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Experimental Research Article

Evaluation of ulcer healing activity of *Mahonia napaulensis* in Swiss albino mice

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ABSTRACT

Introduction: The indigenous population of Nagaland has traditionally utilized the bark of *Mahonia napaulensis* as a remedy for gastrointestinal ailments; however, its therapeutic effectiveness has not been scientifically verified. The objective of the current investigation is to assess the efficacy of the methanolic bark extract of *M. napaulensis* in promoting the healing of peptic ulcers.

Methods: The study involved the induction of ulcers in Swiss albino mice via the oral administration of a single dose of 80% ethanol (1 ml/100g body weight). The effect of the methanolic bark extract of the plant on ethanol-induced peptic ulcer was studied using repeated dosing of 200 mg/kg body weight for ten and fourteen days, respectively. The standard drug utilized in the study was Ranitidine (30 mg/kg body weight). The assessment of ulcer healing activity was conducted through the evaluation of various parameters, including the ulcer index, healing percentage, gross macroscopic lesions, as well as histopathological and ultrastructural observations. The concentrations of malondialdehyde (MDA), glutathione (GSH), protein, and sialic acid were quantified.

Results: The results indicate that the oral consumption of methanolic bark extract of M. napaulensis has pro-healing efficacy on ulcerated mice. Thus, this justifies the use of the plant as a healing agent among the rural population.

Conclusion: The results indicate that the oral consumption of methanolic bark extract of M. napaulensis has pro-healing efficacy on ulcerated mice. Thus, this justifies the use of the plant as a healing agent among the rural population.

Keywords:
Mahonia napaulensis
Methanolic extract
Acute toxicity
Sialic acid
Peptic ulcer

Introduction

Peptic ulcer disease (PUD) is one of the most common diseases in the world. It is mostly caused by two factors: persistent *Helicobacter pylori* infection and non-steroidal anti-inflammatory drugs (NSAIDs) misuse (Kavitt et al., 2019). Besides that, excessive alcohol or caffeine consumption, unhealthy diets, smoking habits, and mental stress are also key contributors to the development of peptic ulcer disease (Ahmad et al., 2019; Tripathi et al., 2021). For evaluation of anti-peptic ulcer potential, one of the most commonly used experimental models is the ethanol-induced ulcer model which closely resembles the physiological and morphological alterations observed in acute human peptic ulcer disease (Fu et al.,

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2018). Ethanol intake exposes the mucosal layer to hydrochloric acid and pepsin, which have proteolytic and hydrolytic effects that destroy cell membranes and result in cell exfoliation, erosion, and ulceration (Shams and Eissa, 2022). Ethanol makes the mast cells, macrophages, and blood cells release vasoactive substances, which increase the overproduction of reactive oxygen species (ROS) and lead to oxidative damage within the cell (Kadasah et al., 2021). The integrity of the gastric mucosa is crucially maintained by endogenous sulfhydryl (SH) and the high content of non-protein sulfhydryl (mostly GSH, γ-glutamyl-cysteinyl glycine) which indicates the potential implication for gastro-protective activity (De Araújo et al., 2021). Protein boosts the stomach wall's resilience to the combined attack of pepsin and hydrochloric acid (Matzner et al., 1938). In addition, the levels of malondialdehyde (MDA) are indicative and reliable parameters of oxidative alterations (Song et al., 2019). Current therapy for peptic ulcers focuses mostly on reducing stomach acid production and reinforcing gastric mucosal development (Khoder et al., 2016). Currently, multiple synthetic anti-ulcer drugs are commercially available and among them, Proton pump inhibitors and H2 blockers are widely used for anti-ulcer treatment. Despite their extensive usage and therapeutic success, synthetic drugs have limitations, including risk factors for asymptomatic hypomagnesemia, hypocalcemia, and hypernatremia. Due to the numerous adverse effects associated with synthetic medications, people are increasingly seeking new healing therapies. Medicinal plants are regarded as a primary source of novel pharmaceuticals, as they typically have minimal side effects for consumers. Currently, approximately 65% of the Indian population is directly reliant on herbal medicine for their preliminary healthcare requirements (Singh et al., 2018). Due to their safety and availability in natural sources, many herbal medicines have historically been used worldwide for the treatment of various illnesses, including peptic ulcers (Mazumder et al., 2021). Also, many secondary plant metabolites, such as alkaloids, flavonoids, and terpenoids have been reported to exert a positive influence on peptic ulcer healing due to their specific chemical structure and mechanism of action (Jain, 2016).

Mahonia napaulensis DC. is a perennial shrub plant which belongs to the family Berberidaceae and is the principal source of isoquinoline alkaloids namely; berberine, jatrorrhizine, coptisine, palmatine, and columbamine (Thusa and Mulmi, 2017). Berberine is the primary chemical element that inhibits hepatoma cell line (HepG2), cholesterol, and triglyceride formation and also helps in the eradication of *H. pylori* (Zhang et al., 2017; Och et al., 2020). Clinical studies indicated that *M. napaulensis* possesses antimicrobial, anti-inflammation, and antifungal potential (Thusa and Mulmi, 2017; Bajpai and Vankar, 2007). Furthermore, it has been reported that decoction of the root and stem is used to treat diarrhea, abscesses, conjunctival congestion, and painful discomfort (He and Mu, 2015). In Nagaland, rural people who do not have the accessibility to modern healthcare facilities are dependent on traditional therapies. Interaction with the traditional healers revealed that our test plant has been used as a therapeutic agent for various gastrointestinal problems for a long time. A review of the available literature suggests that the plant has yet to be scientifically validated for its pro-ulcer healing potential. This observation laid the foundation for our current *in vivo* study. Therefore, the present study aims to examine the anti-peptic ulcer activity of methanolic crude extract of *M. napaulensis* bark against ethanol-induced peptic ulcer in Swiss albino mice.

