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GASTRO-PROTECTIVE ACTIVITY OF LEAVES OF *MANGIFERA INDICA* IN ULCER INDUCED ALBINO RATS

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ABSTRACT

Mangifera indica (Family: Anacardiaceae) is being used in Indian Folk Medicine for the treatment of various diseases including gastric ulcer. In the present study, we investigated the gastro-protective potential of ethanolic & aqueous extracts of *Mangifera indica* leaves against gastric ulcer induced by several experimental methods. The ethanolic extracts (400 mg/kg) of *Mangifera indica* leaves significantly reduced the volume of gastric juice, total acidity, free acidity and ulcer index. Thus, our present study results clearly demonstrate that ethanolic extract of *Mangifera indica* leaves is in possession of good preventive and therapeutic action on the gastric ulcers and supports the traditional uses of the plant.

KEYWORDS: *Mangifera indica* leaves, Pylorus Ligation, Gastro-protective, Aspirin, Albino rat.

INTRODUCTION

Peptic ulcer is the most common disorder among the digestive diseases. It occurs in the duodenum, stomach and rarely in the oesophagus of the gastro-intestinal tract. Numerous causes are involved in the production of peptic ulcer among which hyperacidity stands first. Besides, stress, diet, drugs, habit are the other factors behind occurrence of ulcer.

Though a number of allopathic drugs are available now-a-days, no drug is completely safe in all respect and as such the allopathic medicines are very costly for its routine use, so it becomes a mellifluent need to go back to the nature's medicine i.e. herbal drugs which will act against peptic ulcer.

India is virtually considered as "Herbarium of the World". India possesses all types of climatic conditions with varying temperature and soil texture to develop various types of medicinal plants. So India is also called as "Botanical Garden of the World". In India herbs have been used for treatment of diseases from ancient times, which have been reported in Charaka Sanhita and Susruta Sanhita (1000 B.C.)[1].

If we take a worldwide comparison of patronization of modern and alternative medicine, it is depicted that 75% of world's population preferred or compelled to use alternative system of medicine especially the herbal medicine indigenous to that part of world[2,3].

Therefore the present study aims at establishing the potential of *Mangifera indica* as a gastro-protective (particularly anti-ulcer & anti-secretory) agent.

MATERIALS AND METHODS

Plant Material

Fresh leaves of *Mangifera indica* was collected from matured plants from Utkal University Campus, Bhubaneswar, Odisha and were identified by a botanist of Department of Botany, Utakl University, Bhubaneswar by comparing with the voucher specimen present in the herbarium.

Preparation of Extract[4]

About 500gms of coarse powder of leaves of *Mangifera indica* was taken in the soxhlet apparatus and first extracted with petroleum ether. The defatted material was then extracted with ethanol and finally macerated with aqueous solvent successively. The extraction for each solvent was carried out for 24 to 48 hours and the temperature was maintained between 25-30^{o}C. Before and after every extraction the marc was completely dried and weighed. The extract was concentrated to dryness at 40°C under reduced pressure in a rotary vacuum evaporator. The percentage yield of various extracts is shown in Table No. 1.

Experimental Animals

Healthy adult albino rats of either sex of Wistar strain weighing 150 to 200gms were procured from the Animal House of University Department of Pharmaceutical Sciences, Utkal University, Vanivihar. The animals were maintained in a well-ventilated room with 12:12 hour light/dark cycle in large spacious polypropylene cages. They were fed with Standard pellet feed (Hindustan Lever Limited., Bangalore) and water *ad libitum* through out

experimentation period. Animals were acclimatized to laboratory conditions one week prior to initiation of experiments. This study was permitted by the Institutional Animal Ethical Committee with Regd. No. 678/02/a/CPCSEA.

