. When the defensive factors like mucus, mucosal blood flow, formation of HCO_3^- and prostaglandins like PGE3 gets impaired or overpowered by the aggressive factors such as acid, pepsin, NSAIDs and *Helicobacter pylori* infection. Peptic ulcer may also be induced by variety of other factors such as stress, emotion, anxiety, depression, excessive gastric secretions, mortality of stomach, smoking, psychological stress and also by modern sedentary life style. Hence peptic ulcer is regarded as a multifactorial gastrointestinal disorder which also includes generation of free radicals.

It has been now established that oxygen derived free radicals primarily super oxide (O^{-}) anion and hydroxyl radical (OH^-) play very important role in pathogenesis of acute experimental gastric lesions induced by stress, ethanol and NSAIDs (Nataraj HN *et al*., 2012). It has been postulated that pathophysiology involved in peptic ulcer disorder is the generation of free radicals in addition to increased and prolonged acid secretion (Odabasoglu F *et al*., 2006).

A number of antiulcer drugs like gastric anti-secretory drugs, H2-receptors antagonists, anti- muscarinic drugs, proton pump inhibitors and mucosal protective agents are in most common usage as a remedy for peptic ulcer. In most of cases, incidence of relapses and adverse reactions are seen in the synthetic antiulcer therapy. Hence it is important to introduce a safe drug (or more) of natural origin, to be used for the management of gastric ulcers without side effects (Awaad AS *et al*., 2012)

Upon literature survey, it was observed that *Mangifera indica* Kernels are rich in Polyphenol and flavonoids, which are powerful antioxidants. (Masibo M

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et al., 2008). Since oxidative stress is one important mechanism leading to ulcerative complications, the anti-oxidant property of kernels extract would be helpful in combating ulcer.

Alcohol consumption increases the risk of ulcers in patients taking NSAIDS by impairing zinc absorption. Since zinc is essential for cell proliferation, differentiation and viability, its deficiency can cause poor wound healing and elevation in ROS stress which may induces oxidative damage and alterations in the antioxidant defence system. (Xu XMD *et al*., 2012). The concept of zinc compounds exhibiting antiulcer effects is supported by extensive experimental data. The protective effects of zinc compounds against gastric damage are accomplished through several mechanisms such as prevention of disruption of the mucosal barrier, modifications in PGE levels, mucus production, and improvement of gastric microcirculation. Zinc compounds have also shown additional inhibitory effects on pepsin and acid secretion (Conchillo A *et al*., 1995)

It has been reported that Menadione (Vit K) enhances the mitogenic signal of EGF via MAPK/ERK pathways and there by exists a direct association between increased ERK activity and healing of gastric ulcers. There are reports suggesting pretreatment of rats with Menadione significantly protects the gastric mucosa against pylorus ligation and ethanol- induced gastric lesion and also significant inhibition of gastric acid secretion (Tariq M *et al*., 2005). Ascorbic acid (Vitamin C) is a water-soluble antioxidant. Antiulcer activity on Vitamin C has been reported through both preclinical and clinical studies (Cuevas VM *et al*., 2011).

Therefore, vitamin K, zinc sulphate, vitamin C are taken for study to validate its use in ulcer treatment in rats. There is no scientific data available for usage of *Mangifera indica* kernel alone and in combination with vitamin K (Menadione), zinc sulphate and vitamin C for anti-ulcer activity in rats. Hence the study was planned to screen *Mangifera indica* kernel alone and its combination with vitamin K, zinc sulphate and vitamin C using pylorus ligation and ethanol induced ulcer experimental models in rats.

MATERIAL AND METHODS

Plant

The kernels of *Mangifera indica* Linn were collected from Hopscom, Bangalore (Karnataka). The plant herbarium specimen was identified and authenticated by Dr. MADHAVA CHETTY, Dept. of Botany, Sri Venkateswara University, Tirupati-517502, A.P, India.

Experimental Animals

Healthy female mice weighing (20-25g) were used for acute toxicity studies. Healthy Albino wistar rats of either sex, age between 12 to 13 weeks and weighing between 150-200 g were taken for the evaluating antiulcer study. Animals were procured from Drug testing laboratory, Bangalore. They were housed in polypropylene cages containing bedding material as husk and maintained under controlled conditions of temperature (25±2C), humidity (55±5%), 12h light/dark cycles in animal house facility of Government College of Pharmacy, Bangalore. They were fed with commercial pelleted rat chow (Shri Venkateshvara Enterprises, Bangalore) with water ad Libitum. The study was conducted after obtaining the approval from Institutional Animal Ethical Committee (IAEC) of Government College of Pharmacy, Bangalore (DCD/GCP/ 20/E.C/ ADM/ 2012-13).

Preparation of the extracts

The shadow, air dried kernel seeds of *Mangifera indica* were powdered and used for the extraction. The coarse powder was given to GREEN CHEM, in order to carry out the extraction procedure using ethanol as a solvent. The vacuum concentrated residue was collected from GREEN CHEM and stored in air tight container at room temperature for further use.

Phytochemical Screening (Tiwari P *et al.*, 2011)

The extracts obtained were then subjected to qualitative chemical examination for identification of various plant constituents. The phytochemical examinations were carried out for the extracts as per the standard methods.

Acute Toxicity Study

Acute toxicity study (OECD 420 guidelines) was carried out using female albino mice (20 - 25g). The maximum upper limit dose 2000 mg/kg of *Mangifera indica* was administered orally to 3 female mice. Animals were observed for 48 h to study the general behavior of animals, signs of discomfort and nervous manifestation.

Evaluation of Antiulcer activity

Anti-ulcer activity was assessed using two screening models, namely Ethanol and Pylorus ligation induced ulcer models in rats. The biochemical estimations like stomach LPO and GSH were carried out to validate the potency of the activity in animal models.

Gastric Lesions Induced by Ethanol in Rats

(Cytoprotection Studies) (Kulkarni SK, 2005) Rats were divided into 17 groups of 6 animals in each group.

Group 1: Negative control; received only vehicle.

Group 2: Positive control (1 ml absolute ethanol p.o).

Group 3: Standard group (Lansoprazole 8mg/kg in 2% gum acacia p.o).