Material and Methods

Materials

Plant collection and preparation of methanolic extract

M. napaulensis was collected from Nagaland and was identified and authenticated by a taxonomist from the Botanical Survey of India, Shillong (Accession no. 98166). For the preparation of the crude extract, the stem bark was removed from the plant, cleansed with water, dried under shade, and then processed in a blender to make fine powder. The powder was then soaked in 90% methanol (100 g/l) for a duration of 10 days. The resulting solution was then subjected to filtration using Whatman filter paper No.1 and the solvent from the solution was subsequently separated out through a rotary evaporator. The methanolic crude extract was then stored at 4ºC until further use. Prior to treatment, the doses were prepared by dissolving the extract in a solution of 0.9% Phosphate Buffer Saline (PBS) with a pH range of 7.2-7.4.

Phytochemical analysis of plant extract

The plant materials were subjected to a series of qual-

itative tests in order to identify different phytochemicals such as alkaloids, flavonoids, tannins, terpenoids, and saponins by using standard protocols (Obiamine and Uche, 2008). Descriptions of the different qualitative tests were as follows:

Alkaloids: In a beaker, 100 mg of powdered material was diluted in 5 ml of methanol and then filtered. 1 ml from each of the obtained filtrate was then placed in two different test tubes. A small quantity of Dragendorff's reagent was to one test tube and Mayer's reagent was introduced to another test tube and the occurrence of orange-red and buff-colored precipitate confirms the presence of alkaloid.

Flavonoid: FeCl3 test: A quantity of 0.5 g of the extract was subjected to heat boiling in the presence of distilled water, followed by filtration. A small volume of a 10% solution of ferric chloride was introduced into 2 ml of the filtrate. The observation of a green-blue hue serves as an indicator of the existence of a phenolic hydroxyl moiety.

Tannins: A test tube containing 0.5 g of the plant extract was subjected to boiling in 10 ml of water, followed by filtration. Further a few drops of ferric chloride (0.1%) solution were mixed with 2 ml of the filtrate. The coloration of brownish-green or blue-black suggests the presence of tannin content.

Steroids: The crude extract was dissolved with 2 ml of chloroform. Concentrated H2SO4 was applied in a careful manner at the periphery. The presence of steroids can be inferred when a red coloration is observed in the bottom chloroform layer.

Glycoside: Killer-kilani test: Glacial acetic acid (2 ml) with one or two drops of a 2% FeCl3 solution was added to the crude extract. To this mixture then volume of 2ml concentrated H2So4 was carefully added along the edges of the test tube. The occurrence of a brown ring at the interface implies the existence of glycosides.

Anthroquinone: A volume of 1 ml of plant extract was added with a small quantity of 2% hydrochloric acid (HCl). The observation of a precipitate having a red hue serves as an indication of the existence of anthraquinones.

Saponins: The powdered sample weighing 1g was subjected to boiling in 10 ml of distilled water, followed by filtration. 3 ml of distilled water was added to the filter, and the mixture was violently agitated for five minutes. The occurrence of foam during agitation serves as an indicator of the presence of saponins.

Phlobatannins: A 2 ml volume of an aqueous extract was subjected to boiling in the presence of 2 ml of a 1% hydrochloric acid solution. The manifestation of a reddish hue serves as an indication of the existence of phlobatannins.

Reducing sugars: A quantity of 0.5 g of the plant extract was solubilized in 10 ml of distilled water and afterward subjected to filtration. Following that, the filtrate was subjected to heating in the presence of 5 ml of Fehling's solution A and B, both of which were of identical quantities. The presence of reducing sugars can be determined by the formation of a red precipitate of cuprous oxide.

Terpenoids: Salkowski test: 5 ml of extract was infused with 2 ml of chloroform and then 3 ml concentrated H2So4 was added carefully. The occurrence of a reddish-brown hue near the interface is indicative of the existence of terpenoids.

Experimental animals

Healthy Swiss albino mice (males and females) aged 8-12 weeks and weighing between 25-30 grams were used for this study. The animals were procured from the Pasteur Institute, Shillong, Meghalaya, and were maintained under standard housing circumstances, which included a schedule of 12 hours of light and 12 hours of darkness, and temperature at the range of 22-25°C. They were provided with a conventional laboratory rodent diet and water *ad libitum*. Animals were acclimatized for five days prior before conducting the experiments.

Acute toxicity study

An acute oral toxicity study was carried out according to the guidelines of the Organization for Economic Co-operation and Development (OECD, 2001). Mice (five animals for each step) were administered different concentrations (500, 1000, 2000) of crude plant extract only once (on day 0). Cage-side observations were conducted to document any signs of toxicity, such as salivation, tremors, diarrhea, convulsions, lethargy, sleep, coma, and mortality, if any, over a period of 14 days.

Anti-peptic ulcer study

The Swiss albino mice were randomly placed into four groups, with each group consisting of six mice. Ulcers were induced in all the animals by administer-

ing ethanol, following the standard method (Oates and Hakkinen, 1988), with slight modifications. All animals were fasted for 24 h with free access to water, after which 80% ethanol (1 ml per 100 g of body weight) was administered orally. Group I: considered as ulcerated group, where animals were sacrificed one hour after the induction of ulcers, Group II: considered as positive control, received ranitidine (30 mg/kg bw), Group III: considered as treated group, received methanolic crude extract of *M. napaulensis* (100 and 200 mg/kg bw) and Group IV: considered as self-recovery group, received only distilled water. At the end of the 6^{th} and 14^{th} day, animals were kept for 24 hours fasting before being sacrificed under ether anesthesia.

