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Research Article

ANTIULCER ACTIVITY OF METHANOLIC ROOT EXTRACT OF *BALIOSPERMUM MONTANUM* IN ETHANOL AND INDOMETHACIN INDUCED ULCER MODELS

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Abstract

The objective of this study was to evaluate the antiulcer activity of standardized methanolic extract of *Baliospermum montanum* root and to find out the possible mechanism of this activity. The root extract was evaluated in ethanol and Indomethacin induced ulcer model separately. The ulcer healing effect was studied in wistar albino rats by scoring the ulcer index. The biochemical parameters like reduced Glutathione (GSH), Superoxide dismutase (SOD), Nitrates and Thiobarbituric acid reactive substances (TBARS) were individually studied for both the models at a dose level of 250 mg/kg and 500 mg/kg. The preliminary phytochemical studies show identification of phytochemicals such as alkaloids, tannins, flavonoids and terpenoids. The ulcer index study revealed that the ulcer was reduced significantly in both the models. The biochemical study suggested a marked increase in level of GSH, SOD and Nitrates and decreased lipid peroxidation after the administration of the extract at both dose levels. The result indicated that the extract may exhibited the antiulcer activity due to antioxidant action as it reduced the oxidative stress and consequently improved the integrity of gastric mucosa and enhances the generation of nitric oxide and mucus.

Key Words: *Baliospermum montanum,* Methanolic extract, Ethanol, Indomethacin, Antioxidant

Introduction:

Peptic ulcer disease is a problem of the gastrointestinal tract characterized by mucosal damage secondary to pepsin and gastric acid secretion. It usually occurs in the stomach, proximal duodenum, esophagus, distal duodenum and in the jejunum. Approximately 500,000 persons develop peptic ulcer disease in the United States each year. In 70 % of patients it occurs between the ages of 25 and 64 years [1]. However, the incidence of peptic ulcers is declining, possibly as a result of the increasing use of proton pump inhibitors and decreasing rates of Helicobacter pylori infection [2]. Most GI bleeding comes from ulcers. An ulcer is an area of the lining of the stomach or duodenum that has been destroyed by digestive juices and stomach acid. The actual size of the ulcer can be very small (1-2 cm), but even small lesions can cause tremendous discomfort and pain. The cause of ulceration in patients is mainly due to hyper secretion of gastric juice and also due to hyper secretion of pepsin. The gastric mucosa is continuously exposed to potentially injurious agents such as acid, pepsin, bile acids, food ingredients, bacterial products (Helicobacter pylori) and drugs. These agents have been implicated in the pathogenesis of gastric ulcer, including enhanced gastric acid and pepsin secretion, inhibition of prostaglandin synthesis and cell proliferation growth, diminished gastric blood flow and gastric motility [3].

Herbal alternatives are currently getting tremendous orientations due to their sound efficacy and less toxic effects. *Baliospermum montanum*(Willd.) Muell-Arg Euphorbiaceae, commonly known as Danti is a leafy stout monoecious undershrub, 0.9-1.8 m in height, with many shoots arising from the base. The plant is found in Nepal, Burma, Malaya

and India [4]. In India, it is distributed from Kashmir eastwards to Arunachal Pradesh, up to an elevation of 1,000 m and southwards into peninsular India, ascending to an altitude of 1,800 m in the hills of Kerala [5]. The various parts of *B. montanum*such as roots, leaves and seeds are documented to possess medicinal properties in ethnobotanical surveys conducted by ethnobotanists and in traditional systems of medicine such as Ayurveda. In the current study the root extract was selected for antiulcer study in Ethanol and Indomethacin induced Ulcer models.

Materials And Methods

Omeprazole (OMESEC ®-20), Indomethacin was obtained from Cyper Pharma, Solan as gift sample, Ellman's reagent was purchased from Sigma-Aldrich, Ethanol, Trichloroacetic acid (TCA), Sodium citrate, Sodium lauryl sulphate (SLS), Disodium hydrogen phosphate (Na2HPO4) were procured from Merck India Ltd., Mumbai. All other chemical and reagents were obtained from Merck India Ltd, Mumbai and were of analytical grade. Standardized dried metahnolic extract of *B. montanum*(MEBM) roots was obtained from Amsar Pvt. Ltd., Indore (M.P.).

Procurement of animals

Wistar albino rats of either sex, weighing 180-250gm, were used to evaluate the anti-ulcer activity. The animals were fed on standard diet and water *ad libitum.* They were acclimatized in the animal house of our institute and exposed to natural light and dark cycle. Each experimental group consisted of five animals housed in separate cages. The experimental protocols were approved by the Institutional Animal Ethics Committee and conducted according to the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India(CPCSEA Approval No.:).

Preliminary Phytochemical Screening

The preliminary phytochemical screening of MEBM was carried out according to the methods described by Khandelwal [6]. Phytochemical analysis of the extract was performed for the identification of phytochemicals such as carbohydrates, alkaloids, tannins, saponins, flavonoids, triterpenoids and steroids. The carbohydrates were tested by using Benedict's test, Fehling's test, Molisch test and Barfoed's test. The alkaloids were detected using Dragendroff's test, Wagner's test, Mayer's test and Hager's test. Test for tannins were performed by adding 2-3 drops of ferric chloride to 1ml of extract and the formation of a dark blue or greenish black color product shows the presence of tannins. Flavonoids were detected by means of Shinoda Test. Test for triterpenoids was done by dissolving two or three granules of tin metal in 2 ml thionyl chloride solution and then adding 1 ml of the extract into the test tube. The formation of a pink colour indicates the presence of triterpenoids. The procedure adopted for the identification of saponins was to take 1 ml of extract which is diluted with 20 ml distilled water and then shaken in a graduated cylinder for 15 minutes. A 1 cm layer of foam indicates the presence of saponins. Tests like Legal test, Baijet test, Borntrager's test and Keller Kiliani Test were used for the analysis of glycosides [6,7].

