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Original Article

ANTIOXIDANT AND ANTIULCER POTENTIAL OF ETHANOLIC EXTRACT OF BARK OF *MYRICA ESCULENTA* IN PYLORIC LIGATION ULCER MODEL

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ABSTRACT

Objective: To evaluate the antiulcer and antioxidant potential of ethanolic extract of bark of *Myrica esculenta* in pyloric ligated ulcer model in *albino* rats.

Methods: The rats were subjected to pyloric ligation ulcer model and the ethanolic extract of bark of *Myrica esculenta* were compared with the induced and standard drug Ranitidine. The activity of antioxidant parameters was analysed in the homogenate of stomachs.

Results: The levels of gastric volume, total acidity, free acidity, lipid peroxidation and glutathione (GSH) were reduced significantly and antioxidant, parameters like catalase, nitrite and myeloperoxidase (MPO) were increased significantly in the ethanloic extract of bark of *Myrica esculenta.*

Conclusion: The present study revealed that the ethanolic extract of bark of *Myrica esculenta* improved the anti ulcer and antioxidant potential.

Keywords: Pyloric ligation, *Myrica esculenta*, Antioxidant parameters, Ulcer index, Total acidity, Free acidity, Catalase, nitrite, GSH, MPO.

INTRODUCTION

Peptic ulcer disease represents a serious medical problem. Peptic ulcer disease is one of several disorders of the upper gastrointestinal tract that is caused, at least partially by gastric acid. Patients with peptic ulcer disease may present with a range of symptoms, from mild abdominal discomfort to perforation and bleeding. Reduction of acid secretion and re-inforcement of gastric ulcer, notwithstanding the success of *H. pylori* eradication by antibiotic therapy. The treatment is generally on the inhibition of gastric acid secretion by H_2-receptor antagonists or proton pump blockers as well as acid-independent mucosal protection by sucralfate and bismuth [1-3]. After therapy with H_2-antagonists or proton pump inhibitors, ulcer recurrence is observed in the majority of the cases after suspending the treatment.

Search for newer therapeutic agents from natural sources has been initiated worldwide. Many medicinal plant sources were demonstrated to possess anti ulcer activity in preclinical experimental models. The second source of new chemical entities for potential use as drug molecules has been the natural products. Before the advent of high throughput screening and the post genomic era, more than 80% of drug substances were purely natural products or were inspired by the molecules derived from natural sources.

In the present study, the plant selected is *Myrica esculenta* (Myricaceae). It has been traditionally used for the treatment of various disorders such as liver diseases, fever, asthma, anemia, chronic dysentery, ulcer and inflammation [4]. A number of the chemical constituents of *M. esculenta* have been identified as strong antioxidants [5]. Those drugs which are having antioxidant property can able to cure various kinds of ailments. Hence, the attempt was made to evaluate the ethanolic extract of the above plant for antiulcer and antioxidant activity.

MATERIALS AND METHODS

Preparation of ethanolic bark extract of *M. esculenta*

The bark portion was collected, washed and powdered coarsely. About 200 g of air dried, powdered material were extracted with ethanol in soxhlet extractor for 7 d. The extract was concentrated to dryness under reduced pressure and controlled temperature (40-50 °c) using a rotary evaporator. The yield of the extract was calculated as 26 g. The ethanolic extract yielded a brown sticky mass. The crude extracts were used for further investigation.

Preliminary phytochemical screening

Preliminary phytochemical screening of the extracts was performed for the presence of alkaloids, tannins, reducing sugars, glycosides, saponins, flavonoids, phenols, terpenoids and steroids [6].

Experimental animals

Adult albino rats of Wistar strain of either sex weighing between 150-200 g were used in the present study. The animals were housed in polypropylene cages in a well ventilated room under hygienic conditions and were exposed to 12 h day and night cycle. The animals were fed with commercial rat pellet feed (Gold Mohur, India) and were given water *ad libitum.* The experimental protocol was approved by the Institutional Animal Ethical Committee of TRR College of Pharmacy (Approval No: 1447/PO/a/11/CPCSEA)

Acute toxicity studies

Acute toxicity studies were performed according to OECD (Organization for Economic Co-operation and Development) guidelines 423. Rats were selected for the study was fasted for 3-4 h with free access to water.