Ulcer index and Ulcer healing percentage

The stomach of each animal was operated along the greater curvature, cleaned with water, and then examined to assess the formation of ulcers. After a thorough examination of each stomach, Ulcer index (U_I) was calculated by using the formula: $U_I = U_N + U_S + U_P \times 10^{-1}$(Kulkarni, 2005) Where U_I = Ulcer Index; U_N = Average number of ulcers per animal; U_S = Average number of severity score and U_P = Percentage of animals with ulcers. The severity score was noted as follows, Normal colored stomach = 0, Red coloration = 0.5, Spot ulcer = 1, Hemorrhagic streak = 1.5, Deep ulcer = 2, and Perforation = 3. The percentage of ulcer healing was calculated as follows-

Percentage of ulcer healing = $[(UI_{control} - UI_{treated})/UI_{control}] \times 100$

Biochemical parameters

Tissue preparation

After 6, 10, and 14 days of treatment, stomach tissues from the mice were taken out and washed in ice-cold saline PBS (pH-7.4). Then the tissues were weighed and processed immediately for estimation of protein, sialic acid, GSH, and MDA.

Protein Estimation

The protein concentration of each group was determined using the Bradford Method (Bradford, 1976). The quantification of total protein in the stomach homogenate sample was conducted by assessing the interaction between the Coomassie Blue G250 dye and proteins with greater molecular weights. The 5% homogenate of stomach tissue was prepared in distilled water. 0.5 ml of the sample homogenate was taken and diluted to 1 ml using distilled water. Then, 3 ml of Bradford's solution (diluted, 5x) was added. After 5 minutes, the absorbance was noted at a 595 nm wavelength. The data were presented as µg protein/ mg wet tissue. The experiment was performed in triplicate and mean values are presented.

Sialic acid Estimation

The sialic acid concentration was determined in the stomach tissues of mice under different groups followed by the method as described (Yao and Ubuka, 1987). A 5% tissue homogenate was prepared in PBS (pH 7.4). 0.5 ml of the homogenate was made to 1 ml by adding 0.5 ml of PBS. To the sample 0.3 ml glacial acetic acid and 0.3 ml, acid-ninhydrin reagent-2 were added and mixed thoroughly. The mixture was then incubated in a boiling water bath for 10 minutes and rapidly cooled using tap water. The solution was centrifuged at 4000 rpm for 5 minutes and absorbance at 470 nm was measured using the clear supernatant. The data were presented as µg Sialic acid/ mg wet tissue. The experiment was performed in triplicate and mean values are presented.

Estimation of reduced glutathione

Glutathione (GSH) content as total sulfhydryl contents of the stomach from the entire mice group was determined following the protocol of Sedlak and Lindsay, 1968. The 5% tissue homogenates were made in 0.02 M EDTA having pH 4.7 using a homogenizer. To 100 µl of tissue homogenate, 1 ml of 0.2 M Tris-EDTA buffer (pH 8.2), 0.9 ml of EDTA was added followed by the addition of 20 µl of 0.01 M Ellman's reagent (DTNB), and incubated for 30 minutes at room temperature. The reaction mixtures were then centrifuged at 3,000 rpm for 10 minutes, and the absorbance was measured at 412 nm using a blank reagent in a Systronics Spectrophotometer-106. The data were presented in µmoles of GSH per gram of wet tissue. The experiment was performed in triplicate and mean values are presented.

Lipid peroxidation Assay

The thiobarbituric acid (TBA) test was used to quantify lipid peroxidation (LPO) in the stomach tissues of mice collected from different groups (Buege and Aust, 1978). A 5% tissue homogenate was prepared in 0.15M KCl. 0.5 ml of the tissue homogenate was taken, and to

TABLE 1: Phytochemical screening of *M. napaulensis* bark extract

Phytochemicals	Observations
Alkaloids	+
Flavonoids	+
Tannins	+
Steroids	+
Glycosides	+
Anthraquinones	-
Saponins	+
Phlobatannins	-
Terpenoids	+
Reducing sugars	-

Where "+" denotes "present". "-" denotes "absent".

it, 2 ml of TCA-TBA-HCl reagent (15% Trichloroacetic acid and 0.375% thiobarbituric acid dissolved in 0.25N HCl) was added and mixed thoroughly. The sample was subjected to incubation in a water bath at boiling temperature for 15 minutes, followed by subsequent cooling. The sample was then centrifuged (5000 rpm, 4˚C, 5 min) and the clear supernatant was used to measure malondialdehyde (MDA) concentration of the tissue at 535 nm against the blank. The concentration of MDA in the tissue sample was determined using an extinction coefficient of 1.56 x 10^5 M^{-1} cm^{-1} and results were presented as µmol MDA/ g wet tissue.

Histopathological examination

After dissection, the tissues (stomach) of the experimental groups were collected and fixed in Bouin's fixative and were sectioned using a microtome followed by staining the sections in hematoxylin and eosin, mounting in DPX, and finally viewed under a compound microscope (Leica DM1000).

Ultrastructural studies

For observation of fine surface alterations, scanning electron microscopy was carried out where specimens were fixed in neutral buffer formalin; dehydration was carried out in acetone grades followed by air drying in tetramethylsilane. The gold-coated specimens were ultimately observed using the JEOL JSM 6360 electron microscope at an accelerating voltage of 25 kilovolts (Dey et al., 1989; Roy and Tandon, 1991). For transmission electron microscopic study, the tissue samples collected after 14 days of treatment were first fixed in the modified Karnovsky's fixative, followed by post-fixation in 1% OSO_4 for 4 h, then dehydrated using different acetone grades and then embedded in araldite. The ultrathin sections of the tissue samples were initially stained with uranyl acetate followed by staining with lead citrate. At last, Stomach tissue sections were viewed under JOEL JSM 2100 transmission electron microscope (Hayat, 2000).