Ulcer induction by Ethanol

Ethanol-induced gastric ulceration in rats is considered to be a reliable tool for the evaluation of anti-ulcer activity. This method of inducing gastric lesions is a rapid and convenient way of screening plant extracts for anti-ulcer potency. Ulcers were induced by administering 1 ml absolute ethanol (99%; p.o) to each rat (Ukwe et al., 2010). After One hour all the rats were sacrificed, stomach was cut opened along the greater curvature and gently rinsed under tap water. The stomachs were stretched on a corkboard and the ulcer index was obtained according to scoring method of Suzuki et al. Omeprazole was used as a standard drug (20 mg/kg/p.o) [8, 9].

Ulcer induction by Indomethacin

In this model the animals were fasted for 24 hr and then test drug was administered orally 10 min prior to oral indomethacin in a dose of 20 mg/kg. Six hours later, the rats were sacrificed. The stomachs were removed and examined for ulcer spots and ulcer index [10].

Evaluation of Ulcer

The stomachs were opened along the greater curvature and the mucosa was exposed for evaluation. The ulcerated area was assessed and the ulcer index (UI) was calculated as the arithmetic mean for

each treatment [8]. The mean score for evaluation of ulcer index was tabulated in Table 1.

Table 1 Scoring table of esophagitis index:

Erosion(mm)	1 or less	1-2	2-3	3-4	4-5	>5	Perforated ulcer
Score	1	2	3	4	5	10	25

Ulcer Protection

The percent protection with each test drug dose was calculated by the formula

% Protection = (UI control − UI treated)/ (UI control) × 100

Where, UI stands for ulcer index.

Determination of pH and Total Acidity

Gastric juice was collected from the stomach. The collected gastric juice was centrifuged at 1000 rpm for 10 min. The volume of gastric juice was measured. The gastric juice was used for determining pH and total acidity. The pH of gastric juice was measured by using pH meter. 1 ml of Gastric juice was taken in to a 100 ml conical flask and 2-3 drops of phenolphthalein solution (indicator) was added to the conical flask. Then, the titration was continued until a definite red tinge reappears [11]. The volume of alkali added was noted which corresponds to total acidity. Acidity was calculated by using the formula:

Acidity (millieq/litre/100g) = Volume of NaOH × Normality of NaOH × 100/0.1

Assessment of oxidative stress in tissue

Preparation of Tissue Homogenate

For the determination of biochemical parameters, stomach was homogenized in chilled phosphate buffer (pH 7.4) at a concentration of 10% w/v and centrifuged at 3000 rpm for 10 min at 4oC [12].

Estimation of Reduced Glutathione (GSH)

1ml of homogenate was precipitated with 1ml of 4% sulfosalicilic acid (SSA). The samples were then incubated at 4°C for one hour followed with centrifugation at 1200 rpm for 20 min at 4°C. The assay mixture contained 0.4 ml supernatant, 2.6 ml sodium phosphate buffer (0.1M, pH 7.4) and 0.2 ml DTNB (5, 5′-Dithio-bis (2-nitrobenzoic acid) (100 mM) in a total volume of 3.0 ml. Absorbance was studied at 412 nm immediately after the appearance of yellow colour on a spectrophotometer [13]. Concentration was obtained using the formula: OD×4.41/Protein Absorbance

Where O.D= absorbance

Estimation of superoxide dismutase (SOD)

SOD was estimated in terms of reduced nitroblue tetrazolium (NBT) using method of Wang et al. (1998). The tissue was minced and homogenized in a mixture of 0.1 N sodium hydroxide (NaOH) and 0.1% sodium dodecyl sulphate (SDS) in water containing 40 mg/L of diethylenetriaminepentaacetic acid (DTPA). The mixture was centrifuged at 20,000 g for 20 min and the resultant pellets were suspended in 1.5 ml of pyridine and kept at 80 °C for 1.5 hours to extract formazan, an adduct formed after reaction of reduced NBT with superoxide anions. The mixture was again centrifuged at 10,000 g for 10 min and the absorbance of formazan was determined spectrophotometrically at 540 nm [13]. The amount of reduced NBT was calculated using the following formula:

Amount of reduced NBT = A × V/T × Wt × ε × L

Where A is absorbance, V is volume of solution (1.5 ml), T is time for which the tissue was incubated with NBT (90 min), Wt is blotted wet weight of tissue, ε is extinction coefficient (0.72 L/mmol/mm) and L is length of light path (10 mm).

Estimation of Nitrate

The estimation of nitrate in the supernatant was determined using a colorimetric assay with the Griess reagent as described by Green et al. Equal volumes of supernatant and the Griess reagent (0.1% N-(1-naphthyl) ethylenediaminedihydrochloride (NEDA), 1% sulfanilamide and 2.5% phosphoric acid) were mixed. Then, the mixture was incubated for 10 min at room temperature in the

dark, and the absorbance was measured at 540 nm [14,15].
Concentration was obtained using the formula: OD × 3.38/Protein Absorbance
Where O.D= absorbance.