Extracts of above plant parts at a dose of 5, 50, 300, 2000 mg/kg were given *p. o*. at 48 h intervals simultaneously to the respective groups. Animals were observed individually after dosing for signs of toxicity and mortality rates.

Antiulcer activity

Pylorus ligation model

Gastric ulcers were induced by pylorus ligation model [7]. Animals were divided into six groups (n=5). Group I receive no treatment, serves as normal animals. The animals of all groups are starved for 48 h with free access to drinking water. Under light ether anesthesia, the pylorus of rat was ligated. Group II received vehicle (distilled water 2 ml/animal) that serves as a vehicle control. Group III received standard drug ranitidine (38 mg/kg, per oral) one hour prior to pylorus ligation. Group IV and V received the plant extract (100 mg/kg and 200 mg/kg., p. o) respectively. 19 h later the ligated rats were sacrificed by decapitation. The abdomen was opened and the stomach was removed after ligating the cardiac end and opened along the greater curvature. The contents were drained into a centrifuge tube and centrifuged at 2000 rpm, 3 min for assessing parameters like acid volume, gastric pH, total acidity, free acidity,

ulcer index. The stomach was thoroughly washed under running tap water and pinned onto a cork plate. The no. of ulcers and severity was scored [8].

The parameters monitored in pylorus ligation model are:

a) Gastric pH [9]

b) Acid volume [10]

c) Total acidity [11]

A known amount of gastric residue was titrated with 0.1 N NaOH. The total acidity, however was determined by titration using phenolphthalein (1% alcoholic) as an indicator.

Reading was taken (ml NaOH) for total acidity.

Y = ml of 0.1 N NaOH x 10

Where Y = Total acidity (meq/l).

d) Free acidity [11]

A known amount of gastric residue was titrated with 0.1 N NaOH. Add two drops of Methyl orange reagent which changes to a salmon color when all the free hydrochloric acid was neutralized.

Reading was taken (ml NaOH) for free acidity.

Y = ml of 0.1 N NaOH x 10

Where Y = Free acidity (meq/l).

e) MUN-Mean of number of ulcers per animal.

f) MUS–Mean of severity score per animal.

g) Ulcer Index.

Severity score

0 = Normal coloured stomach.

0.5 = Red colouration.

1 = Spot ulcer.

1.5 = Hemorrhagic streaks.

2 = ulcers ≥ but ≤ 5.

3 = ulcers>5.

Calculation:

Ulcer index (UI) was calculated as:

$$\text{Ulcer Index} = UN + US + UP \times 10^{-1}$$

Where,

UN = Average of ulcers per animal.

US = Average of severity score.

Evaluation of antioxidant activity

Stomach was homogenized in chilled phosphate buffer (pH 7.4) using a Homogenizer. The homogenates were centrifuged at 800 rpm for 5 min at 4 °C to separate the molecular debris. The supernatant so obtained was centrifuged at 10,000 rpm for 20 min at 4° C to get the post mitochondrial supernantant (PMS)[12], which was used to assay, the *in vivo* antioxidant parameters like lipid peroxidation (LPO)[13], glutathione (GSH)[14], catalase (CAT) [15] and nitrite [16].

Estimation of lipid peroxidation (LPO) from PMS

Procedure: 0.5 ml of PMS was taken and to it was added 0.5 ml of Tris hydrogen chloride buffer and incubated at 37° C for 2 h and then 1 ml of ice cold trichloroacetic acid was added, centrifuged at 1000 rpm for 10 min. From the above, 1 ml of supernatant was taken and added 1 ml of thiobarbituric acid and the tubes were kept in boiling water bath for 10 min. The tubes were removed and brought up to room temperature and 1 ml of distilled water was added. Absorbance was measured at 532 nm by using a UV–VIS-spectrophotometer.

Blank: It was prepared without tissue homogenate.

