JJBS

Volume 16, Number 4,December 2023
ISSN 1995-6673
Pages 599 – 609
https://doi.org/10.54319/jjbs/160405

Jordan Journal of Biological Sciences

Studies on Toxicity and Peptic Ulcer Healing Potential of Crude Extract of *Osbeckia crinita* in Swiss Albino Mice

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Received: November 17, 2022; Revised: March 21, 2023; Accepted: March 29, 2023

Abstract

Background: For gastrointestinal diseases *Osbeckia crinita* (*O. crinita*) has been traditionally used among different parts of Northeast India. The present study aims to evaluate the toxicity and antiulcer activity of *O. crinita* methanolic leaf extract.

Method: For sub-acute toxicity study, repeated oral doses of 150 mg, 300 mg and 600 mg extract of the plant *O. crinita* per kg body weight of mice were administered for 28 days, and analyzed the hematological and biochemical parameters. The effect of crude methanolic extract of the plant on ethanol-induce peptic ulcer was studied using repeated dosing (200 mg/kg bw) for 6, 10 and 14 days, respectively. Ranitidine (30 mg/kg bw) was used as standard drug. Antiulcer activity was assessed by measuring Ulcer index, healing percentage, gross macroscopic lesions, protein and carbohydrate content along with histopathology and ultra-structural observation.

Result: LD_{50} value was found to be more than 2000 mg/kg body weight. The sub-acute toxicity study showed that at a dose of 600 mg/kg bw of mice significant changes were observed in hematological and biochemical parameters. Six, ten and fourteen day's treatment with the plant extract exhibited significant increase in ulcer protection by 62.09%, 81.04% and 90.06%, respectively. As compared to negative ulcerated mice, *O. crinita* treated mice showed higher level of protein and carbohydrate content. The histological and scanning electron microscopic observations showed treatment with *O. crinita* resulted in comparatively better gastric healing in a time dependent manner.

Conclusion: Based on the results, it was concluded that *O. crinita* extract possesses peptic ulcer protective potency comparable to ranitidine, which justifies the use of this plant for ulcer treatment.

Keywords: *Osbeckia crinita*; antiulcer;toxicity; Ranitidine; ethanol; methanol; protein.

1. Introduction

Peptic ulcer is a lesion of gastric or duodenal mucosa that occurs due to imbalance between the aggressive factors (acid, pepsin, free radicals, etc) and the mucosal protective factors, (mucus, prostaglandins) and is responsible for high rate of morbidity affecting up to 10% of the world's population (Kuna *et al*., 2019). Various factors are implicated that play a pivotal role in the pathogenesis of the ulceration like exposure to *Helicobacter pylori*, smoking, consumption of alcohol, poor diet, stress, and the abuse of non-steroidal anti-inflammatory drugs (NSAIDs) (Tijani *et al*., 2021 and Mahmoud *et al*., 2023).The epidemiology revealed that infection with *Helicobacter pylori* is the primary cause of peptic ulcers. It causes 70% of stomach ulcers and 95% of duodenal ulcers (Tripathi *et al*., 2021).The protective gastric mucosal factors are a group of several neurohormonal and physiological systems which prevent the mucosa against noxious and harmful stimuli (Yandrapu and Sarosiek, 2015).The ethanol-induced gastric ulcer model has been frequently utilized to test anti-ulcer activity in the laboratory because of its close resemblance to acute gastric ulcer in humans (Song *et al*., 2018). Excessive alcohol consumption is the most prevalent cause of ulcer which damages the gastric mucosal integrity through lowering gastric mucosal blood flow (Yu *et al*., 2020)

Drug therapy of peptic ulcer has been commonly targeted at either counteracting the aggressive factors or stimulating the defensive ones. Various synthetic antiulcer drugs presently available in the market include antacids, proton pump inhibitors, anticholinergics, H2- receptor antagonists and cytoprotective agents which are being used to prevent and treat various types of ulcers. However, most of the drugs confer simpler to several side effects like arrhythmias, impotence, hematopoietic changes etc. (Freedberg *et al*., 2017; Sharifi-Rad *et al.,* 2018); these complications enforce for the development of new antiulcer drug and search for novel molecules from drug basket of nature, which are the herbal resources.