Group 4 and 5: Test group; received EEMI 200mg/kg and 400mg/kg p.o. respectively.
Group 6, 7 and 8: Test groups; received Vitamin K (Menadione) at dose of 5, 15 and 45 mg/kg i.p respectively.
Group 9, 10 and 11: Test groups; received Vitamin C at the dose of 30, 50, 70 mg/kg i.p respectively.
Group 12, 13 and 14: Test groups; received Zinc sulphate at the dose of 20, 40, 80 mg/kg i.p respectively.
Group 15: Test group; received EEMI (400mg/kg p.o.) + Menadione (15 mg/kg i.p).
Group 16: Test group; received EEMI (400mg/kg p.o.) + Zinc sulphate (40 mg/kg i.p).
Group 17: Test group; received EEMI (400mg/kg p.o.)+ Vitamin C (40 mg/kg i. p).

On the day of experiment, the animals were fasted for 24 hour with free access to water. Animals were given the ethanolic extract of *Mangifera indica* standard Lansoprazole/Vitamin K/ Vitamin C/Zinc sulphate as mentioned above. Absolute alcohol at the dose of 1ml/200 gm was administered into each animals on the day of the experiment 1 hour after the administration of extracts and standard. The animals were sacrificed 1 hour after administration of ethanol and the stomach was removed and opened along the greater curvature. Lesions were examined with the help of hand lens (10X) and sample was sent for further histopathological study.
Scoring was done for all the models as given below:

0 = normal stomach, 0.5 = red coloration.
1.0 = spot ulcers. 1.5 = hemorrhagic streaks.
2.0 = ulcer > 3 but < 5, 3.0 = ulcer > 5.

In this method following parameters was studied-1. Ulcer index. 2. % Protection.

Mean ulcer score for each animal is expressed as ulcer index. The percentage protection was calculated using the formula,

$$\text{Percentage protection} = \frac{100 - Ut \times (100)}{Uc}$$

Where, Ut = ulcer index of treated group. Uc = ulcer index of control group

Pylorus Ligation Induced Ulcer Model in rat (Sanyal AK *et al.,* 1982; Virupakasha JH *et al.,* 2007)
Rats were divided into 11 groups of 6 animals in each group and treated as follows:
Group 1: Negative control, received only vehicle.
Group 2: Positive control.
Group 3: Standard group; received (Lansoprazole 8mg/kg in 2% gum acacia p.o.).
Group 4 and 5: Test groups; received EEMI 200 and 400mg/kg p.o. respectively.
Group 6, 7 and 8: Test group; received Menadione (15mg/kg i.p), Vitamin C (50 mg/kg I p) and Zinc sulphate (40 mg/kg i.p) respectively.
Group 9: Test group; received EEMI (400mg/kg p.o.) + Menadione (15 mg/kg I p). Group 10: Test group; EEMI (400mg/kg p.o.) + Vitamin C (50 mg/kg i.p).
Group 11: Test group; received EEMI (400mg/kg p.o.). + Zinc sulphate (40 mg/kg i.p).

In this method, albino rats were fasted in individual cages for 24 hr. Ethanolic extract of *Mangifera indica*/standard lansoprazole / Vitamin K/Vitamin C/Zinc sulphate administered 30 min prior to pyloric ligation. Under light ether anesthesia, the abdomen was opened and pylorus was ligated. The abdomen was then sutured. At the end of 6 hours after ligation the animals were sacrificed with excess of anesthetic ether. The stomach was dissected out, gastric juice was collected, were drained into tubes and centrifuged at 1000 rpm for 10 minutes and the volume was noted. Then the contents were subjected to analysis for free and total acidity. The stomach was then washed with running water to see ulcers in the glandular portion of the stomach. The number of ulcers per stomach was noted and severity of the ulcers scored microscopically with the help of hand lens (10X) and scoring was done as following.
0 = normal stomach, 0.5 = red coloration,
1.0 = spot ulcers, 1.5 = hemorrhagic streaks,
2.0 = ulcer > 3 but < 5, 3.0 = ulcer > 5.

Mean ulcer score for each animal is expressed as ulcer index.
The percentage protection was calculated using the formula,

$$\text{Percentage protection} = \frac{100 - Ut \times (100)}{Uc}$$

Where, Ut = ulcer index of treated group. Uc = ulcer index of control group.

Determination of free acidity and total acidity

One ml of gastric juice was pipetted into 100ml conical flask, 2 to 3 drops of topfer's reagent was added and titrated with 0.01N NaOH until all traces of red color disappears and the color of the solution turns to yellowish orange. The volume of the alkali added was noted. This volume corresponds to free acidity. Then 2 to 3 drops of phenolphthalein solution was added and titration was continued until a defined red tinge reappears. Again the total volume of alkali added was noted. The volume corresponds to total acidity. Acidity was calculated by following formula:

$$\text{Acidity} = \frac{\text{Vol. of NAOH} \times \text{Normality of NaOH} \times 100 \text{ meq /L/100gm}}{0.1}$$

***In-vivo* anti-oxidant studies**

Tissue preparation

After scoring the ulcers, stomach was perfused with an ice-cold saline. The whole stomach dissected out, blotted dry and immediately weighed. A 10% stomach homogenate was prepared with ice-cold phosphate

buffered saline using Teflon glass homogenizer. The homogenate was centrifuged at 10,000 rpm at -40º C for 15 min and the pellet discarded. The supernatant obtained was used for biochemical estimations.

Lipid peroxidation (LPO)

Briefly, to a test tube containing 0.1ml homogenate, 1 ml of TBA reagent containing equal proportions of 0.375% TBA, 15% TCA and 0.25 N HCl was added and placed in a boiling water bath for 30 min. Then the mixture was placed in crushed ice for 10 min followed by centrifugation at 6000 rpm for 5 min. The absorbance of the clear pink color supernatant was measured at 532 nm against appropriate blank. The amount of lipid peroxidation was determined by using = 1.56 x105 M-1 cm-1 and the results were expressed as nmoles MDA/mg protein.