Calculation

$$\frac{3 \times \text{Absorbance of sample}}{50.156 \times (\text{mg of tissue taken})} = \mu m \ / \ mg \ tissue$$

Estimation of reduced glutathione (GSH) from PMS

Procedure: 0.75 ml of PMS was mixed with 0.75 ml of 4% sulfosalicylic acid and then centrifuged at 1200 rpm for 5 min at 4° C (REMI CM-12). From the above, 0.5 ml of supernatant was taken and added to 4.5 ml of 0.01M DTNB. Absorbance was measured at 412 nm by using a UV–VIS-spectrophotometer.

Blank: It was prepared without PMS.

Calculation:

$$\frac{3 \times \text{Absorbance of sample}}{13.6 \times (\text{mg of tissue taken})} = \mu m \ of \ GSH / mg \ tissue$$

Estimation of catalase (CAT) from PMS

Procedure: 0.4 ml of the homogenate was diluted 20 times with phosphate buffer (pH 7.0).

Test: 2 ml of diluted homogenate+1 ml of H_2O_2.

Blank: To 4 ml of the above diluted homogenate+1 ml of phosphate buffer pH 7.0 was mixed.

Absorbance was measured for both blank and tests at 240 nm for 2 min with 60 s interval by using a UV–VIS-spectrophotometer.

Estimation of Nitrite from PMS

Procedure: 0.5 ml of PMS was mixed with 0.5 ml of Griess reagent (1% w/v sulfanilamide, 2% w/v H_3PO_4 (phosphoric acid) and 0.1% w/v napthylethylenediamine dihydrochloride. Absorbance of the chromophore was measured at 546 nm using a UV–VIS-spectrophotometer.

Blank: It was prepared without PMS.

Estimation of Myeloperoxidase

The activity of myeloperoxidase was assessed using the method [17]. The sciatic nerve was homogenized in phosphate buffer containing 0.5% hexadecyl trimethyl ammonium bromide using homogenizer to produce a 10 % w/v homogenate. After freeze-thawing for three times, the samples were centrifuged at 15 000 rpm for 30 min at 40 °C and resulting supernatant was assayed spectrophotometrically for MPO. To 40 µl of the sample, 960 µl of phosphate buffer containing O-dianisidine dihydrochloride and hydrogen peroxide was mixed and shaken vigorously. The change in the absorbance of this mixture was measured at 460 nm for 3 min at an interval of 60 s. One unit of enzyme activity defined as the amount of MPO that causes a change in absorbance measured at 460 nm for 3 min. Myeloperoxidase activity was expressed as units/gm tissue.

Statistical analysis

Results were expressed as mean±SEM. Data were analyzed by one way ANOVA followed by Dunnett's multiple comparison test using Graphpad PRISM software-4. $P<0.05$ was considered significant.

RESULTS AND DISCUSSION

Phytochemical screening

Ethanolic extract of bark of *M. esculenta* showed the presence of alkaloids, glycosides, carbohydrates, saponins, and flavonoids.