Traditionally used natural plant products and their derivates have long been recognized as a viable alternative source of therapeutic agents (Koparde *et al*., 2019). *O. crinita* belongs to the family Melastomataceae, known to possess wound healing property and being used for treatment of snake bites and nose bleeding (Rao *et al*., 1981). Methanolic leaf extract of *O. crinita* exhibits anti-inflammatory and anti-oxidant properties. In addition, the plant is reported to contain a plenty of phytochemicals such as alkaloids, flavonoids, tannin and saponin etc.

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(Kalita *et al*., 2022). Local people of Nagaland use this plant to cure gastrointestinal disease. Since scientific validation of the plant is not carried out, the present study aims to assess the anti-peptic ulcer activity of methanolic crude extract of *O. crinita* leaves against ethanol induced peptic ulcer in mice. Acute and sub-acute tests were also carried out to assess the extent of toxicity of the methanolic crude extract of *O. crinita* leaves.

2. Materials and method

2.1. Experimental Animals

Healthy Swiss albino mice (males and females) of 8-12 weeks old and weighing 25-30 grams were used for this study. The animals were procured from the Pasteur Research Institute, Shillong, Meghalaya, and maintained under housing conditions: lighting cycles of 12 h light/12 h dark and temperature of 22-25°C. They were fed with a standard laboratory Rodents' diet and an unlimited supply of drinking water was allowed. The animals were acclimatized for five days before conducting the experiments. The final approval to carry out the study was granted by the Institutional Animal Ethics Committee (IEC), North-Eastern Hill University, Shillong, Meghalaya, India (IEC/MS/Misc./05)

2.2. Plant collection and preparation of methanolic extract

The plant *O. crinita* was collected during the months of July to August, 2019, from Nagaland and was identified and authenticated by a taxonomist from the Botanical Survey of India, Shillong (Accession No. of *O. crinita*: NEHU – 98166). For preparation of the crude extract, the leaves were separated from the plant, washed with water, dried under shade and then grinded into fine powder using a blender. The powder was then soaked in 90% methanol (100 g/l) for 10 days, filtered using Whatman filter paper No.1 and the solvent from the solution was separated out using a rotary evaporator. The methanolic crude extract of leaves yielded 12.7% and was stored at 4ºC until further use. Before treatment, the doses were prepared by dissolving the extract in 0.9% Phosphate Buffer Saline (PBS, pH 7.2-7.4).

2.3. Phytochemical analysis of plant extracts

The plant materials were subjected to various qualitative tests to detect the presence of different phytochemicals such as alkaloids, terpenoids, tannins, saponins, flavonoids etc. using standard protocols (Obiamine and Uche, 2008).

2.4. Acute toxicity study

Acute oral toxicity study was conducted according to the guidelines of Organization for Economic Co-operation and Development (OECD, 2002). Fifteen mice were divided into three groups with five animals in each group. Following an overnight fasting, the mice were administered with different concentrations (500, 1000, 2000 mg/kg bw) of crude plant extract only once (on day 0), and cage side observations were carried out to documented any sign of toxicity (tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma) and mortality, if any, for next 14 days. Number of dead mice was recorded and used for calculation of the median lethal dose (LD_{50}).

2.5. Sub-acute toxicity study

Sub-acute toxicity study was conducted according to the guidelines of Organization for Economic Co-operation and Development (OECD, 2008).Twenty four animals were divided into four groups of six animals each. Before the experiments mice were fasted for 24 h. Group I (control) was administered orally with vehicle (PBS) only, and the remaining three groups (Group II, III and IV) were administered orally with different desired sub-acute concentrations(150, 300, 600 mg/kg bw) of crude plant extracts daily for 28 days. Food and water intake was recorded daily, whereas body weight was recorded once in a week throughout the study period.