Statistical analysis

The data were subjected to analysis using Origin software and were presented as mean ± Standard Error Mean. Furthermore, Statistical significance was determined by one-way analysis of variance (ANOVA), Student's t-test followed by a post hoc (Tukeys' test) to analyze the difference among multiple groups. Each experiment was repeated thrice i.e., n=3. The values were considered significant at $P<0.05$ compared to the healthy and ulcerated group.

Results

Phytochemical analysis

The methanolic *M. napaulensis* bark extract showed the presence of alkaloids, flavonoids, saponins, glycosides, terpenoids steroids, and tannin (Table 1).

Acute toxicity

Zero mortality was observed in mice that received a dose of 2000 mg/kg of methanolic bark extract of *M. napaulensis*. During the 14-day observation period, all

TABLE 2: Effect of *M. napaulensis*stem bark (methanolic extract) on ulcer index and healing activity in ethanol-induced peptic ulcer in mice

Groups	Ulcer index		Healing %	
Ulcerated group	1.85±*0.02*		-	
	6 days		**14 days**	
	Ulcer index	**Healing %**	**Ulcer index**	**Healing %**
Ulcer treated with ranitidine (30 mg/kg)	*0.61±0.05*$^{a*\ b*}$	67.02	*0.079±0.02*$^{a*\ b*}$	95.72
Ulcer treated with *M. napaulensis* (100 mg/kg)	0.78±0.06^{a*}	57.83	0.27**±0.07**^{a*b*}	82.70
Ulcer treated with *M. napaulensis* (200 mg/kg)	0.65±0.04^{a*b*}	64.56	0.098±0.02^{a*b*}	94.65
Self-recovery group	0.99±0.10^{a*}	47.34	0.55±0.14^{a*}	70.20

Values are presented as mean ± SEM, a*P<0.05 when compared with the ulcerated group, and b*P<0.05, when compared with a self-recovery group. One-way analysis of variance (ANOVA) was used in the statistical analysis, followed by the Tukey test.

the animals were found to have normal morphological characteristics related to fur, skin, eyes, mucous membranes, etc. No excessive salivation, diarrhea, lethargy, or bizarre actions were noticed.

Anti-ulcer study

Ulcer index and percentage of healing

Oral administration of Ethanol caused gastric damage with an ulcer index of 1.85±0.02. Animals post-treated with *M. napaulensis* extract (100 and 200 mg/kg bw) and ranitidine (30 mg/kg) for 6 and 14 days exhibited a significant (P<0.05) reduction in ulcer index in comparison to ulcerative and self-recovery group. Compared with ranitidine, *M. napaulensis* extract equivalently heals the ulcer in a time-dependent manner as revealed in the percentage healing offered by *M. napaulensis* (57.83%, 82.70%, and 64.56%, 94.65%) at 100 and 200 mg/kg body weight, respectively and ranitidine (67.02%, 95.72%) at 30 mg/kg body weight. (Table 2). Therefore, based on the findings, we have determined that a dose of 200 mg/kg bw is the optimal dose for the further investigation of ethanol-induced stomach ulcers.

Biochemical study

The amount of GSH, MDA, sialic acid, and protein in the stomach tissues was quantitatively assessed after 6 and 14 days of ulcer treatment. Mice (ulcerated group) administered with 80% ethanol alone showed a significant decrease in protein, sialic acid, and GSH content compared to the control group (Fig.1A, B, C). Total protein has been found to be considerably higher in *M. napaulensis* and ranitidine-treated groups. Compared to the ulcerated group, the treatment with *M. napaulensis* (200 mg/kg bw) and ranitidine (30 mg/kg bw) for 6 and 14 days to ethanol-induced ulcerated mice exhibited a significant (P<0.05) boost in protein levels. However the self-recovery group also showed a significant elevation of protein levels compared to the ulcerated group (Fig.1A). Sialic acid content in the stomach tissues of the ulcerated group was found to be significantly (P<0.05) less as compared to healthy mice i.e., 0.38±0.09 µg/mg tissue. After 6 and 14 days of treatment of the ulcer with *M. napaulensis*, a significant increase in sialic content compared to the ulcerated group was observed (Fig.1B).

Similar to the protein and sialic acid content, a significant (P<0.05) rise of GSH has also been observed for the experimental group treated with *M. napaulensis* both after 6 and 14 days of treatment. However, ranitidine-treated mice showed better healing (Fig.1C). In contrast, ulcerated mice showed a significant rise in MDA level (0.19±0.01 µmoles/g tissue) in comparison to the healthy mice. Further, those mice that were given both therapies and were allowed to heal themselves showed a significant decrease in MDA level as compared to ulcerated mice (Fig.1D).