Acute toxicity studies

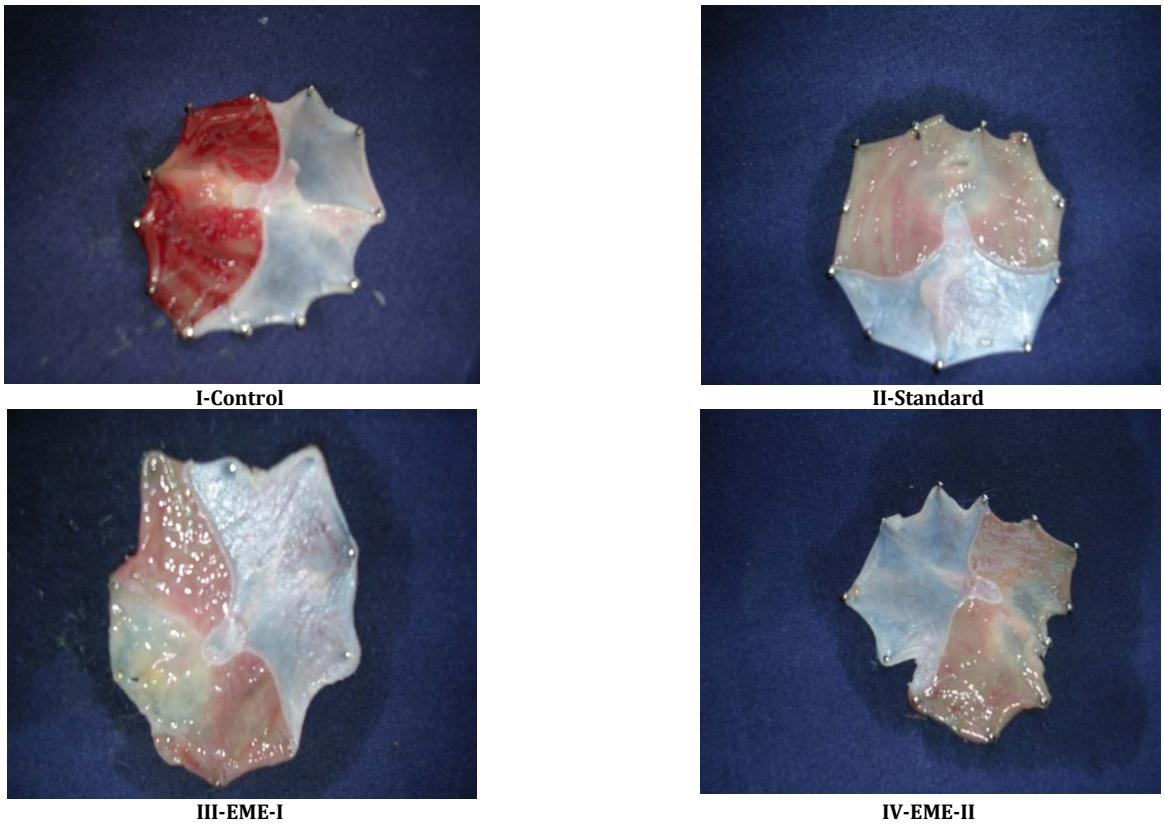
Acute toxicity studies were carried out for the ethanolic extract of bark of *M. esculenta.* It was observed that all the animals were found to be safe, no other signs of toxicity and no mortality rate was observed up to a maximum dose of 2000 mg/kg *p. o*.

Pylorus ligation induces ulcers that serve as a useful model for investigating the efficacy of drugs on gastric secretions. The ligation of the pyloric end of the stomach causes accumulation of gastric acid in the stomach that produces ulcers. These ulcers result from autodigestion of the gastric mucosa leading to a breakdown of the gastric mucosal barrier. So, basically an increase in acid-pepsin accumulation due to pylorus obstruction may cause subsequent mucosal digestion. This model is useful for evaluating the effects of anti-secretory drugs that reduce the secretion of aggressive factors such as acid and pepsin. And also useful for assessing the cytoprotective effect of drugs that increase the secretion of mucus [18]. In the present study significant reduction in ulcer index, total acidity, free acidity and gastric volume are observed with the ethanolic bark extract of *Myrica esculenta* when compared with the control group.

Table 1: Effect of Ethanolic extract of *Myrica esculenta* for pyloric ligation ulcer model

Groups	Treatment	Protection (%)	MUI	pH	Gastric juice (ml)	Free acidity (mEq/l)	Total acidity(mEq/l)
I	Control	-	6.7±0.1	1.42±0.08	2.2±0.10	27.25±0.85	47.5±0.64
II	Ranitidine (38 mg/kg.,p. o)	69	2.1±0.09[a]	5.35±0.06[a]	3.42±0.4[a]	12.5±0.64[a]	24.5±0.64[a]
III	EME-I (100 mg/kg.,p. o)	64	2.4±0.08[a]	4.90±0.03[a]	2.26±0.13[a]	14.37±0.47[a]	29.52±0.32[a]
IV	EME-2 (200 mg/kg.,p. o)	71	1.9±0.09[a]	5.12±0.05[a]	2.65±0.12[a]	11.43±0.01[a]	22.16±0.35[a]

Values are expressed as mean±SEM, [a]p<0.01 considered statistically significant as compared with control group.