2.5.1. Hematological and biochemical analysis

Twenty four hours after termination of experiment, the blood sample was drawn from the vein of hind leg of mice with a syringe under anesthesia and collected into vials containing the anticoagulant to count RBCs, WBCs, and platelets and to determine haemoglobin content (Davie and Lewis, 1975). Biochemical indices for liver function test and renal function test was carried out using a semi-automated biochemical analyzer using standard kits. Organs like liver, kidney, stomach, heart and spleen were excised and washed with 0.9% saline and weighed. The relative organ weight (ROW) was calculated as follows:

ROW = Absolute organ weight (g) X 100/Body weight (g) on the day of sacrifice (Geetha and Vijayalakshmi, 2013).

2.6. Anti-peptic ulcer study

Swiss albino mice were randomly divided into four groups of six animals each. Ulcer was induced in all the animals by administration of ethanol following Oates and Hakkinen, (1988) with slight modification. All animals were fasted for 24 hours with free excess to water, and then 80% ethanol was administered at a dose of 1 ml/100 g of mice orally. Group I was considered as ulcerated group, where animals were sacrificed one hour after induction of ulcer, Group II, considered as negative control, received only distilled water; Group III considered as positive control, received ranitidine (30 mg/kg bw). The reference drug was procured from Cadila pharmaceuticals limited. Group IV, considered as treated group, received methanolic crude extract of *O. crinita* (200 mg/kg bw). At the end of the 6th, 10th and 14th day, animals were kept for 24h fasting and then were sacrificed under ether anesthesia.

2.6.1. Scoring of ulcers

Stomach of each animal was opened along the greater curvature, rinsed in water and examined to assess the formation of ulcer (Photographs were obtained using digital camera). Scoring of ulcer was recorded following Dashputre and Naikwade, (2011), as mentioned below:

Normal colored stomach = 0, Red coloration = 0.5, Spot ulcer = 1, Hemorrhagic streak = 1.5, Deep ulcer = 2, Perforation = 3. Mean ulcer score for each animal was expressed as ulcer index. The percentage of ulcer healing was determined as follows:

Ulcer index (U_I) was measured by using following formula: $U_I = U_N + U_S + U_P \times 10^{-1}$

Where, U_I = Ulcer Index; U_N = Average number of ulcers per animal; U_S = Average number of severity score; U_P = Percentage of animals with ulcers. Percentage inhibition of ulceration was calculated as given below:

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

2.6.2. Histopathological examination

After dissection, the tissues (stomach) of the experimental groups were collected and fixed in Bouin's fixative and were processed for microtomy followed by staining the sections in hematoxylin and eosin, mounting in DPX and viewed under compound microscope (Leica DM1000)

2.6.3. 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 following Dey *et al.*(1989) modified by Roy and Tandon, (1991). The gold coated specimens were viewed in the JEOL JSM 6360 scanning electron microscope at 25 kV.

2.6.4. Estimation of protein and carbohydrate

For estimation of protein and carbohydrate content the stomach tissues were collected after 6, 10 and 14 days of treatment, and 5% homogenate was prepared in PBS ($_P$H-7.4) using homogenizer. Total Protein and carbohydrate content of the different groups were estimated following Bradford, (1976) and Albalasmeh *et al*. (2013).

2.7. Statistical analysis

Data were analyzed using SPSS software and were expressed as mean ± Standard Error Mean, and analyzed using one-way analysis of variance (ANOVA). Tukey's test was applied for post hoc analysis. $p<0.05$ was considered as statistically significant.