Histological observation

The healthy normal stomach tissue of mice was macroscopically examined and revealed entirely normal, pink-colored gastric mucosa with normal mucosal thickness. Healthy mice showed normal gastric epithelium with intact mucosa and submucosa with no inflammation (Fig.2A). The Ulcerated group showed the occurrence of an ulcer after one hour of 80% ethanol exposure. Compared to the healthy mice, histology of the ulcer induced by ethanol alone showed extensive

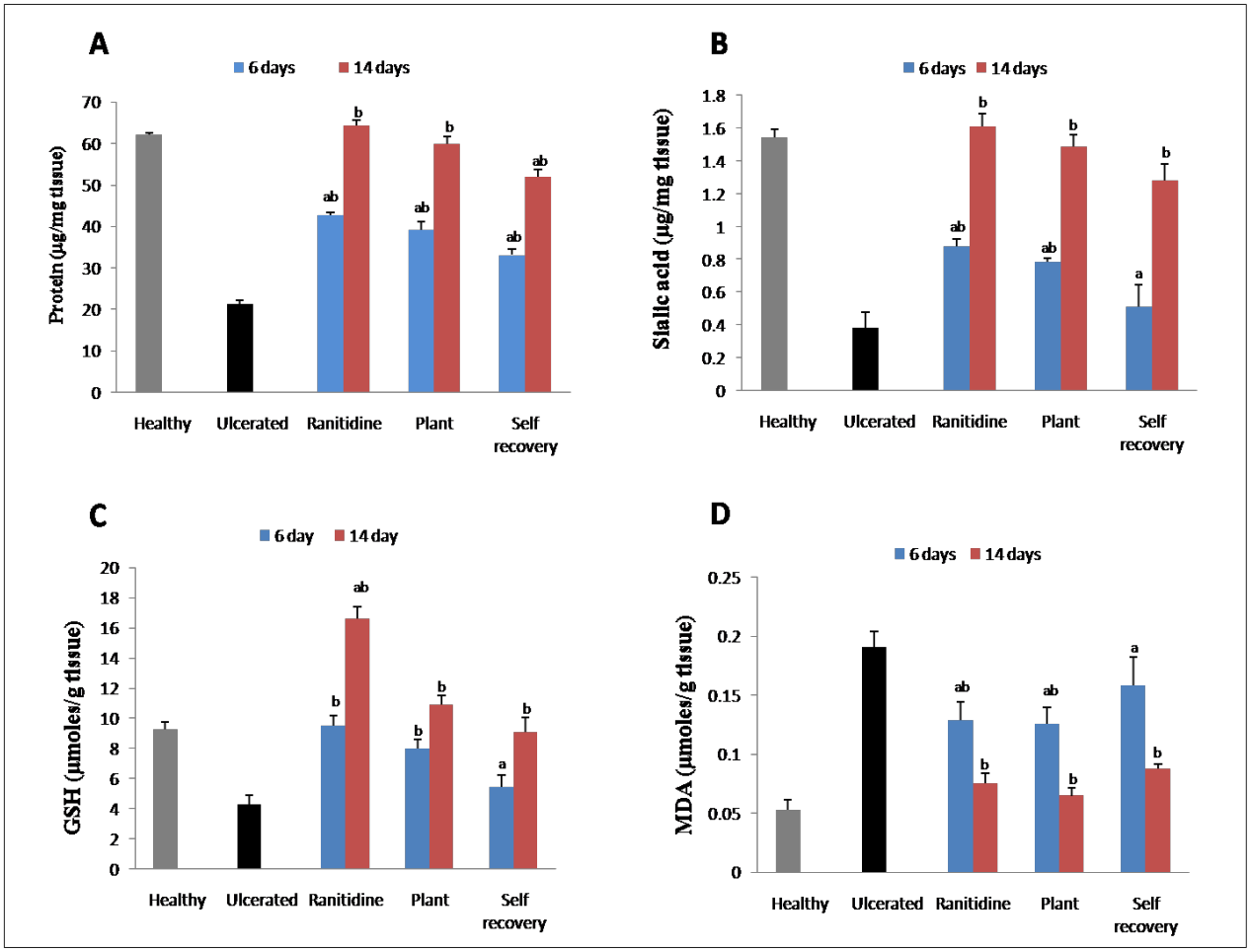

FIGURE 1. Effect of methanolic stem bark extract of M. napaulensis on level of (A) Protein (B) Sialic acid (C) Reduced glutathione (GSH) (D) Malondialdehyde (MDA) in ethanol-induced gastric ulcer mice. Bars with different superscripts for each parameter are considered significantly different (P<0.05). 'a' denotes significantly different from the healthy normal group (P<0.05); 'b' denotes significantly different from the ethanol ulcerated group (P< 0.05)

damage to the stomach mucosa coupled with necrotic lesions and leukocyte infiltration. The necrotic lesions of gastric mucosa penetrated deeply into the basement membrane and lamina propria (Fig.2B). Ulcer treatment with *M. napaulensis* (200 mg/kg) and ranitidine (30 mg/kg) showed a considerable reduction of mucosal damage as compared to self-recovery group (Fig.2C, D). The entire gastric mucosal tissue and surface epithelial cells were restored and regenerated as well.

Ultrastructural observation

Micrographs observed through scanning electron microscope revealed stomach surface topography of mice (Fig.3). The healthy group showed intact epithelial cells having regular arrangement (Fig.3A). As compared to normal mice, ulcerated group depicted significant damage in architecture and orientation of epithelial layers along with gastric pit erosion (Fig.3B). Treatment with both *M. napaulensis* and ranitidine showed intact epithelial cells with slight erosion (Figs.3C, E). The self-recovery group showed a moderate amount of erosion in the epithelial layer (Fig.3D).

Transmission electron microscopy revealed the normal cytomorphology of primary and parietal cells in the healthy group (Fig.4A). Also, the endoplasmic reticulum, mitochondria, and nucleus exhibited regular shape. In addition, Rich zymogen particles were visible in the chief cells of the cytoplasm. The ulcerated group (Fig.4B) showed an irregular picknotic nucleus with fragmented rough endoplasmic reticulum and mitochondria. The zymogen particles were not clear. Ulcerated mice treated with ranitidine (30 mg/kg bw) and *M. napaulensis* (200 mg/kg bw) showed regular intact nuclei with well-developed rough endoplasmic reticulum.