Estimation of Lipid Peroxidation
The estimation of TBARS (Thiobarbituric acid reactive substances) was determined by the method of Ohkawa et al. [15]. The reaction mixture contained 0.1 ml of sample, 0.2 ml of 8.1% SDS, 1.5 ml of 20% acetic acid solution and 1.5 ml of 0.8% aqueous solution of TBA (Thiobarbituric acid). The pH of 20% acetic acid solution was adjusted with sodium hydroxide (NaOH) above pH 3.0. The mixture was finally made up to 4.0 ml with distilled water (DW), and heated at 95°C for 60 min. After cooling under tap water, 1.0 ml of DW and 5.0 ml of the mixture of n-butanol and pyridine (15:1, v/v) were added, and the mixture was shaken vigorously. After centrifugation at 4000 rpm for 10 min, the absorbance of the organic layer was measured at 532 nm.

Concentration was obtained by using the formula: OD × 76.92/Protein Absorbance
Where O.D= absorbance.

Statistical Analysis
All data were offered as mean ± SEM and analyzed by one way ANOVA followed by Bonferroni test for the possible significance identification between the various groups.

Results
Effect of *B. montanum* on Ethanol-induced ulcers
Ulcer study
Ulcer index (UI) is shown in (**Figure .1)** Per-oral administration of 1 ml of absolute ethanol produced multiple mucosal lesions in the rat stomach. Pre-treatment with omeprazole (20 mg/kg/p.o.) and MEBM (250 mg/kg/p.o. and 500 mg/kg/p.o.) significantly reduced ulcer index in ethanol-induced gastric mucosal injury. MEBM at a dose of 500 mg/kg/p.o. showed more efficiency as compared to 250 mg/kg/p.o.

Effect of B. montanum on Reduced Glutathione
Induction of gastric ulcers in ethanol control group produced a significant decrease in colonic GSH level ($p < 0.001$) as compared to the normal group. However, MEBM (250 mg/kg and 500 mg/kg) and Omeprazole (20 mg/kg) pre-treatment significantly increased GSH content as compared to the ethanol control group (**Figure 2**). A dose dependent response was obtained with 500 mg/kg dose of MEBM and 250 mg/kg dose of MEBM.

Effect of B. montanum on Superoxide Dismutase activity
The rats administered ethanol showed marked reduction in the level of SOD when compared with the normal rats. However, MEBM (250 mg/kg/p.o. and 500 mg/kg/p.o.) and omeprazole (20 mg/kg/p.o.) administration produced markedly increased SOD concentration in rats when compared with ethanol treated rats (**Figure 3**).

Effect of B. montanum on tissue Nitrate/nitrite
The rats administered 1 mL of absolute ethanol decrease in the tissue nitrate level in ethanol treated group as compared to normal rats. Whereas, rats treated with omeprazole (20 mg/kg/p.o.) and MEBM at a dose of 250 mg/kg/p.o. and 500 mg/kg/p.o. showed a significant increase in the nitrate levels as compared with ethanol administered. Both doses of MEBM 250 mg/kg/p.o. and 500 mg/kg/p.o. Showed dose dependent response **(Figure 4).**

Effect of B. montanum on tissue TBARS level in ethanol-induced ulcer
1ml ethanol administration caused a significant increase in the level of TBARS in rats ($p < 0.001$). However treatment with omeprazole (20 mg/kg/p.o.) and MEBM at a dose of 250 mg/kg/p.o. and 500 mg/kg/p.o. markedly maintained the level of TBARS. A dose dependent response was obtained with 500 mg/kg dose of MEBM and 250 mg/kg dose of MEBM (**Figure 5**).

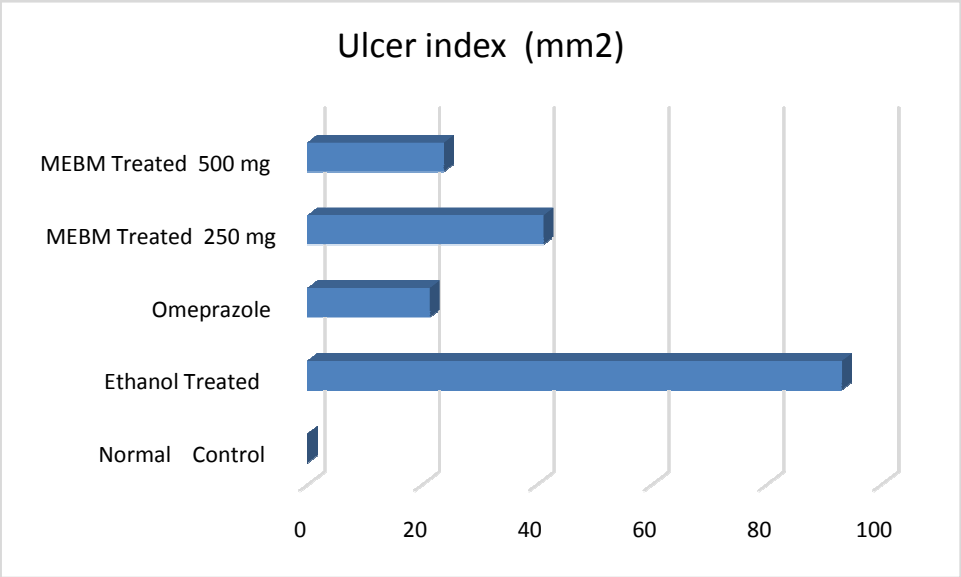


Figure 1. Effect of MEBM on ulcer index (mm²) in ethanol induced gastric ulcers.