Reduced Glutathione (GSH)

Briefly, 0.5 ml of homogenate is mixed with 0.1 ml of 25% thiochloroacetic acid to precipitate proteins and centrifuged at 4000 rpm for 5 min. Then 0.3ml of the supernatant was mixed with 0.5 ml of 0.1 M phosphate buffer (pH 7.4) and 0.2 ml of 10 mM 5,5- dithiobis(2-nitrobenzoic acid). This mixture was incubated for 10 min and the absorbance was measured at 412 nm against appropriate blanks. The glutathione content was calculated by using extension coefficient 13.6 x 103 M-1 cm-1. The values are expressed as nmoles/mg protein.

Histopathological studies (Luna LG, 1986)

The stomach tissues were processed and embedded in paraffin wax. The central part of damaged or ulcerated tissue (if present) was cut on half along the long diameter. If the stomach was protected from the damage, then the section was taken from basal part using a rotary microtome, sections of thickness of about 5 µm were cut and stained with heamatoxylin and eosin. These were examined under the microscope for histopathological changes and photographs were taken.

Statistical analysis

The values were expressed as mean ± S.E.M. The statistical analysis was carried out by one way analysis of variance (ANOVA) followed by Tukey post hoc test. P values < 0.05 were considered as significant.

RESULTS

Acute toxicity studies

Acute toxicity studies of Ethanolic extract of *Mangifera indica* kernel were determined in mice as per OECD Guidelines No. 420. The extract was administered at a dose of 2000 mg/Kg and no mortality in treated group was observed. Hence, 1/10th (200mg/kg) and 1/5th (400 mg/kg) doses from the maximum LD50 dose tested were selected for the present study.

Preliminary Phytochemical Screening

To screen the phytochemical constituents of the selected kernel part, the obtained extract was subjected to appropriate preliminary chemical analysis. Some of the active constituents were analysed such as glycosides, alkaloids, triterpenoids, saponins, steroids, mucilage, tannins, phenolic compounds and flavonoids. It was observed that the important active constituents are found in ethanolic extract, hence EEMI was selected for the intended study.

ANTIULCER ACTIVITY

Histopathological observations

The histopathological sectional studies of gastric mucosa in negative control, positive control, standard and all the treated groups of animal show intact architecture. The mucosal layer of negative control and standard group has shown secreting lining of epithelial cells but the lack of secreting lining of epithelial cells has been produced in positive control group. Groups of animals which have had been given the lower dose of treatment, most of mucosal layer shows degenerated epithelial cells along with few intact secreting epithelial cells and moderate inflammatory infiltrations whereas groups of animals treated with higher dose of treatment and combinational treatment, the mucosal layer shows secreting lining of epithelial cells along with few degenerated epithelial cells and scant inflammatory infiltrations.

The submucosal layer appears to be intact in negative control and standard group, but there is severe edema with congested vascular spaces and moderate edema in the submucosal layer of positive control and lower dose treatment group respectively. The EEMI (400mg/kg), menadione 45mg/ kg, ZnSO4 80mg/kg, vitamin C 50mg/kg and combinational treatment has produced reduction in edematous state of submucosal layer. The muscular and serosal layers appear unremarkable in all the groups.

DISCUSSION

The study was planned to evaluate the anti- ulcer and anti-oxidant effect of EEMI kernel on various ulcer models like ethanol and pylorus ligation induced ulcer in rats. Since Menadione, Zinc sulphate, Vitamin C has been reported to possess antiulcer activity, hence the study was planned to validate the effect of Menadione, Zinc sulphate, Vitamin C alone at graded doses and also in combination with EEMI. The cytoprotective activity was assessed in rats using ethanol induced ulcer models. EEMI has produced significant decrease in ulcer index with a dose of 400 mg/kg b.w (2.66) and 200 mg/kg b.w (3.46) compared to control group of animals (4.37) and increase in percentage protection with a dose of 400 mg/kg b.w (48.04%) and 200 mg/kg b.w (26.75%) as shown in table No 1 and fig No 1. The dose of 400 mg/kg of EEMI

against ethanol induced ulcer model has showed better cytoprotective activity than 200 mg/kg.

Table 1. Effect of oral administration of EEMI and I.P. administration of Menadione, Zinc Sulphate, Vitamin C on ulcer index and their percentage protection in ethanol induced ulcer in rats (n=6).

Group	Treatment	Dose mg/kg	Mean Ulcer Index ±SEM	% age Protection
1	Positive control (Ethanol)	-	5.12± 0.24	-
2	Standard (Lansoprazole)	8	1.5± 0.166 ***	70.70
3	EEMI	200	3.75± 0.478 *	26.75
4	EEMI	400	2.66± 0.333***	48.04
5	Menadione	5	5.0± 0.0122 ns	2.3
6	Menadione	15	3.12± 0.440**	39.06
7	Menadione	45	1.16± 0.333***	77.34
8	Zinc Sulphate	20	3.2± 0.012**	37.5
9	Zinc Sulphate	40	2.66± 0.667***	48.04
10	Zinc Sulphate	80	1.66± 0.167***	67.57
11	Vitamin C	30	3.7± 0.233*	27.73
12	Vitamin C	50	2.5± 0.441***	51.17
13	Vitamin C	70	3.16± 0.005 ns	18.75
14	EEMI+Menadione	(400 + 15)	3.12± 0.726**	39.06
15	EEMI+Zinc Sulphate	(400 + 40)	2.6± 0.333***	49.21
16	EEMI+Vitamin C	(400 + 50)	4.0± 0.441ns	21.87

Each value is expressed as mean ± SEM (n = 6), where, NS represents non-significant; ***P<0.001 – highly significant; **P<0.01- very significant; *P<0.05- significant, when treated groups are compared to control group rats. One-way ANOVA followed by Tukey's multiple comparison tests. EEMI-Ethanolic extract of *Mangifera indica* kernel.