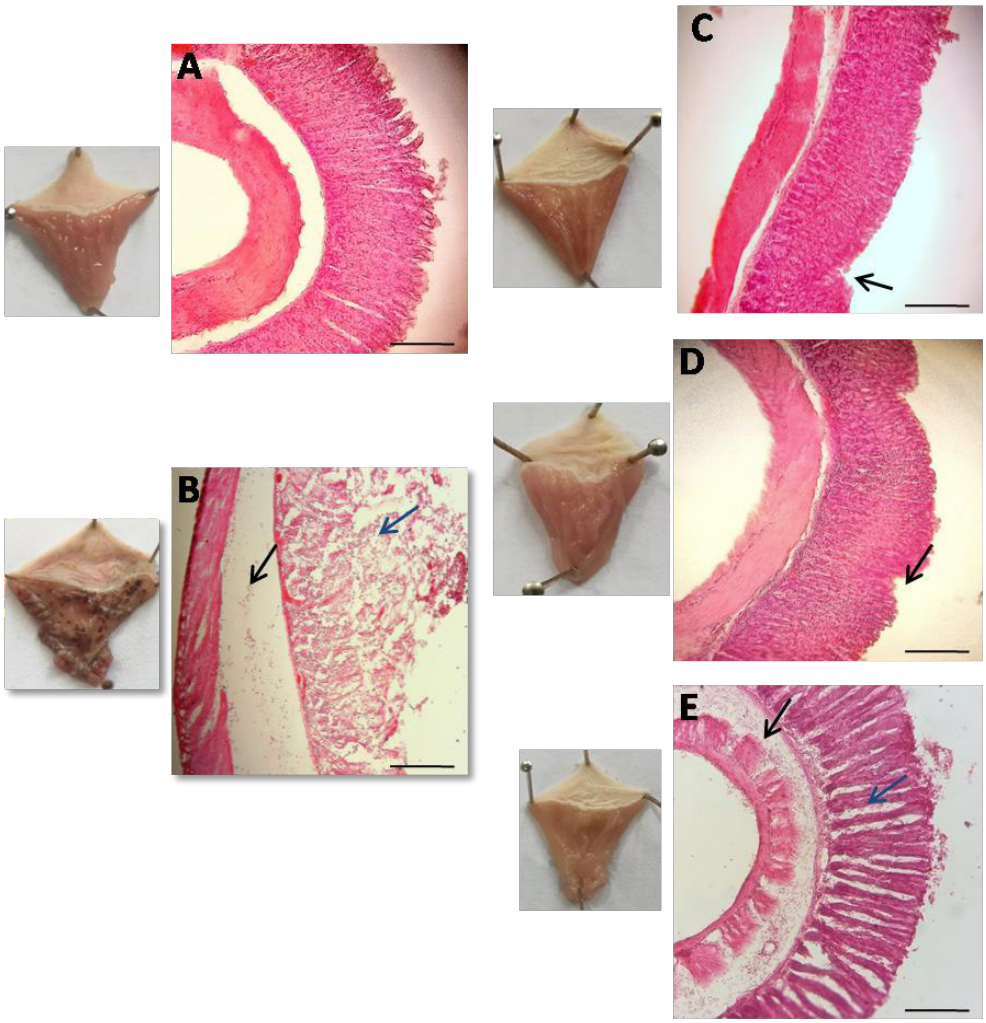

FIGURE 2. Effect of methanolic stem bark extract of M. napaulensis on histology of gastric mucosa in Swiss albino mice. Healthy mice (A) showing normal stomach architecture of gastric epithelium. (B) Stomach of ulcerated mice showing several mucosal injuries (blue arrow), edema with leucocytes, and inflammation of sub mucosal layer (black arrow). (C) Represents ranitidine (30 mg/kg) treated mice on 14th day (D) Represents 14th day treatment with M. napaulensis (200 mg/kg) (E) Represents self-recovery mice on 14th day, all Scale bar: 100µm.

An increase in the number and density of mitochondria was also observed. (Fig.4C & E)

Discussion

Numerous plant species have been documented for their efficacy in the treatment of peptic ulcers (Sharifi-Rad et al., 2018). Ethanol-induced peptic ulcers have been a widely used experimental method for the evaluation of traditional plants' anti-ulcer potency because ethanol simply infiltrates the stomach epithelium and causes damage to the stomach (Mousa et al., 2019, Fahmy et al., 2020).

The phytochemical analysis of the methanolic bark extract of *M. napaulensis* demonstrated the presence of flavonoids, alkaloids, steroids, glycosides, saponins, terpenoids, and tannins which is similar to the previous observation (Paudel et al., 2020). Several studies have demonstrated that berberine, terpenoids, and alkaloids, which are the main active constituents of *M. napaulensis,* protect the gastric mucosa from ulcer lesions via their anti-oxidant and anti-inflammatory effects (Salem et al., 2023). Flavonoids may have reduced the concentration of platelet-activating factor in the mucosa of ethanol-induced ulcerated stomachs, leading to faster healing (Bonamin et al., 2014). In the present study, treatment with *M. napaulensis* bark extract suggests the positive response of plant extract in the ulcer healing process. Furthermore, in ethanol-induced ulcerated mice, berberine prevents the excessive production of nitric oxide (NO) through inhibition of iNOS expression