3. Results

3.1. Preliminary phytochemical test

Preliminary phytochemical analysis of the *O. crinita* leaves extract revealed the presence of alkaloids, flavonoids, saponins, steroids, glycosides, terpenoids and tannin (Fig 1)

Alkaloids (Dragendroffs reagent)		Alkaloids (Mayer's reagent)		Anthroquinone		Terpenoids (salkowski test)		Steroids		Glycoside (keller- kilani test)		Glyceroids (libersmens test)		Tanins	
C	+	C	+	C	-	C	+	C	+	C	+	C	+	C	+

Flavonoids (Fecl3 test)		Flavonoids (Alkaline reagent test)		Saponin		Phlobatanins		Reducing sugars	
C	+	C	+	C	+	C	-	C	-

Figure 1. Photograph showing phytochemical analysis of methanolic leaves extract of *Osbeckia crinita*. C: control, "+": present, "-": absent

3.2. Effect of plant extracts on acute toxicity studies

The result of the acute toxicity study recorded zero mortality of mice receiving 2000 mg/kg bw methanolic extract of *O. crinita*. No lethal effects were noted throughout the short and long-term observation period. Therefore, the extract was revealed to be safe at a dose level 2000 mg/kg bw of mice and LD_{50} was considered to be more than 2000 mg/kg bw (Table 1).

Table 1. Clinical sign of acute toxicity test of methanolic leaves extract of *O. crinita* treated mice at dose 2000 mg/kg bw

Observation on the mice	30mins		4 hrs		24 hrs		72 hrs		1week		2 weeks	
	C	T	C	T	C	T	C	T	C	T	C	T
Skin and fur	N	N	N	N	N	N	N	N	N	N	N	N
eyes	N	N	N	N	N	N	N	N	N	N	N	N
Mucus membrane	N	N	N	N	N	N	N	N	N	N	N	N
Salivation	N	X	N	N	N	N	N	N	N	N	N	N
Lethargy	N	X	N	N	N	N	N	N	N	N	N	N
Sleep	N	X	N	N	N	N	N	N	N	N	N	N
convulsion	N	X	N	N	N	N	N	N	N	N	N	N
Tremors	N	X	N	N	N	N	N	N	N	N	N	N
Diarrhea	N	N	N	N	N	N	N	N	N	N	N	N
Mortality	N	N	N	N	N	N	N	N	N	N	N	N

Where C= Control, T= Treated, N= Normal and X=Change

3.3. Effect of plant extracts on sub-acute toxicity studies

All the treated mice of both sexes at the doses of 150, 300 and 600 mg/kg survived throughout the 28 days of treatment. No observable toxicity signs were noticed in the extract treated mice compared to the control.

3.3.1. Body weight and relative organ weight

The mean body weight changes of tested mice for 28 days of treatment are shown in Table 2. At the end of the 28 days study period, there was no significant change observed between control and treated mice at doses 150 and 300 mg/kg body weight; however, a significant reduction in mean body weight was observed at 600mg/kg body weight, although after 28 days of sub-acute treatment, significant changes were not recorded in the organ's weight compared to control group (Table 3).

Table 2. Effect of methanolic leaves extract of *Osbeckia crinita* on Body weight in sub-acute toxicity (28 days)

Body weight (g)	control	150mg/kg	300mg/kg	600mg/kg
Initial	29.26±0.37	30.88±0.46	30.59±0.68	29.31±0.67
Final	32.68±1.35	32.78±1.09	31.70±1.82	27.39±1.39
Mean weight change	3.42±1.15	1.89±1.38	1.11±1.15	-1.92±0.44*

Values are expressed as mean ±S.E.M. (n=6) * significantly different from control (p<0.05).