***In-vivo* models for Anti-Oxidant activity**

Table 2. Effect of oral administration of EEMI and I.P. administration of Menadione, Zinc Sulphate, Vitamin C on reduced Glutathione and Lipid peroxidation in ethanol induced ulcers in rats (n=6)

Group	Treatment	Dose mg/kg	GSH Mean ± SEM	LPO Mean ± SEM
1.	Positive control(Ethanol)	-	0.04367±0.002	4.374±0.099***
2.	Standard(Lansoprazole)	8	0.3352±0.015***	1.265±0.046***
3.	EEMI	200	0.0975±0.00 *	3.465±0.127**
4.	EEMI	400	0.2325±0.014***	1.105±0.020***
5.	Menadione	5	0.0603±0.001 ns	3.579±0.107***
6	Menadione	15	0.0691±0.001 ns	2.989±0.029***
7	Menadione	45	0.1247±0.007***	1.342±0.023***
8	Zinc Sulphate	20	0.1013±0.002*	3.483±0.115***
9	Zinc Sulphate	40	0.1475±0.015***	2.846±0.151***
10	Zinc Sulphate	80	0.2785±0.008***	1.232±0.041***
11	Vitamin C	30	0.09433±0.002 ns	2.385±0.107
12	Vitamin C	50	0.2135±0.005**	2.197±0.067***
13	Vitamin C	70	0.2747±0.009***	2.462±0.0619*
14	EEMI+Menadione	(400 + 15)	0.1925±0.009**	3.285±0.172***
15	EEMI+Zinc Sulphate	(400 + 40)	0.2972±0.021***	1.272±0.013***
16	EEMI+Vitamin C	(400 + 50)	0.1963±0.006**	2.163±0.033***

Each value is expressed as mean ± SEM (n = 6), where, NS represents non-significant; ***P<0.001 – highly significant; **P<0.01- very significant; *P<0.05- significant, when treated groups are compared to control group rats. One-way ANOVA followed by Tukey's multiple comparison tests. EEMI-Ethanolic extract of *Mangifera indica* kernel.

Table 3. Effect of oral administration of EEMI and I.P. administration of Menadione, Zinc Sulphate, Vitamin C on ulcer index and their % protection in Pylorus ligation induced ulcer in rats(n=6).

Group	Treatment	Dose (mg/kg)	Mean Ulcer Index ±SEM	% age Protection
1	Positive control	-	3.984±0.1169	-
2	Standard(Lansoprazole)	8	0.6000±0.1304***	68
3	EEMI	200	2.730±0.0800**	25.6
4	EEMI	400	1.624±0.03487***	47.6
5	Menadione	15	2.220±0.08602**	35.5
6	Zinc Sulphate	40	1.606±0.05115***	48.01
7	Vitamin C	50	1.520±0.06621**	43
8	EEMI+Menadione	(400 + 15)	2.168±0.06621**	35.06
9	EEMI+Zinc Sulphate	(400 + 40)	1.610±0.03225***	48.9
10	EEMI+Vitamin C	(400 + 50)	3.088±0.07546**	41.6

Each value is expressed as mean ± SEM (n = 6), where, NS represents non-significant; ***P<0.001 – highly significant; **P<0.01- very significant; *P<0.05- significant, when treated groups are compared to control group rats. One-way ANOVA followed by Tukey's multiple comparison tests.EEMI-Ethanolic extract of *Mangifera indica* kernel.

Table 4. Effect of oral administration of EEMI and I.P. administration of Menadione, Zinc Sulphate, Vitamin C on gastric secretion following pylorus ligation in rats(n=6).

Group No.	Treatment	Dose (mg/kg)	Vol. of gastric juice (ml)	Free acidity (mEq/L) 100gm	Total acidity (mEq/L) 100 gm
1	Positive control	-	9.620±0.0374	64.00±0.442	132.4±0.509
2	Std (Lansoprazole)	8	3.000±0.0312***	24.60±0.50***	53.02±0.316***
3	EEMI	200	7.520±0.165*	40.40±0.509*	85.25±0.008*
4	EEMI	400	3.940±0.0672***	34.60±0.92***	59.46±0.574***
5	Menadione	15	4.500±0.1612**	37.40±0.509**	83.27±0.148**
6	Zinc Sulphate	40	3.260±0.0509***	29.60±0.24***	71.22±0.179***
7	Vitamin C	50	4.520±0.0860**	37.51±0.158**	80.44±0.288***
8	EEMI+Menadione	(400 +15)	4.200±0.031**	57.10±0.374**	123.1±0.118**
9	EEMI+ZnSo4	(400 +40)	2.500±0.158***	26.80±0.37***	58.38±0.135***
10	EEMI+Vitamin C	(400 +50)	5.560±0.0509*	62.30±0.200*	128.4±1.030**

Each value is expressed as mean ± SEM (n = 6), where, NS represents non-significant; ***P<0.001 – highly significant; **P<0.01- very significant; *P<0.05- significant, when treated groups are compared to control group rats. One-way ANOVA followed by Tukey's multiple comparison tests.EEMI-Ethanolic extract of *Mangifera indica* kernel.

In-vivo models for Anti-Oxidant activity

Table 5. Effect of oral administration of EEMI and I.P. administration of Menadione, Zinc Sulphate, Vitamin C on reduced Glutathione and Lipid peroxidation in pylorus ligation induced ulcers in rats (n=6).

Group No	Treatment	Dose (mg/kg)	GSH Mean ± SEM	LPO Mean ±SEM
1	Positive control	-	0.1120±0.003	0.7118±0.007296
2	Standard (Lansoprazole)	8	0.7325±0.014***	0.2010±0.006890*
3	EEMI	200	0.2170±0.011 ns	0.5892±0.01063***
4	EEMI	400	0.5080±0.0042***	0.2538±0.01027***
5	Menadione	15	0.3030±0.016 **	0.3627±0.008853**
6	Zinc Sulphate	40	0.6668 ± .220***	0.1980±0.002887***
7	Vitamin C	50	0.8413±0.0994***	0.1920±0.005304***
8	EEMI+Menadione	(400 + 15)	0.2817±0.00707*	0.4458±0.01780**

9	EEMI+Zinc Sulphate	(400 + 40)	0.7260±0.01030***	0.1710±0.009441***
10	EEMI+Vitamin C	(400 + 50)	0.3225±0.00895**	0.2303±0.008838***

Each value is expressed as mean ± SEM (n = 6), where, NS represents non-significant; ***P<0.001 – highly significant; **P<0.01- very significant; *P<0.05- significant when treated groups are compared to control group rats. . One-way ANOVA followed by Tukey's multiple comparison tests.EEMI-Ethanolic extract of *Mangifera indica* kernel.