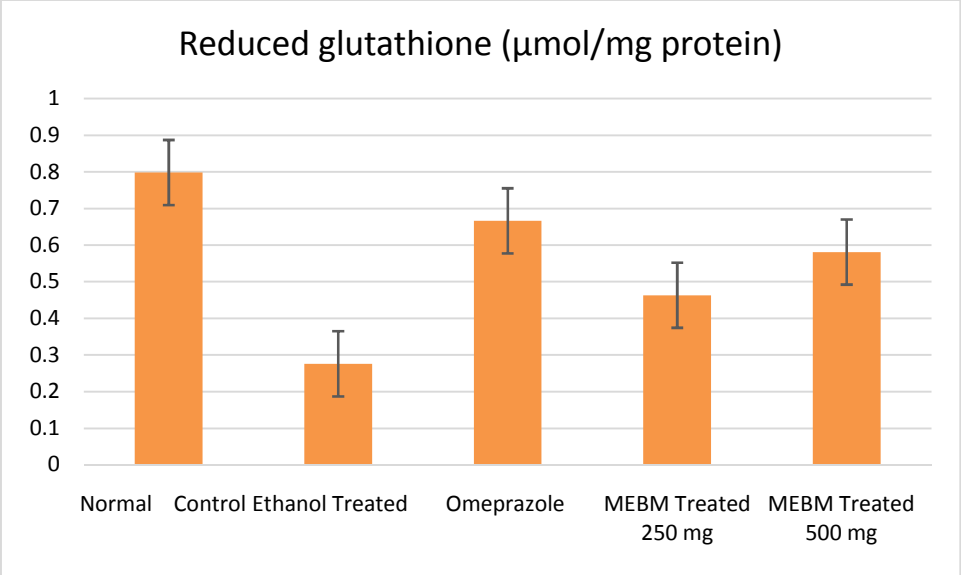


Figure 2. Effect of MEBM on GSH level in Ethanol induced ulcer in rats

All the values are expressed as Mean ± SEM. a*** represent $p < 0.001$ *vs* normal control ; b** represent $p < 0.01$, b*** represent $p < 0.001$, *vs* ethanol control.

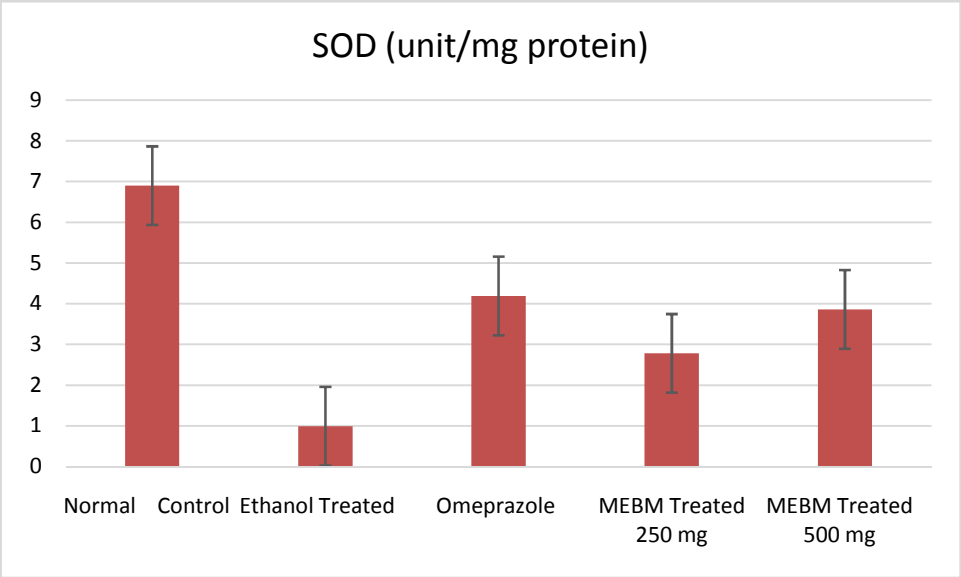


Figure 3. Effect of MEBM on SOD level in Ethanol induced ulcer in rats

All the values are expressed as Mean ± SEM a*** represent $p < 0.001$ *vs* normal control; b** represent $p < 0.01$, b*** represent $p < 0.001$, *vs* ethanol control.