Table 3. Effect of methanolic leaves extract of *Osbeckia crinita* on Relative Organ Weight (ROW) in sub-acute toxicity (28 days)

ROW (g)	Control	150mg/kg	300mg/kg	600mg/kg
Liver	4.39 ± 0.11	4.44 ± 0.25	4.59±0.19	4.67±0.09
Kidney	1.22 ± 0.08	1.32± 0.05	1.32±0.06	1.35±0.08
Stomach	1.22 ± 0.16	1.27 ± 0.06	1.24±0.18	1.43±0.11
Heart	0.54 ± 0.04	0.62 ± 0.05	0.52±0.03	0.58±0.02
Spleen	0.37 ± 0.04	0.46 ± 0.02	0.35±0.04	0.41±0.07

Values are expressed as mean ±S.E.M. (n=6)

3.3.2. Hematological and biochemical studies

Table 4. represents the results of hematological parameters of control and *O. crinita* treated groups for 28 days. After 28 days, the results revealed that there is a significant increase in blood parameters such as Hb and RBC at dose150 mg/kg bw when compared with the control group. *O. crinita* treatment at 300 mg/kg caused no significant changes in the blood parameters. However, when the dose of plant extract was increased to 600 mg/kg bw a significant decline in Hb, RBC and WBC count was recorded.

Table 4. Effect of methanolic leaves extract of *Osbeckia crinita* on hematological parameters in sub acute toxicity (28 days)

Parameters	control	150mg/kg	300mg/kg	600mg/kg
Hb (g%)	14.48±0.25	15.78±0.28*	14.13±0.46	12.24±0.47*
RBC (10^5/mm^3)	8.57±0.28	9.78±0.29*	8.06±0.16	6.93±0.18*
WBC (10^3/mm^3)	5.80±0.12	6.36±0.27	6.54±0.29	4.1±0.23*
Plt (10^5/mm^3)	9.15±0.51	8.47±0.54	8.35±0.39	7.65±0.51

Values are expressed as mean ±S.E.M. (n=6) * significantly different from control (p<0.05). Hb: Hemoglobin, RBC: Red Blood Cells, WBC: White Blood Cells, Plt: Platelets.

Table 5. represents the results on biochemical parameters of control and *O. crinita* treated groups for 28 days. Interestingly, after 28 days of treatment, results showed the changes in ALT and ALP level in all *O. crinita* treatment group but were not significant from that of the control group, while there was a significant increase in AST at dose 600 mg/kg bw compared to control group. Treatment with *O. crinita* at a dose of 600 mg/kg bw for 28 days, showed a significant increase in bilirubin(T) level. On the other hand, no significant changes were observed in the levels of bilirubin(D) in mice treated with 150, 300 and 600 mg *O. crinita*/ kg bw. The creatinine level increased significantly in experimental groups treated with *O. crinita* at concentration of 150 and 600 mg/kg bw. Treatment with *O. crinita* showed a significant elevation in urea level in both 300 and 600 mg/kg bw treated mice groups.

Table5. Effect of methanolic leaves extract of *Osbeckia crinita* on biochemical parameters in sub acute toxicity (28 days)

Parameters	control	150mg/kg	300mg/kg	600mg/kg
ALT (U/L)	17.53±0.38	18.58±1.85	17.97±1.08	15.38±0.58
AST (U/L)	32.69±0.84	32.49±1.04	30.70±0.85	44.46±0.31*
ALP (U/L)	64.73±0.32	59.58±3.53	62.20±1.54	66.73±0.52
Bilirubin(T) (mg/dl)	0.68±0.01	0.68±0.03	0.69±0.02	0.82±0.02*
Bilirubin(D) (mg/dl)	0.34±0.02	0.34±0.04	0.35±0.03	0.39±0.02
Creatinine (mg/dl)	0.43±0.01	0.52±0.02*	0.47±0.01	0.53±0.01*
Urea (mg/dl)	70.49±0.53	70.21±0.89	79.78±0.86*	82.07±1.18*

Values are expressed as mean ±S.E.M. (n=6) * significantly different from control (p<0.05). ALT: Alanine transaminase, AST: Aspartate transaminase, ALP: Alkaline phosphatase, Bilirubin(T): bilirubin total, Bilirubin(D): bilirubin direct

3.4. Anti-ulcer activity of the plant extract

3.4.1. Ulcer scoring

Table 6. represents the ulcer index and healing activity of control, negative control, ranitidine and *O. crinita* treated groups. Treatment with *O. crinita* extract at 200 mg/kg and with ranitidine 30 mg/kg bw significantly (p<0.05) reduced the ulcer index when compared with negative control group. Consequently, it also stimulated the healing process of peptic ulcers provoked by 80% ethanol. However, ranitidine (30 mg/kg bw) treated mice showed highest healing property.