Fig 1. Effect of oral administration of EEMI and I.P. administration of Menadione, Zinc Sulphate, Vitamin C on ulcer index and their percentage protection in ethanol induced ulcer in rats (n=6)

P- Positive control, S-Standard, E_1-Extract 200 mg, E_2-Extract 400mg, K-Vit-K 5mg, K-Vit k 15mg, K_3- Vit K 45 mg Z_1-Zinc Sulphate 20mg, Z_2- Zinc Sulphate 40 mg, Z_3- Zinc Sulphate 80 mg, C_1 Vit C 30mg, C_2-vit C 50mg, C_3- vit C 70mg, M_1-Extract 400mg + Vit K 15 mg, M_2-Extract+Zinc Sulphate 40 mg, M_3-Extract 400mg + vit C 50mg.

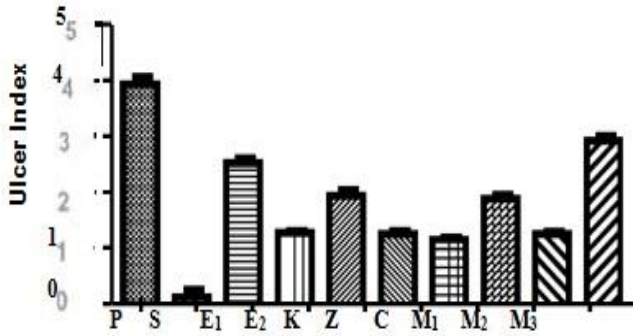
Fig 2(a). Effect of oral administration of EEMI and I.P. administration of Menadione, Zinc Sulphate, Vitamin C on reduced Glutathione in ethanol induced ulcers in rats (n=6)

N-Negative control, P- Positive control, S-Standard, E_1-Extract 200 mg, E_2-Extract 400mg, K-Vit-K 5mg, K-Vit 15mg, K_3- Vit K 45 mg Z_1-Zinc Sulphate 20mg, Z_2- Zinc Sulphate 40 mg, Z_3- Zinc Sulphate 80 mg, C_1 Vit C 30mg, C_2-vit C 50mg, C_3- vit C 70mg, M_1-Extract 400mg + Vit K 15 mg, M_2-Extract+Zinc Sulphate 40 mg, M_3-Extract 400mg +vit C 50mg.

Fig 2(b). Effect of oral administration of EEMI and I.P. administration of Menadione, Zinc Sulphate, Vitamin C on Lipid peroxidation in ethanol induced ulcers in rats (n=6).

N-Negative control, P- Positive control, S-Standard, E_1-Extract 200 mg, E_2-Extract 400mg, K-Vit-K 5mg, K-Vit k 15mg, K_3- Vit K 45 mg Z_1-Zinc Sulphate 20mg, Z_2- Zinc Sulphate 40 mg, Z_3- Zinc Sulphate 80 mg, C_1 Vit C 30mg, C_2- vit C 50mg, C_3- vit C 70mg, M_1-Extract 400mg + Vit K 15 mg, M_2-Extract+Zinc Sulphate 40 mg, M_3-Extract 400mg + vit C 50mg

Fig 3. Effect of oral administration of EEMI and I.P. administration of Menadione, Zinc Sulphate, Vitamin C on ulcer index in Pylorus ligation induced ulcer in rats (n=6).



P- Positive control, S-Standard, E_1-Extract 200 mg, E_2-Extract 400mg, K-Vit k 15mg, Z-Zinc Sulphate 40 mg, C- vit C50mg,, M_1-Extract 400mg + Vit K 15 mg, M_2-Extract+Zinc Sulphate 40 mg, M_3-Extract 400mg + vit C 50mg.

Fig 4(a). Effect of oral administration of EEMI and I.P. administration of Menadione, ZincSulphate, Vitamin C on gastric secretion following pyloric ligation in rats(n=6).

positive control, S-Standard, E_1-Extract 200 mg, E_2-Extract 400mg, K-Vit k 15mg, Z-Zinc Sulphate 40 mg, C- vit c 50mg,, M_1-Extract 400mg + Vit K 15 mg, M_2-Extract+Zinc Sulphate 40 mg, M_3-Extract 400mg + vit C 50mg.

Fig 4(b). Effect of oral administration of EEMI and I.P. administration of Menadione, Zinc Sulphate, Vitamin C on free acidity in Pylorus ligation induced ulcer in rats (n=6)

P- Positive control, S-Standard, E_1-Extract 200 mg, E_2-Extract 400mg, K-Vit k 15mg, Z-Zinc Sulphate 40 mg, C- vit C50mg,, M_1-Extract 400mg + Vit K 15 mg, M_2-Extract+Zinc Sulphate 40 mg, M_3-Extract 400mg + vit C 50mg.

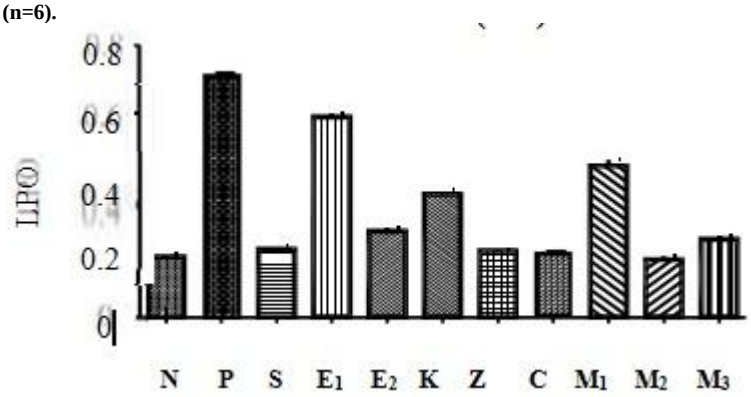
Fig 4(c. effect of oral administration of EEMI and I.P. administration of Menadione, Zinc Sulphate, min C on total acidity in Pylorous ligation induced ulcer in rats (n=6)

P- Positive control, S-Standard, E1-Extract 200 mg, E2-Extract 400mg, K-Vit k 15mg, Z-Zinc Sulphate 40 mg, C- vit C50mg, M1-Extract 400mg + Vit K 15 mg, M2-Extract+Zinc Sulphate 40 mg, M3-Extract 400mg + vit C 50mg.