Drugs and Chemicals

All the chemicals and solvents used were of Analytical grade like, Sodium CMC (S.D.Fine Chemicals, Maharastra), Petroleum Ether, RBC & WBC diluting fluid (Central Drug House Pvt. Ltd., New Delhi), Ethanol (Bengal Chemicals and Pharmaceuticals, Ltd.) Collodion Flexible (Loba Chemie, Mumbai) and Topfer's Reagent (Qualigen Fine Chemicals, Mumbai). The different extracts of leaves of *Mangifera indica* was suspended in 0.5%w/v Sodium CMC as per the dose requirement and used in our various screening studies.

Acute Toxicity Study

The toxicity study was determined in mice by modified method of Lorke.[5] Overnight fasted mice were randomly divided into groups of 4 mice per group and were administered orally with the extract in doses ranging from 500-4000 mg/kg. The results are tabulated in Table No. 2.

Modified Pylorus Ligated (Shay) Rat Model

The test was performed in adult albino rats of either sex weighing 150 to 200gms as suggested by Shay et al., 1945[6] and modified by Okbes et al., 1982[7]. The selected animals were divided into six groups of six animals each. The test groups received two doses (300mg/kg, 400mg/kg) of EEMI & AEMI each. The control group animals were received 0.5%w/v Sodium CMC in distilled water while standard group animals were administered Ranitidine (20mg/kg).

Each group of animals received the test drug and Ranitidine once daily orally for three days prior to and one hour before the pyloric ligation. All the animals were overnight fasted before pyloric ligation and allowed to take only water *ad libitum*. Under light ether anaesthesia the abdomen was opened by a small midline incision, pyloric portion of stomach was slightly lifted out and ligated. The stomach was replaced carefully and the abdomen wall closed by interrupted sutures followed by application of Collodion solution. The animals were deprived of both food and water during the post-operative period and were sacrificed after 4 hour. The stomach was isolated, gastric juice was collected. The gastric content were centrifuged at 200 rpm for 10 minutes. The supernatant fluid (1ml) was diluted with 9ml distilled water and then

titrated against 0.01N NaOH solution using Topfer's reagent till the solution turns to orange colour. The volume of NaOH required corresponds to free acidity. The solution was further titrated till the solution turns pink colour. The volume of NaOH required corresponds to total acidity. Each stomach was examined for severity of ulcers. The ulcers were graded as suggested by Kunchandy et al, 1985[8] and Kulkarni, 2002[9]. The results are tabulated in Table No. 3.

Aspirin Induced Ulceration

Albino rats of either sex weighing 150-200gms were divided randomly into six groups containing six animals each. The test groups received two doses (300mg/kg, 400mg/kg) of EEMI & AEMI each. The control group animals were received 0.5%w/v Sodium CMC in distilled water while standard group animals were administered Ranitidine (20mg/kg).

Aspirin was suspended in 0.5%w/v Sodium CMC in water and administered orally at a dose of 200mg/kg in non-fasted rats once daily for five days. Drug treatment was carried out 30 minutes prior to aspirin treatment on each day. On sixth day pyloric ligation was carried out on 12 hour fasted rats under light ether anaesthesia. Under light ether anaesthesia the abdomen was opened by a small midline incision, pyloric portion of stomach was slightly lifted out and ligated. The stomach was replaced carefully and the abdomen wall closed by interrupted sutures followed by application of Collodion solution. The animals were deprived of both food and water during the post-operative period and were sacrificed after 4 hour. The stomach was isolated, gastric juice was collected. The gastric content were centrifuged at 200 rpm for 10 minutes. The supernatant fluid (1ml) was diluted with 9ml distilled water and then titrated against 0.01N NaOH solution using Topfer's reagent till the solution turns to orange colour. The volume of NaOH required corresponds to free acidity. The solution was further titrated till the solution turns pink colour. The volume of NaOH required corresponds to total acidity. Each stomach was examined for severity of ulcers. The ulcers were graded as suggested by Kunchandy et al, 1985 [8] and Kulkarni, 2002[9]. The results are tabulated in Table No. 4.