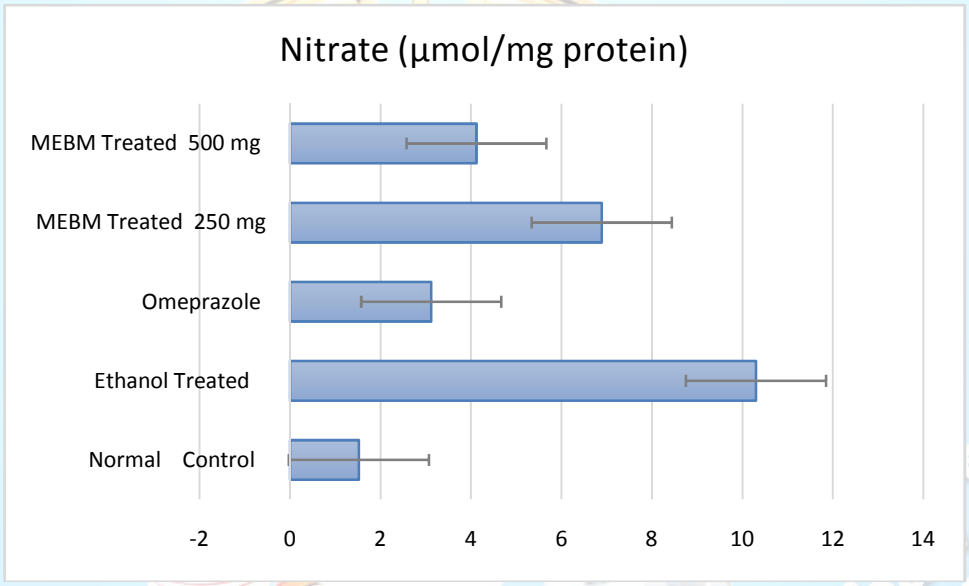


Figure 4. Effect of MEBM on nitrate level in Ethanol induced gastric ulcers.

All the values are expressed as Mean ± SEM a*** represent $p < 0.001$ *vs* normal control; b** represent $p < 0.01$, b*** represent $p < 0.001$ *vs* ethanol control.

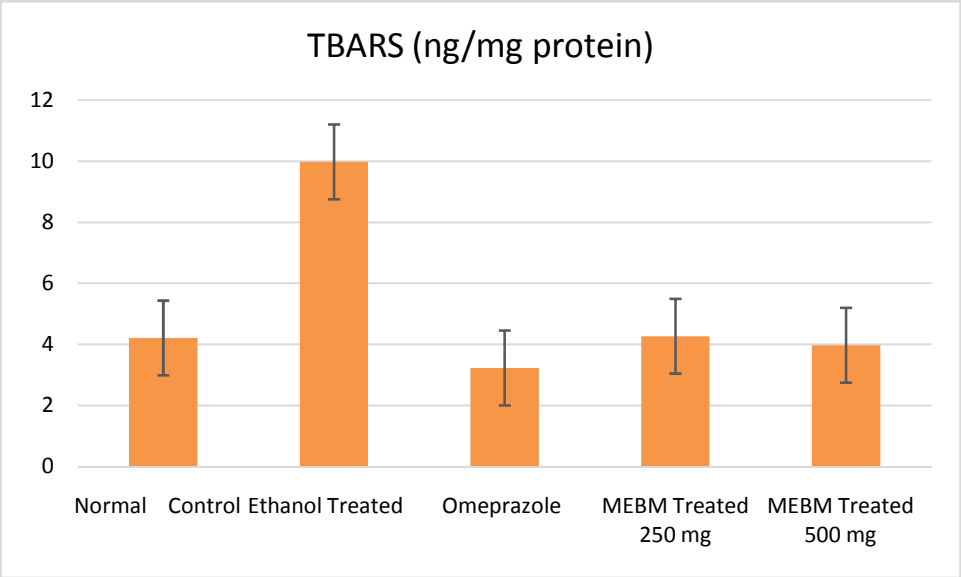


Figure 5. Effect of MEBM on TBARS level in Ethanol induced ulcer in rats

All the values are expressed as Mean ± SEM a*** represent $p < 0.001$ *vs* normal control ; b** represent $p < 0.01$, b*** represent $p < 0.001$ *vs* ethanol control.

Effect of *B. montanum* on Indomethacin-induced ulcers

Ulcer study

Ulcer index (UI) is shown in (**Figure 6)** Per-oral administration of 1 ml of absolute Indomethacin produced multiple mucosal lesions in the rat stomach. Pre-treatment with omeprazole (20 mg/kg/p.o.) and MEBM (250 mg/kg/p.o. and 500 mg/kg/p.o.) significantly reduced ulcer index in Indomethacin-induced gastric mucosal injury. MEBM at a dose of 500 mg/kg/p.o. showed more efficiency as compared to 250 mg/kg/p.o.

Effect of B. montanum on Reduced Glutathione

Induction of gastric ulcers in Indomethacin control group produced a significant decrease in colonic GSH level ($p < 0.001$) as compared to the normal group. However, MEBM (250 mg/kg and 500 mg/kg) and Omeprazole (20 mg/kg) pre-treatment significantly increased GSH content as compared to the Indomethacin control group (**Figure 7**). A dose dependent response was obtained with 500 mg/kg dose of MEBM and 250 mg/kg dose of MEBM.

Effect of B. montanum on Superoxide Dismutase activity

The rats administered Indomethacin showed marked reduction in the level of SOD when compared with the normal rats. However, MEBM (250 mg/kg/p.o. and 500 mg/kg/p.o.) and omeprazole (20 mg/kg/p.o.) administration produced markedly increased SOD concentration in rats when compared with Indomethacin treated rats (**Figure 8**).