Table 6. Effect of treatment with *Osbeckia crinita* methanolic leave extract in the ulcer index and healing activity in ethanol induced peptic ulcer in mice

Groups	Ulcer index	Healing %				
Ulcerated	1.53±0.03	-				
	6 days		10 days		14 days	
	Ulcer index	Healing %	Ulcer index	Healing %	Ulcer index	Healing %
NC	0.95±0.02^{a*}	37.90	0.75±0.03^{a*}	51	0.57±0.02^{a*}	62.15
PC (30 mg/kg)	0.52±0.02^{a*b*}	66.01	0.20±0.06^{a*b*}	86.47	0.07±0.03^{a*b*}	95.09
O. crinita (200 mg/kg)	0.58±0.04^{a*b*}	62.09	0.29±0.06^{a*b*}	81.04	0.15±0.04^{a*b*}	90.06

Values are expressed as mean ± S.E.M. (n=6), a*p<0.05, when compared with ulcerated group b*p<0.05, when compared with negative control group. Statistically analyzed by one way analysis of variance (ANOVA) followed by tukey's test. NC: Negative control, PC: positive control, *O. crinita*: *Osbeckia crinita*

Macroscopic view of gastric mucosa of control and experimental groups of mice were presented in Fig 2. Gastric mucosa of healthy mice showed clear and proper arrangement of cells. Whereas ethanol induced gastric ulcerated mice mucosa showed several reddish lesions with hemorrhagic streaks. Total stomach signs of mice administered with *O. crinita* and ranitidine showed remarkable decrease in reddish lesion and hemorrhagic streaks as compared to negative control and ulcerated mice.

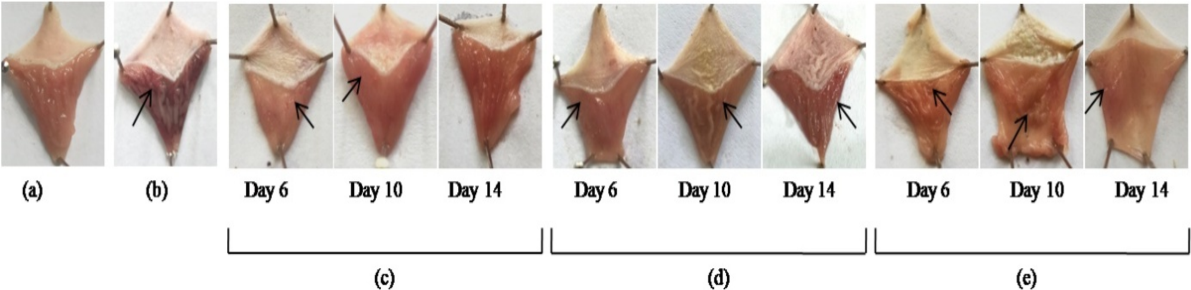

Figure 2. Photographs showing ethanol induced ulceration in the stomach of mice. Gastric mucosa of (a) Healthy normal animals showing absence of ulcer; (b) ethanol administered mice showing severe ulceration as a reddish lesions (c) Ranitidine (30 mg/kg bw) treated animals showing less intense ulcer as compared to ulcerated mice in 6,10 and 14 days respectively; (d) *O. crinita* (200 mg/kg bw) treated mice showing moderate ulcers in 6 days and minor mucosa lesion in 10 and 14 days as compared to ulcerated animals; (e) animals treated with normal saline (negative control) showing major mucosa lesion in day 6, however lesion reduces in day 10 and 14 respectively.