Fig 5(a). Effect of oral administration of EEMI and I.P. administration of Menadione, Zinc Sulphate, Vitamin C on reduced Glutathione in pylorus ligation induced ulcers in rats (n=6)

P- Positive control, S-Standard, E1-Extract 200 mg, E2-Extract 400mg, K-Vit k 15mg, Z-Zinc Sulphate 40 mg, C- vit C 50mg,, M1-Extract 400mg + Vit K 15 mg, M2-Extract+Zinc Sulphate 40 mg, M3-Extract 400mg + vit C 50mg.

Fig 5(b). Effect of oral administration of EEMI and I.P. administration of Menadione, Zinc Sulphate, Vitamin C on Lipid peroxidation in pylorus ligation induced ulcers in rats (n=6).



N- Negative control P- Positive control, S-Standard, E1-Extract 200 mg, E2-Extract 400mg, K-Vit k 15mg, Z-Zinc Sulphate 40 mg, C- vit C 50mg,, M1-Extract 400mg + Vit K 15 mg, M2-Extract+Zinc Sulphate 40 mg, M3-Extract 400mg + vit C 50mg.

Fig 6. Histopathological study of EEMI, Menadione, Zinc sulphate, Vitamin C alone and in combination in Ethanol induced ulcer model in rats(n=6).

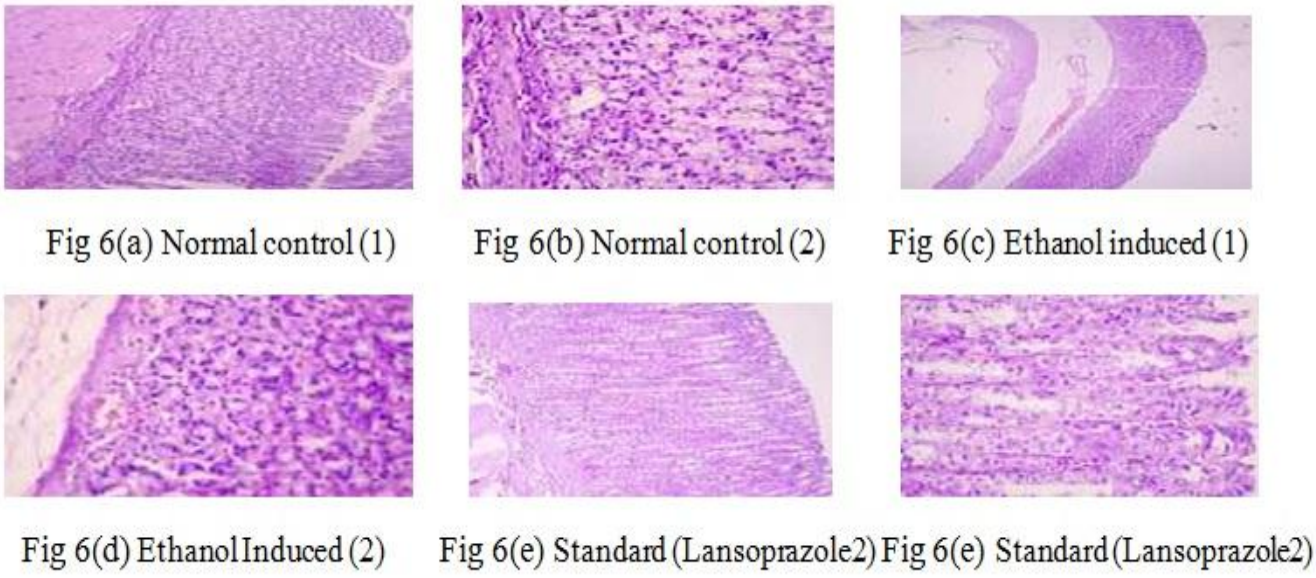


Fig 6(a) Normal control (1) Fig 6(b) Normal control (2) Fig 6(c) Ethanol induced (1)

Fig 6(d) Ethanol Induced (2) Fig 6(e) Standard (Lansoprazole2) Fig 6(e) Standard (Lansoprazole2)

Fig No 6(g)
EEMI-200 mg/kg (1)

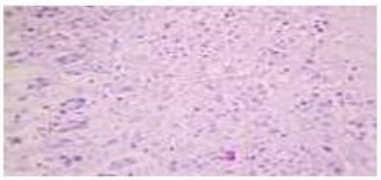


Fig No 6(h)
EEMI-200 mg/kg (2)

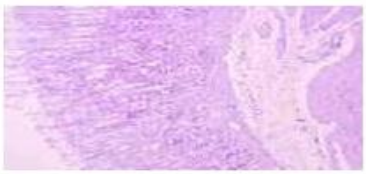


Fig No 6(i)
EEMI-400 mg/kg (1)

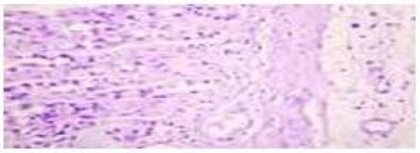


Fig No 6(j)
EEMI-400 mg/kg (2)

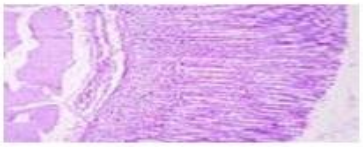


Fig No 6(k)
Menadione 45 mg/kg (1)

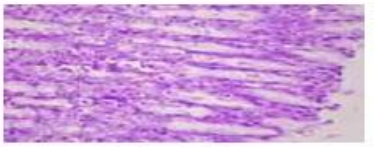


Fig No 6(l)
Menadione 45 mg/kg (2)

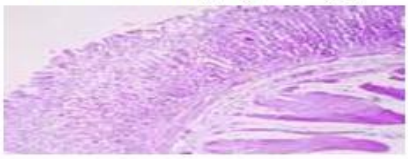


Fig No 6(j)
EEMI-400 mg/kg (2)