Effect of B. montanum on tissue Nitrate/nitrite

The rats administered 1 mL of absolute Indomethacin decrease in the tissue nitrate level in Indomethacin treated group as compared to normal rats. Whereas, rats treated with omeprazole (20 mg/kg/p.o.) and MEBM at a dose of 250 mg/kg/p.o. and 500 mg/kg/p.o. showed a significant increase in the nitrate levels as compared with Indomethacin administered. Both doses of MEBM 250 mg/kg/p.o. and 500 mg/kg/p.o. showed dose dependent response **(Figure 9)**

Effect of B. montanum on tissue TBARS level in Indomethacin-induced ulcer

1ml Indomethacin administration caused a significant increase in the level of TBARS in rats ($p < 0.001$). However treatment with omeprazole (20 mg/kg/p.o.) and MEBM at a dose of 250 mg/kg/p.o. and 500 mg/kg/p.o. markedly maintained the level of TBARS. A dose dependent response was obtained with 500 mg/kg dose of MEBM and 250

mg/kg dose of MEBM (**Figure 10**).

The treatment with methenolic extract of *B. montanum* (MEBM) (250 mg/kg/p.o. and 500 mg/kg/p.o.) per se to normal rats did not produce any significant effects on various parameters performed in the present study. The effect was similar to normal control that's why per se groups are not showed in results.

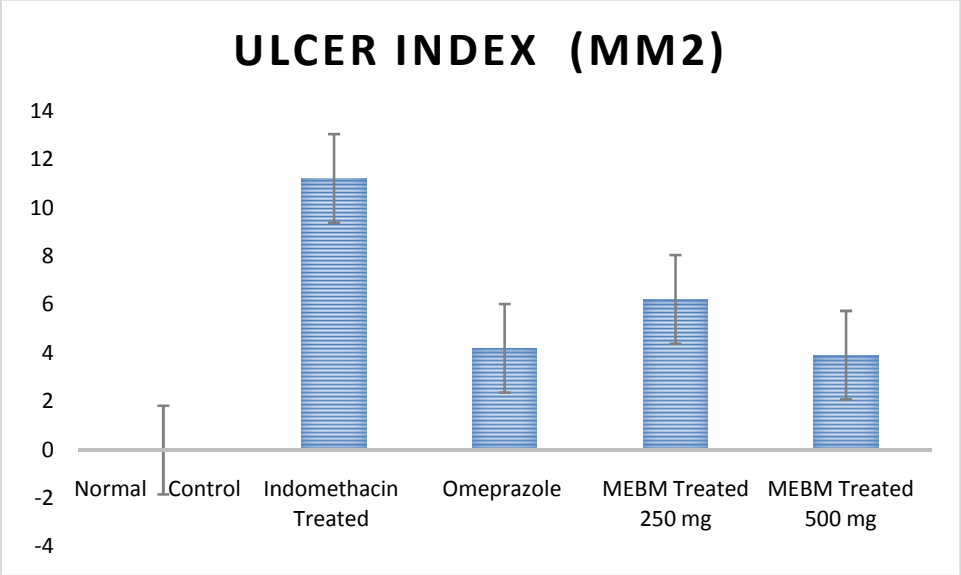


Figure 6. Effect of MEBM on ulcer index (mm²) in indomethacin induced gastric ulcers.
All the values are expressed as Mean ± SEM where a*** represent $p < 0.001$ *vs* normal control; b*** represent $p < 0.001$ *vs*indomethacincontrol.

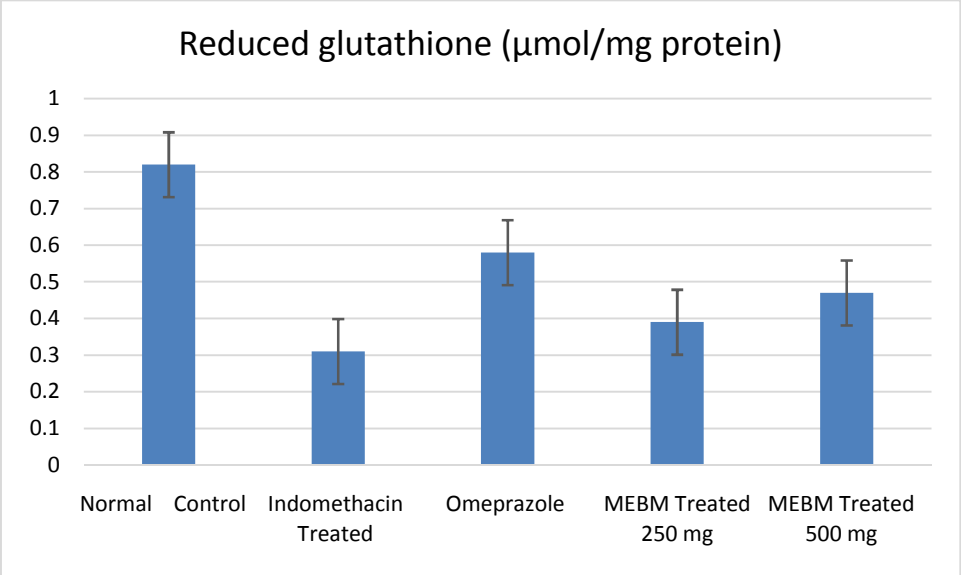


Figure 7. Effect of MEBM on GSH level in indomethacin induced ulcer in rats
All the values are expressed as Mean ± SEM a*** represent $p < 0.001$ *vs* normal control; b** represent $p < 0.01$, b*** represent $p < 0.001$, *vs*indomethacin control.