Ethanol Induced Ulcer in Rats[10]

Albino rats of either sex weighing 150-200gms were divided randomly into six groups containing six animals each. The test groups received two doses (300mg/kg, 400mg/kg) of EEMI & AEMI each. The control group animals were received 0.5%w/v Sodium CMC in distilled water while standard group animals were administered Sucralfate (0.27mg/kg).

Acute gastric ulceraltion was induced by absolute ethanol (1ml/animal) which was administered intra-gastrically to control and rats pretreated, 30 minute and 24 hours before with the extract. The animals were sacrificed 1 hour after ethanol administration. The ulcer index was found out by opening the stomach. The results are tabulated in Table No. 5.

Statistical analysis

The data were expressed as mean ± standard error of mean (S.E.M). The Significance of differences among the group was assessed using one way analysis of variance (ANOVA). The test followed by Dunnet's T-test, p value < 0.05 were considered as significant.

RESULTS

Percentage Yield of Ethanolic & Aqueous Extracts of *Mangifera indica*

Table No. 1

Sl. No.	Extracts	Percentage Yield (%w/v)
1	Ethanolic	15.79
2	Aqueous	10.81

Acute toxicity study

There was no mortality amongst the graded dose groups of mice up to a dose of 4000 mg/kg for duration of 72 h. The animals were observed for further 14 days period for all toxicity signs. There was no considerable change in body weight before and after treatment and no sign of toxicity were observed.

Table No. 2

Group	Dose (mg/kg)	Dead Total
I	500	0
II	1000	0
III	2000	0
IV	3000	0
V	4000	0

Modified Pylorus Ligated (Shay) Rat Model

In pylorus ligated model, the findings of the study reveals that ethanol and aqueous extracts of leaves of *Mangifera indica* posses significant gastro-protective activity when compared their total acidity, free acidity and ulcer index with solvent control (0.5%w/v Sodium CMC).

The order of reduction of ulcer score observed was:

Ranitidine > Ethanol Extract (400 mg/kg) > Ethanol Extract (300 mg/kg) = Aqueous Extract (400 mg/kg) > Aqueous Extract (300 mg/kg).

Table No. 3: Biochemical estimation of different extracts of leaves of *Mangifera indica* treated in Modified Pylorus Ligated (Shay) Rat Ulcer Model.

Groups	Treatment	Dose	Volume of Gastric Juice (ml)	Total Acidity (mEq/l/100g)	Free Acidity (mEq/l/100g)	Ulcer Index
I	Solvent	2 ml/kg	4.12 ± 0.252	26.50 ± 0.428	17.5 ± 0.563	3.83 ± 0.067
II	Ranitidine	20 mg/kg	2.00 ± 0.115*	11.17 ± 0.477*	5.83 ± 0.401*	0.67 ± 0.150*
III	Ethanol Extract	300 mg/kg	3.17 ± 0.193*	16.17 ± 0.307*	9.33 ± 0.422*	1.92 ± 0.154*
IV	Ethanol Extract	400 mg/kg	3.08 ± 0.217*	12.17 ± 0.477*	6.17 ± 0.307*	0.92 ± 0.154*
V	Aqueous Extract	300 mg/kg	3.25 ± 0.527**	20.50 ± 0.224*	12.17 ± 0.307*	2.17 ± 0.211*
VI	Aqueous Extract	400 mg/kg	3.25 ± 0.431**	16.67 ± 0.333*	9.67 ± 0.494*	1.92 ± 0.154*

*n=6 in each group. Values are expressed as mean ±SEM of six observations. *P <0.001 and **P<0.01 when compared to the vehicle by Dunnet's T-test.*

Aspirin Plus Pylorus Ligated Rat Ulcer Model

The results reveal that ethanolic and aqueous extracts of leaves of *Mangifera indica* posses remarkable gastro-protective activity which are confirmed by comparing the volume of gastric juice, total acidity, free acidity and ulcer index of the different extracts with solvent control (0.5%w/v Sodium CMC).