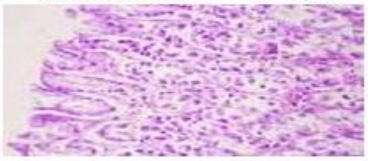


Fig No 6(k)
Menadione 45 mg/kg (1)

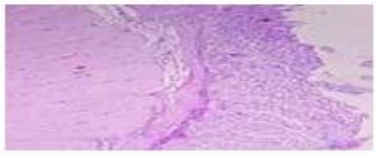


Fig No 6(l)
Menadione 45 mg/kg (2)

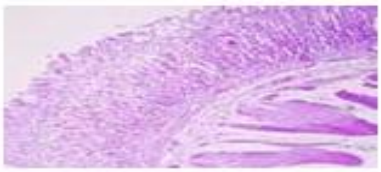


Fig No 6(m)
Zinc Sulphate 80 mg/kg (1)

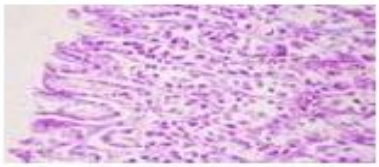


Fig No 6(n)
Zinc Sulphate 80 mg/kg (2)

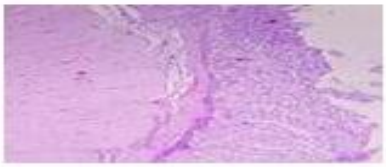


Fig No 6(o)
Vitamin C 50 mg/kg (1)

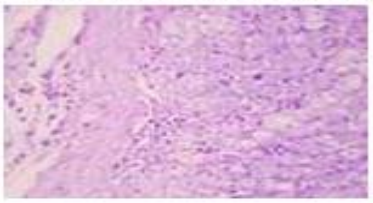


Fig No 6(p)
Vitamin C 50 mg/kg (2)

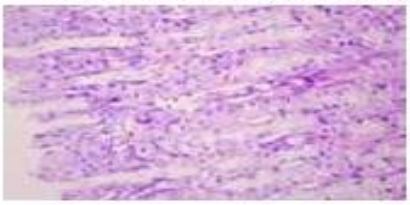


Fig No 6(q)
EEMI (400mg/kg)
+ Menadione 15mg/kg (1

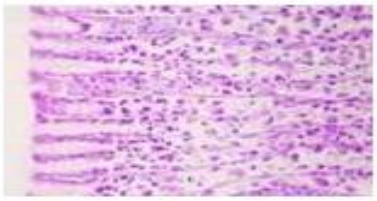


Fig No 6(r)
EEMI (400mg/kg)
+ Menadione 15mg/kg (2)

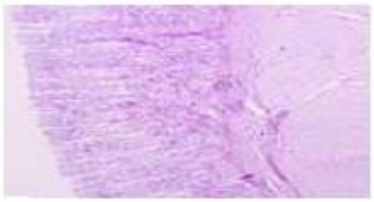


Fig No 6(s)
EMI (400mg/kg) +
ZnSo4 40mg/kg (1)

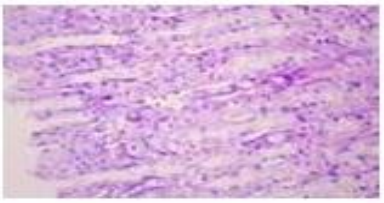


Fig No 6(t)
EEMI (400mg/kg +
ZnSo4 40mg/kg (2)

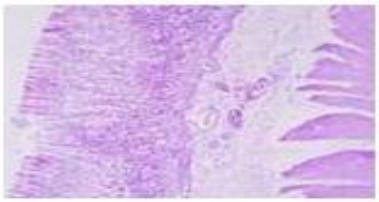


Fig No 6(u)
EEMI (400mg/kg) +
Vitamin C 50mg/kg (1)

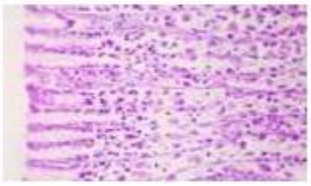


Fig No 6(v)
EEMI (400mg/kg)+ Vitamin C 50mg/kg (2)

It has already been reported that *Mangifera indica* kernel is composed of important phytoconstituents like polyphenols and flavonoids. The potential effect of EEMI may be due to increase in the blood flow to necrotic area thereby enhancing mucus secretion. Another possible mechanism may likely to be because of its anti-oxidant property. Menadione, Zinc sulphate, and Vitamin C administered by IP route significantly decreased ulcer index and increased percentage protection compared to control animals as shown in Table No 1 and Fig No 1. Co-administration of EEMI 400 mg/kg b.w. with Menadione (15 mg/kg), Zinc sulphate (40 mg/kg), Vitamin C(50 mg/kg) has also significantly decreased ulcer index [3.12, 2.6 0 & 4.4] and increased percentage protection [39.06%, 49.21% & 21.87%] respectively compared to control animals [ulcer index (4.374±0.09)] as shown in table No 1 and fig No 1. Hence co-administration of EEMI with above said vitamins [Menadione & Vitamin C] and micronutrient [Zinc sulphate] did not show any additive or synergistic effect in reducing ulcer index & increasing percentage protection.

The GSH & LPO were observed in the ethanol induced ulcer model in rats.

The ethanolic extract of *Mangifera indica* kernel (EEMI) at a dose of 400 mg/kg has produced significant increase in GSH [0.232] and decrease in LPO [1.10] compared to ethanol induced positive control group of animals [GSH 0.0436, LPO 4.37]. Menadione , Zinc Sulphate and Vitamin C has also produced significant increase in GSH [0.069, 0.147, 0.213] and decrease in LPO [2.98, 2.84, 2.19] respectively compared to ethanol induced positive control group of animals [GSH 0.0436, LPO 4.37].