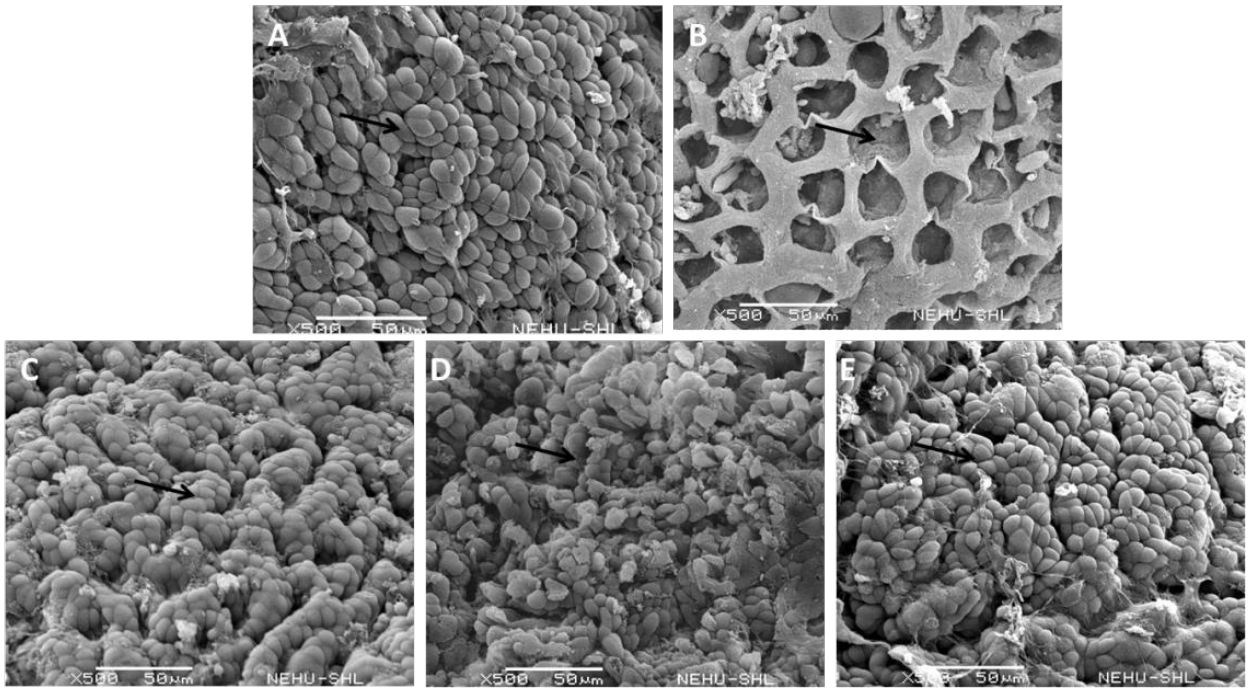

FIGURE 3. Scanning electron micrographs of the stomach of Swiss albino mice. Black arrow indicates epithelium cells with gastric pits. (A) Healthy mice showing uniform gastric epithelium with gastric pits (B) Ulcerated group mice showing damaged epithelial cells and gastric pits (C) Ranitidine (30 mg/kg) treated mice 14th day, showing normal gastric epithelium. (D) Self-recovery (treated with saline only) mice on 14th day. (E) M. napaulensis (200 mg/kg) treated on 14th day showed epithelium cells with slight erosions. Magnification: 20kv x 500, all scale bar: 50µm.