The order of reduction of ulcer score observed was:

Ranitidine > Ethanol Extract (400 mg/kg) > Aqueous Extract (400 mg/kg) > Ethanol Extract (300 mg/kg) > Aqueous Extract (300 mg/kg).

Table No. 4: Biochemical estimation of different extracts of leaves of *Mangifera indica* treated in Aspirin Plus Pylorus Ligated Rat Ulcer Model.

Groups	Treatment	Dose	Volume of Gastric Juice (ml)	Total Acidity (mEq/l/100g)	Free Acidity (mEq/l/100g)	Ulcer Index
I	Solvent	2 ml/kg	4.90 ± 0.221	38.67 ± 0.494	24.83 ± 0.307	5.92 ± 0.154
II	Ranitidine	20 mg/kg	2.89 ± 0.163*	14.33 ± 0.333*	8.17 ± 0.401*	0.83 ± 0.105*
III	Ethanol Extract	300 mg/kg	3.84 ± 0.531**	24.33 ± 0.422*	15.33 ± 0.333*	2.67 ± 0.167*
IV	Ethanol Extract	400 mg/kg	3.25 ± 0.125*	17.50 ± 0.224*	10.67 ± 0.422*	1.17 ± 0.105*
V	Aqueous Extract	300 mg/kg	4.02 ± 0.452**	30.33 ± 0.422*	19.17 ± 0.478*	2.75 ± 0.112*
VI	Aqueous Extract	400 mg/kg	3.55 ± 0.282*	24.50 ± 0.500*	15.33 ± 0.333*	2.50 ± 0.224*

*n=6 in each group. Values are expressed as mean ±SEM of six observations. *P <0.001 and **P<0.01 when compared to the vehicle by Dunnet's T-test.*

Ethanol Induced Ulcer in Rats

The gastro-protective activity of leaves of *Mangifera indica* once again justified by ethanol induced gastric ulcer model in rats. In this model the ulcer index was taken as the parameter for study of gastro-protective activity.

The order of reduction of ulcer score observed was:
Sucralfate > Ethanol Extract (400 mg/kg) > Aqueous Extract (400 mg/kg) > Ethanol Extract (300 mg/kg) > Aqueous Extract (300 mg/kg).

Table No. 5: Effect of different extracts of leaves of *Mangifera indica* on Ethanol induced Rat Ulcer Model.

Groups	Treatment	Dose	Ulcer Index
I	Solvent	2 ml/kg	6.33 ± 0.307
II	Sucralfate	0.27 mg/kg	1.42 ± 0.154*
III	Ethanol Extract	300 mg/kg	2.33 ± 0.167*
IV	Ethanol Extract	400 mg/kg	1.75 ± 0.214*
V	Aqueous Extract	300 mg/kg	2.83 ± 0.211*
VI	Aqueous Extract	400 mg/kg	2.25 ± 0.171*

*n=6 in each group. Values are expressed as mean ±SEM of six observations. *P <0.001 when compared to the vehicle by Dunnet's T-test.*

DISCUSSION

In the present study for the screening of ethanolic and aqueous extract of leaves of *Mangifera indica* for gastro-protective activity, three standard methods have been employed. It is found that ethanolic extract (400 mg/kg) of leaves of *Mangifera indica* markedly possess gastro-protective and anti-secretory activity in albino rats.

It is proposed that GABA and Baclofen, a GABA mimetic agent, exert marked anti-ulcerogenic effects against pylorus ligated ulcers. It may not be due to attenuation of offensive acid pepsin factor since GABA has been shown to induce an increase in gastric acid output.[11] As the volume of gastric juice, total acidity, free acidity and ulcer index are remarkably reduced in ethanolic extract (400 mg/kg and 300 mg/kg) and aqueous extract (400 mg/kg and 300 mg/kg) when compared with solvent control group in pylorus ligated model and Aspirin plus pylorus ligated model, it may be suggested that, the leaves of

Mangifera indica possess gastro-protective activity. Development of ulcer due to pylorus ligation model can be attributed to increased metabolism of carbohydrate and other compensatory mechanisms.[12] The gastro-protective activity of leaves of *Mangifera indica* may be due to inhibition of metabolism of carbohydrate or decreased synthesis of nucleic acid. The various phyto-chemical constituents isolated from leaves of *Mangifera indica* may be responsible for healing of gastric ulcer induced by pylorus ligation model.