In the study, co-administration of EEMI 400 mg/kg b.w. with Menadione, Zinc sulphate , Vitamin C has produced significant increase in GSH [0.192, 0.297 & 0.196] and decrease in LPO [3.285, 1.272 & 2.163] compared to ethanol induced positive control group of animals [GSH 0.0436 LPO 4.374]. Pretreatment with EEMI, Menadione, Vitamin C, Zinc sulphate has significantly increased tissue GSH levels and decreased LPO levels when compared to control animals. This result may be attributed to anti-oxidant property of EEMI (Polyphenols & flavonoids), Menadione, Vitamin C and Zinc sulphate.

In pylorus ligation ulcer model in rats, there will be increase in gastric secretion, free acidity and total acidity. Pretreatment with 200 mg/kg and 400mg/kg of EEMI , selected dose of Menadione 15 mg/kg, Zinc sulphate 40 mg/kg and Vitamin C 50 mg/kg has produced significant reduction in the volume of gastric secretion (7.520, 3.940, 4.5, 3.26 & 4.52), free acidity (40.40, 34.60, 37.4, 29.6 & 37.5) and total acidity (85.25, 59.46, 83.27, 71.2 & 80.44), ulcer index (2.730,1.624, 2.22, 1.6 & 1.52) respectively as compared to volume of gastric secretion (9.620), free acidity (64.0) and total acidity (132.4), ulcer index (3.984) in control group animals as shown in Table No 3, 4 and Fig No 3 ,4(a), 4(b), 4(c). Combination of EEMI with vitamins and micronutrient [Zinc sulphate] were challenged to assess synergistic effect by measuring various parameters in pylorus ligation induced ulcer models in rats.

Co-administration of EEMI 400 mg/kg b.w. with Menadione (15 mg/kg), Zinc sulphate(40 mg/kg) and Vitamin C(50 mg/kg) have also produced significant decrease in ulcer index [2.16, 1.61 & 3.08], the volume of gastric secretion (4.20, 2.50 & 5.56), free acidity (57.1, 26.8 & 62.3) , total acidity (123.1, 58.38 & 128.4) and increased percentage protection [35.06%, 48.9% & 41.6%] as compared to volume of gastric secretion (9.62), free acidity (64.0) and total acidity (132.4), ulcer index (3.98) in control group animals. However co-administration did not produce any additive or synergistic effect as showed in Table No 3, 4 and Fig No 3, 4(a), 4(b), 4(c).

In pylorus ligation ulcer model, gastric ulceration occurs due to increased secretion of gastric acid and acidic pepsin. The Gastroprotective activity exhibited by EEMI, Menadione, Zinc sulphate, Vitamin C may be due to reduction in gastric acid secretion which further minimizes the activity of pepsin, reduces gastric volume and hence prevents the auto-digestion of mucosal barrier. The GSH & LPO were also observed in pylorus ligation induced ulcer model in rats. The EEMI at a dose of 400 mg/kg has produced significant increase in GSH [0.217] and decrease in LPO [0.5892] levels compared to pylorus ligation induced positive control group of animals [GSH 0.0436 & LPO 4.37] as showed in Table No

5 and Fig No 5(a), 5(b). The vitamins [Menadione & Vitamin C] and micronutrient [Zinc sulphate] were challenged to assess GSH & LPO levels.

Pretreatment with selected dose of Menadione 15 mg/kg, Zinc sulphate 40 mg/kg, Vitamin C 50 mg/kg has produced significant increase in GSH [0.303, 0.66 & 0.84] and decrease in LPO [0.362, 0.198 & 0.192] levels compared to pylorus ligation induced positive control group of animals [GSH 0.0436 & LPO 4.37] as showed in Table No 5 and Fig No 5(a), 5(b). The combination of the vitamins [Menadione & Vitamin C] and micronutrient [Zinc sulphate] with EEMI was also challenged to assess the synergistic/additive effect.

In the study, co-administration of EEMI 400 mg/kg b.w. with Menadione (15 mg/kg), Zinc sulphate (40 mg/kg), Vitamin C (50 mg/kg) has produced significant increase in GSH [0.2817, 0.726 & 0.322] and decrease in LPO [0.44, 0.171 & 0.230] levels compared to pylorus ligation induced positive control group of animals [GSH 0.0436 LPO 4.374] as showed in Table No 5 and Fig No 5(a), 5(b). Pretreatment with EEMI, Menadione, Vitamin C, Zinc sulphate has significantly increased tissue GSH levels and decreased LPO levels when compared to control animals. This result may be attributed to anti-oxidant property of EEMI (polyphenols & flavonoids), Menadione, Vitamin C, Zinc sulphate and prevented excessive peroxidation.

In the histopathological study of stomach in ethanol induced ulcer models, the gastric mucosa appears very much intact in all the groups of animals including positive control. The secreting lining epithelial cells are seen in negative control but variations in the structure of these cells are seen with other groups of animals. The submucosal layer showed severe edema in positive control (ethanol induced) but there was reduction in the pattern of edema in treated and standard group of animals.

The possible mechanism of protective effect may be due to the presence of essential phytoconstituents (polyphenols and flavanoids) present in the extract and antioxidant property of Vitamin C, Menadione, Zinc Sulphate and EEMI.

CONCLUSION

The ethanolic extract of *Mangifera indica* kernel (EEMI) with a dose of 400mg/kg b.w p.o. has shown better gastroprotective activity than EEMI 200mg/kg b.w p.o. in both ethanol and pylorus ligation induced ulcer models in rats.

The possible mechanism for all the protective action brought by the EEMI may be due to increase in mucus secretion, reduction in gastric acid secretion which minimizes the activity of pepsin and prevents the auto- digestion of mucosal barrier. Other possible mechanism may be because of the anti- oxidant property of the essential phytoconstituents (Polyphenols and flavonoids) responsible for increased GSH and decreased LPO levels in treated animal tissues.

The significant gastroprotective and antioxidant activity shown by Menadione, Zinc sulphate and Vitamin C was in a dose dependent manner but treatment in combination of EEMI with Menadione, Zinc sulphate, Vitamin C have shown no significant higher gastroprotective potential than the individual treatment.

In the present study EEMI was subjected for preclinical studies using both the species i,e rats and mice. There is a scope for clinical studies to validate these activities further in detailed manner in human volunteers to find out more detailed mechanism of action of this plant extract and also assess the isolated phytoconstituents present in them.

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