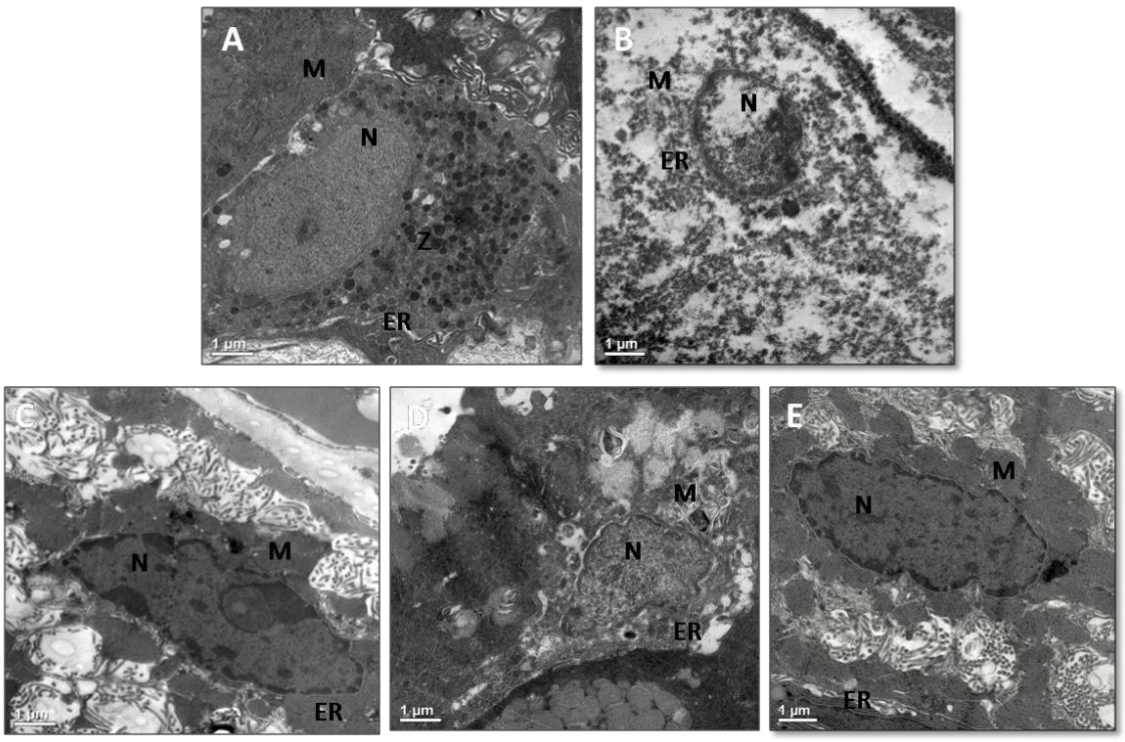

FIGURE 4. Transmission electron micrographs of the stomach of Swiss albino mice. (A) Healthy mice show regular intact nucleus (N), mitochondria (M), intact rough endoplasmic reticulum (ER), and numerous zymogen (Z). (B) Gastric mucosa of ulcerated mice showing irregular pyknotic nucleus (N), ruptured mitochondria (M), and unclear endoplasmic reticulum (ER) (C) Ranitidine (30 mg/kg) treated mice at 14th day showing intact nucleus (N), large number of mitochondria (M) and endoplasmic reticulum (ER) (D) Self-recovery (treated with saline only) mice on 14th day, showing irregular nucleus (N), and unclear mitochondria (M). All scale bar: 1µm.

that exacerbates gastric mucosal injury and ultimately improves ulcer healing (Pan et al., 2005).

According to the observations of the present study, oral ethanol administration to mice caused considerable ulceration in the stomach glandular region, which was confirmed by gross macroscopic analysis showing a sharp rise in the ulcer index. These results are consistent with previous studies (Raish et al., 2021), which showed that ethanol produces visible ulcers in experimental animals. Treatment with *M. napaulensis* bark extract and ranitidine revealed a significant decrease in ulcer index. Furthermore, the percentage of ulcer healing dramatically elevated in both treatment groups in a time-dependent manner. Similar to our observation Ahmed et al., (2022) also recorded a significant reduction in ulcer index in repeated treatment with *Urtica simensis* extract in ethanol-induced rats. The aqueous extract of *Commicarpus chinensis* significantly reduced the ulcer index and enhanced the percentage of protection in a dose-dependent manner (Nitin and Sayeed, 2014).

Protein is very crucial for the healing of epithelial tissue as well as tissue cell regeneration (Dev et al., 2022). The current study showed a significant decrease in protein levels in gastric tissue as compared to healthy mice, which is consistent with the earlier study (Halabi et al., 2014). After 6 and 14 days of treatment, ulcerated mice treated with *M. napaulensis*and and ranitidine showed elevated protein levels in gastric tissue. The self-recovery group also showed an elevation in protein levels but to a lesser extent than the treatment groups. A similar kind of observation was also reported by Fahmi et al., (2019), where the extract of *Pulicaria crispa* showed a significant elevation of total protein concentration in a time-dependent manner as compared to the ulcerated and self-healing group.

Consumption of ethanol increases oxidative stress and lipid peroxidation in gastric tissue which consequently leads to significant damage to stomach mucosal layers with an increase in MDA content and a decrease in gastric GSH content (Rahman et al., 2020; Li et al., 2021). Determining GSH and MDA levels might reflect and signify oxidative stress in the tissue. In our study, a marked rise in MDA and a decline in GSH level were recorded in mice stomach tissue after the administration of ethanol however, treatment with *M. napaulensis* bark showed a significant elevation of GSH and a decline in the level of MDA in mice stomach. These results suggested that *M. napaulensis* stem bark extract may act as an antioxidant agent for healing ethanol-induced ulcers. Consistent with our study, treatment with *Braophyllum pinnatum* extract against ethanol-induced ulcer model showed improvement in the antioxidant defense system by stimulating GSH and decreasing MDA level (De Araújo et al., 2021).

The gastric mucosa is crucial to the defense mechanism of the stomach. By creating a mucous-bicarbonate barrier, it preserves the structural integrity of the stomach and guards the gastric wall against harmful and irritating substances (Guzmán-Gómez et al., 2018). Histological and ultrastructural analysis showed that treatment of ulcerated mice with *M. napaulensis* extract at 200mg/kg bw for fourteen days, significantly attenuated the gastric damage than the self-recovery group and accelerated the healing of gastric lesions brought by ethanol. Although healing ability is comparable to that of the ranitidine-treated group. This implies that the healing effect promoted by the *M. napaulensis* stem bark extract is due to increasing gastric mucosal secretion, which helps in the regeneration of the gastric mucosa. Leukocyte infiltration and tissue granulation in the self-recovery group may indicate that auto-healing has occurred. This process involves macrophages and neutrophils, which are responsible for clearing away debris from damaged tissues (Fahmi et al., 2019). Similar to our findings, Shredah and El deeb, (2017), showed that 12 days of continuous treatment with curcumin healed the ulcer by enhancing the regeneration of gastric mucosa. In the present study, treatment with *M. napaulensis* bark extract suggests a positive effect of the plant extract on the ulcer healing process.

Conclusion

M. napaulensis bark methanolic extract successfully healed the peptic ulcer induced by ethanol in mice. The amelioration in ulcer indices, biochemical parameters, and the histological and ultra-structural images of the stomach mucosa have demonstrated the therapeutic potential of *M. napaulensis*. The changes in these biomarkers not only helped detect the presence of stomach ulcers but also illuminated the recovery process. The finding of our present study justifies the use of this plant as a healing agent for peptic ulcer disease by rural people. However, additional investigation is required to identify the specific mechanism of action in the recovery of

gastric ulcers as well as to isolate the active ingredients responsible for the anti-ulcer efficacy.

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Conflict of interest

The authors declare that there is no conflict of interest

Ethics approval

The experimental study was carried out in compliance with the ethical guidelines issued by the committee for the purpose of control and supervision of experiments on animals (CPCSEA), Government of India. The experimental protocol was approved (IEC/MS/Misc./05) by the Institutional Ethics Committee (Animal models), North Eastern Hill University, Shillong, Meghalaya, India, on 28th July 2021

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