In the aspirin plus pylorus ligation model the initial damage by aspirin aggravates the damage further, which further increases acid secretion and decrease mucus production with increased pepsin activity. Therefore, in this model, the volume of gastric juice, total acidity and free acidity comparatively showed increased value than pylorus ligation model.

Ethanol induced gastric ulcer (lesion) formation may be due to stasis in gastric blood flow which contributes to the development of haemorrhage and necrotic aspects of tissue injury.[13] The available literature information on possible mechanism of action of sucralfate reveals that, it accelerates ulcer healing by forming ulcer adherent complex with proteinaceous exudates, as a result of which pepsin activity is inhibited. Therefore, it is proposed that the test sample protect the gastric ulcer induced by ethanol by increasing the blood flow in gastric mucosal membrane.

CONCLUSION

From the present study, it was concluded that the ethanolic and aqueous extract of leaves of *Mangifera indica* exhibited remarkable gastro-protective activity in pylorus ligation, aspirin plus pylorus ligation and ethanol induced gastric ulcer models. The results of the present investigation indicates that *Mangifera indica* may have equal extent of therapeutic effect (gastro-protective effect) with largely used well known anti gastric ulcer drug Ranitidine. Further studies are in progress to isolate the active constituent, the exact mechanism of gastro-protective activity and chances of teratogenicity.

REFERENCES

1. Sukh Dev. An Ancient Modern Concordance in Ayurvedic Plants, Some Examples Environmental Health Prospective. 1999; 107: 783-784.
2. Jain SK. Medicinal Plants. National Book Trust, India; 1968; 318-320.
3. Mukharjee PK, Sahu M, Suresh B. The Eastern Pharmacist, 1998; XLI(490): 21-23.
4. Harbone JB. Phytochemical methods, Edn. 2nd, Jackmann and Hall, Londaon, 1973; 5-6.

5. Lorke D. A new approach to practical acute toxicity testing. Arch Toxicol, 1983; 54: 275-287.
6. Shay H, Komarow SA, Fels SS, Meranze D, Gruenstein M, Siplet HA. Gastroenterology, 1945; 5: 43-47.
7. Okbes S, Tabata K, Kawakani M. Effects of Prolonged Treatment of Piperazine HCL on Gastric Secretion and Plasma Gastrin Levels in Rats. Arzneim Forisch, 1982; 32: 664-668.
8. Kunchandy J, Khanna S, Kulkarni SK. Arch. Int. Pharmacodyn. 1985; 275: 123-126.
9. Kulkarni SK. Hand Book of Experimental Pharmocology. Delhi; Vallabha Prakashan: 2002; 148-150.
10. Robert A, Nezamis JE, Lancaster and Hanchar AJ. Cytoprotection by Prostaglandins in Rats, prevention of gastric Necrosis Produced by Alcohol, HCl, NaOH, hypertonic NaCl and thermal injury. Gastroentenol, 1979; 77: 233-243.
11. Goyal RK, Abbas WR, Maite RW, Bhattacharya SK. Effects of GABA and Baclofen on Gastric Mucosal Protective Factors. Ind J Exp Biol., 1998; 36: 182-186.
12. Blum AI. Therapeutic Approach to Ulcer Healing. Am J Med., 1985; 79: 8-12.
13. Guth PH, Paulsen G, Nagata H. Histologic and microcirculatory changes in alcohol-induced gastric lesions in the rat: effect of prostaglandin cytoprotection. Gastroenterology, 1984; 87: 1083-1090.