I-Control **II-Standard**

III-EME-I **IV-EME-II**

Fig. 2: Effect of Ethanolic extract of *Myrica esculenta* for pyloric ligation ulcer model

Table 2: Effect of ethanolic extract of *Myrica esculenta* on *in vivo* anti oxidant parameters on pyloric ligated gastric ulcer

Group	LPO(µm/g tissue)	Catalase(µm/g tissue)	Nitrite(µm/g tissue)	GSH(µm/g tissue)	MPO(µm/g tissue)
I-Normal	0.028±0.002[a]	5.50±0.13[a]	45.34±0.93[a]	0.42±0.05 [a]	0.44±0.01[a]
II-Control	0.045±0.003	3.05±0.15	31.67±0.50	0.16±0.06	1.26±0.03
III-Standard	0.031±0.004[a]	4.93±0.18[a]	42.94±1.38[a]	0.38±0.07[a]	0.55±0.01[a]
IV-EME-1 (100 mg/kg.,p. o)	0.033±0.003[a]	3.96±0.70[b]	41.26±3.16[a]	0.35±0.03[a]	0.63±0.01[a]
V-EME-2 (200 mg/kg.,p. o)	0.031±0.001[a]	4.60±0.33[b]	43.86±1.26[a]	0.37±0.05[a]	0.59±0.03[a]

Values are expressed as mean±SEM, [a]p<0.01, [b]p<0.05 found to be significant when compared with normal control

In our present work, we also observed an increase in the oxidative free radicals, lipid peroxides and nitric oxide, leading to oxidative damage in pyloric ligation and ethanol induced ulcers. The principle free radical in tissues is superoxide anion (O_2^-). O_2^-can be produced by both endothelial cells through xanthine oxidase and activated neutrophils through NADPH (reduced nicotinamide adenine dinucleotide phosphate) oxidase, which reduces molecular oxygen to the O_2^-radical and through the enzyme MPO. Superoxide ion (O_2^-) if not scavenged by the CAT causes lipid peroxidation by an increase in the generation of hydroxyl free radicals resulting in tissue damage [19]. The above effect could be further aggravated by the decreased activity of CAT and GSH during pyloric ligation induced ulcers.

Catalase is an endogenous antioxidant enzyme present in peroxisomes reduces hydrogen peroxide produced by the dismutation reaction and prevents the generation of hydroxyl radicals there protecting the cellular constituents from oxidative damage [20]. The lowered activity of catalase in ulcer condition indicates intense peroxidase and suppression of the antioxidant process.

In ulcer pathogenesis, an important role belongs to hydroxyl free radicals, which emerge under conditions of oxidative stress in which glutathione content and peroxidase activity. GSH is a well known antioxidant, which is usually present as the most abundant low molecular mass thiol in most organisms. It has various functions in the defense against oxidative stress and xenobitic toxicity. In the present study significant increase in catalase and nitrite and glutathione was observed with the ethanolic bark extract of *Myrica esculenta* when compared with control animals.

Myeloperoxidase (MPO) is a peroxidase enzyme that in humans is encoded by the MPO gene. Myeloperoxidase is most abundantly expressed in neutrophil granulocytes. It is a lysosomal protein stored in azurophilic granules of the neutrophil. The MPO has a heme pigment, which causes its green color in secretions rich in neutrophils, such as pus and some forms of mucus. The MPO has a key role in the initiation and propagation of acute and chronic inflammatory disease and is strongly involved in the regulation of cellular homeostasis [21]. There is a significant reduction in LPO and MPO was observed with the extract when compared with control animals.

CONCLUSION

The results of the study with albino rats, confirm that the ethanolic extract of bark of *Myrica esculenta* can improve the antiulcer potential and antioxidant status of the tissue by increasing the levels of different antioxidants.