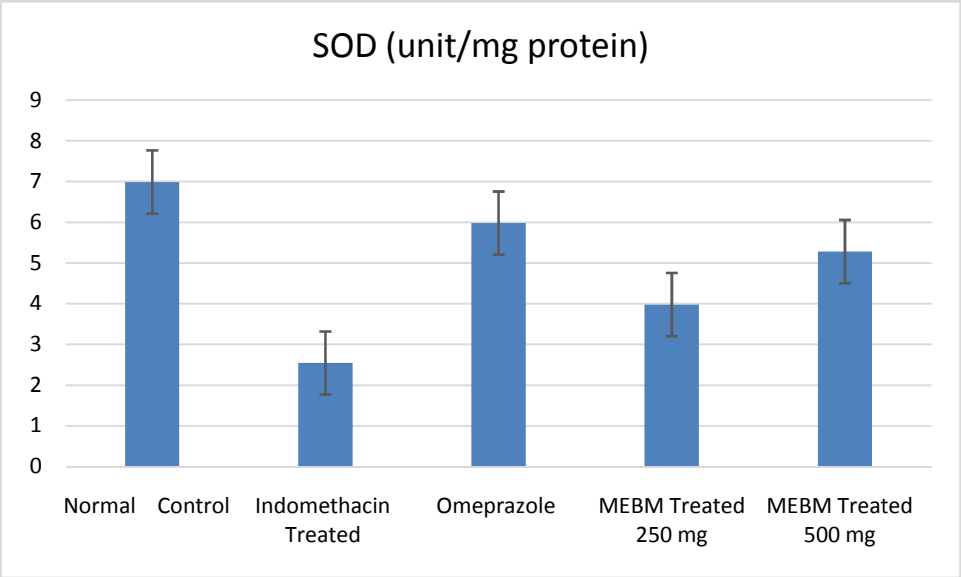


Figure 8. Effect of MEBM on SOD level in indomethacin induced ulcer in rats

All the values are expressed as Mean ± SEM a*** represent $p < 0.001$ *vs* normal control; b** represent $p < 0.01$, b*** represent $p < 0.001$, *vs*indomethacin control.

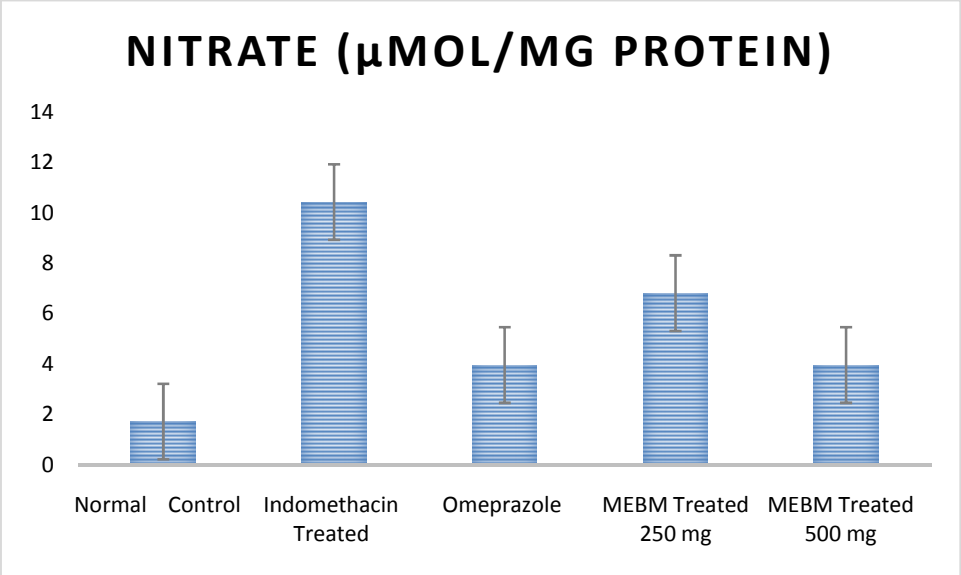


Figure 9. Effect of MEBM on nitrate level in indomethacin induced gastric ulcers.

All the values are expressed as Mean ± SEM a*** represent $p < 0.001$ *vs* normal control; b** represent $p < 0.01$, b*** represent $p < 0.001$ *vs*indomethacin control.

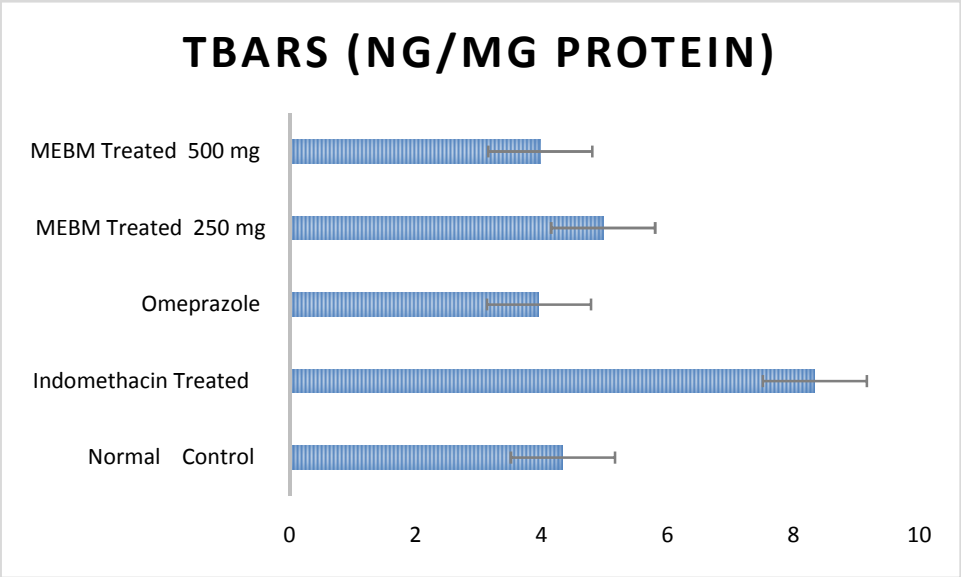


Figure 10. Effect of MEBM on TBARS level in indomethacin induced ulcer in rats

All the values are expressed as Mean ± SEM a*** represent $p < 0.001$ *vs* normal control ; b** represent $p < 0.01$, b*** represent $p < 0.001$ *vs*indomethacin control.