CONFLICT OF INTERESTS

Declare None

REFERENCES

1. Brunner G. Proton-pump Inhibitors are the treatment of choice in acid related disease. Eur J Gastronterol 1996;14:188-98.
2. Cozzarini W, Rath J, Bauer A, Gyorog I, Prenner M, Trianto T, *et al*. Mucosa protective therapy with long-term nonsteroidal antirheumatic drugs. Wien Med Wochenschr 2003;153:295-303.
3. Leontiadis GI, Sharma VK, Howden CW. Proton pump inhibitor treatment for acute peptic ulcer bleeding. Cochrane Database of Systematic Rev 2006;1:CD002094.
4. Nadkarni KM. Indian Materia Medica I. Bombay: Popular Book Depot; 1954. p. 828-9.
5. Malterud KE, Diep OH, Sund RB. C-Methylated dihydrochalcones from *Myrica gale* L: Effects as antioxidants and as scavengers of 1,1-diphenyl-2-picryl hydrazyl. Annu Rev Pharmacol Toxicol 1996;78:111–6.
6. Kokate CK, Purohit AP, Gokhale SB. Text book of Pharmacognosy. 34th ed. Pune: Nirali prakashan; 2006. p. 337.
7. Shay M, Komarov SA, Fels D, Meranze D, Gruenstein H, Siplet H. A simple method for the uniform production of gastric ulceration in the rat. Gastroenterology 1945;5:43-61.
8. Rao CM, Ramesh KV, Biary KL, Kulkarni DR. Zinc complexes of NSAIDs abolish gastric ulceration propensity of parent drugs. Indian Drugs 1990;28:64-7.
9. Parmar NS, Desai JK. A review of the current methodology for the evaluation of gastric and duodenal anti-ulcer agents. Indian J Pharmacol 1993;25:120-35.
10. Susan G, Sathimoorthy A, Sathimoorthy SS. Effect of α-tocopherol on gastric ulcers induced by pylorus ligation in rats. Ind J Pharmacol 1990;31:431-3.
11. Mukherjee KL. Medical Laboratory Technology. 3rd ed. New Delhi: Tata. Mc Graw-Hill; 1989. p. 1086-8.
12. Naveen T, Sangeeta P, Anurag K, Kanwaljit Chopra. Hesperidine, a citrus bioflavonoid, decreases the oxidative stress produced by carbon tetra chloride in rat liver and kidney. BMC Pharmacol Toxicol 2005;471:221-30.
13. Niehaus WG Jr, Samuelsson B. Formation of malondialdehyde from phospholipids arachidonate during microsomal lipid peroxidation. Eur J Biochem 1968;6:126-30.
14. Jollow D, Mitchell L, Zampaglione N, Gillete J. Bromobenzene induced liver necrosis: protective role of glutathione and evidence for 3, 4-bromobenzenoxide as the hepatotoxic intermediate. Pharmacol 1974;11:151-69.
15. Aebi H. Oxidoreductases acting on groups other than CHOH: Catalase. Methods Enzymol 1984;105:121-5.
16. Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR. Analysis of nitrate, nitrite and [^{15}N] nitrate in biological fluids. Anal Biochem 1982;126:131-8.
17. Mullen KM, Bruce Smith ND. Myeloperoxidase activity as a quantitative assessment of neutrofil infiltration into ischemic myocardium. J Pharmacol Methods 1995;14:157-67.
18. Mandal S, Das DN, De K, Ray K, Roy G, Chaudhuri SB, *et al*. *Ocimum sanctum* Linn–A study on gastric ulceration and gastric secretion in rats. Ind J Physiol Pharmacol 1993;37:91-2.
19. Das D, Bandyopadhyay D, Bhattacharjee M, Banerjee RK. Hydroxyl radical is the major causative factor in stress-induced gastric ulceration. Free Radic Biol Med 1997;23:8-18.
20. John M. C Gutteridge and Barry Halliwell. Free radical and antioxidant in the year 2000: A historical look to the future. Ann N Y Acad Sci 2000;899:136-47.
21. Lau D, Baldus S. Myeloperoxidase and its contributory role in inflammatory vascular disease. Pharmacol Ther 2006;111:16-26.