Discussion

The present study was planned to evaluate the anti-ulcer activity of methanolic extract of *B. montanum*. The plant was explored for its effect on ethanol induce ulcer and indomethacin induce ulcer in rats. The aim of the study was to verify the gastric parameters (ulcer index, gastric fluid volume, pH of gastric fluid, total acidity) and anti-oxidant parameters (TBARS, GSH, nitrates and SOD). The preliminary phytochemical studies show identification of phytochemicals such as alkaloids, tannins, flavonoids and terpenoids. In ethanol (1ml/p.o.) and indomethacin induced ulcer models, MEBM at both dose levels showed significant gastroprotective activity. They also reduce gastric fluid volume, total acidity of gastric fluid and increase the pH of the gastric fluid. So, we can conclude that MEBM shows antisecretory and gastroprotective property. Also it is observed that, ethanol and indomethacin found to increase lipid peroxidation and decrease SOD, decrease Nitrate and reduced glutathione in the disease control group, thus leading to oxidative stress. With the administration of MEBM, at the both doses there was significant reduction in lipid peroxidation and an increase in SOD, increase the level of Nitrate and glutathione. No death and side effects were observed at both dose levels of MEBM (250mg/kg/p.o. and 500 mg/kg/p.o.).

Conclusion

The present study thus concludes that the extract of the roots of *B. montanumis* an effective anti-ulcer agent. Study also proves that the anti-ulcer effect may be due to its antioxidant mechanism of action as it reduces the oxidative stress and consequently improves the integrity of gastric mucosa and enhances the generation of nitric oxide and mucus in experimentally-induced gastric ulcers.

References

1. Sonnenberg A, Everhart JE. (1996) The prevalence of self-reported peptic ulcer in the United States. Am J Public Health.86,200-205.
2. Kang JY, Tinto A, Higham J, Majeed A (2002) Peptic ulceration in general practice in England and Wales 1994-98: period prevalence and drug management. Aliment Pharmacol Ther. 16, 1067-74.
3. Grossman M (2009) Peptic ulcer: A guide for the practicing physician. Am J Pharm Toxicol. Vol.4. Chicago: Year Book Medical Publishers. 79, 89-93.
4. R. Best, D.A. Lewis & N. Nasser., (1984) Br J Pharmacol. 82,107.
5. Anonymous. The Wealth of India: A dictionary of Indian RawMaterials and Industrial Products. New Delhi: Publication andInformation Directorate; 1988.

6. Khandelwal, KR., (2004) Techniques and experiments.Practical Pharmacognosy. 11 ed., Nirali Prakashan, 149-156.
7. Kokate CK (2005). Practical Pharmacognosy. New Delhi: Vallabh Prakashan, p.107-8, 115-20, 122-3.
8. Suzuki, Y., Hayashi, M., Ito, M., Yamagami, I., (1976) Antiulcer effects of 40-(2-carboxyethyl) phenyl trans-4-aminomethyl cyclohexane carboxylate hydrochloride (Cetraxate) on various experimental gastric ulcers in rats. Jpn. J. Pharmacol. 26, 471–480.
9. Patil PB (2011). Evaluation of antiulcer activity of aqueous and ethanolic extract of Oxalis corniculata leaf in experimental rats. IJPRD. 3, 98-104.
10. Urushidani, T, Kasuya U and Okabe S. (1979). The mechanism of aggravation of indomethacin-induced gastric ulcers by adrenalectomy in the rat. Jpn. J. Pharmacol., 29: 775-780
11. Karthik P, Amudha P, Srikanth J (2010). Study on phytochemical profile and anti-ulcerogenic effect of Cayratia pedatata Lam in albino wistar rats. Pharmacologyonline. 2, 1017-1029.
12. Baghaei A, Esmaily H, Abdolghaffari AH, Baeeri M, Gharibdoost F, Abdollahi M (2010). Efficacy of Setarud (IMOD@), a novel drug with potent anti-toxic stress potential in rat inflammatory bowel disease and compaison with dexamethasone and infliximab. Indian J Biochem Biophys. 47(4): 219-226.
13. W. Suwannaphet, A. Meeprom, S. Yibchok-Anun, and S. Adisakwattana, "Preventive effect of grape seed extract against highfructose diet-induced insulin resistance and oxidative stress in rats," *Food and Chemical Toxicology*, vol. 48, no. 7, pp. 1853–1857,2010.
14. Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannebaum SR (1982). Analysis of nitrate, nitrite, and [15N] nitrate in biological fluids. Anal. Biochem. 126, 131–138.
15. Ohkawa H, Ohishi N, Yagi K (1979). Assay for Lipid Peroxides in Animal Tissues by Thiobarbituric Acid Reaction. Anal. Biochem. 95: 35-l-358