3.4.2. Evaluation of total protein and carbohydrate

After 6, 10, and 14 days of treatment, the amount of protein and carbohydrate in the stomach tissues were measured quantitatively. The analysis of total protein revealed that in *O. crinita* and ranitidine treated groups, total protein content was found to be significantly higher (p<0.05) than in the ulcerated groups. Administration of *O. crinita* (200 mg/kg bw) and ranitidine (30 mg/kg bw) for 6, 10 and 14 days to ethanol induced ulcerated mice showed significant (p<0.05) elevation in protein and carbohydrates levels when compared with ulcerated groups. No changes were observed in protein levels *in O. crinita* and ranitidine treated mice when compared with healthy mice. Fourteen days of treatment with *O. crinita* depicts the elevation of protein concentration to the normal level as that of healthy mice (Fig 3 and Fig 4).

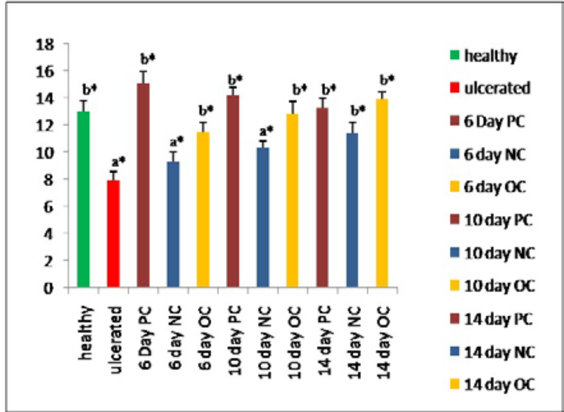

Figure 3. Effect of *O. crinita* on protein concentration in the stomach tissue after ulcer induction. Values are expressed as mean SEM (n=6), a*p<0.05, when compared with control group, b*p<0.05 when compared with healthy group. Statistically analyzed by one way analysis of variance (ANOVA) followed by tukey's test. PC: Positive control, NC: Negative control, OC: *Osbeckia crinita*

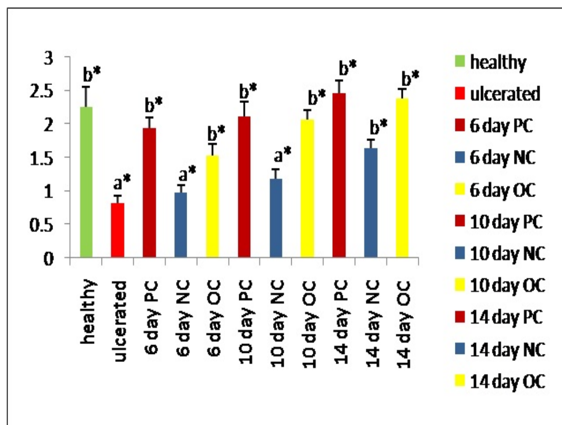

Figure 4. Effect of *O. crinita* on carbohydrate concentration in the stomach tissue after ulcer induction. Values are expressed as mean SEM (n=6), a*p<0.05, when compared with healthy group, b*p<0.05, when compared with ulcerated group. Statistically analyzed by one way analysis of variance (ANOVA) followed by tukey's test. PC: Positive control, NC: Negative control, OC: *Osbeckia crinita*

3.4.3. Histological and ultrastucture Observations

Histology of a healthy stomach showed intactgastric epithelium with normal architecture of stomach. Contrary in the ulcerated group, histological analysis revealed fairly substantial damage to the stomach mucosa, with necrotic lesions which penetrate deeply into the mucosa and extensive edema along with leukocyte infiltration. Compared to negative control group, treatment with ranitidine (30 mg/kg) and *O. crinita* (200 mg/kg), a significant reduction of mucosal damage in a time dependent manner was recorded. It also reduced inflammatory cell infiltration in sub mucosal region as well. However, ranitidine (30 mg/kg) restores the mucosal injury faster than *O. crinita*. Furthermore, *O. crinita* (200 mg/kg) and ranitidine (30 mg/kg) allowed regeneration in time dependent manner (Fig 5).

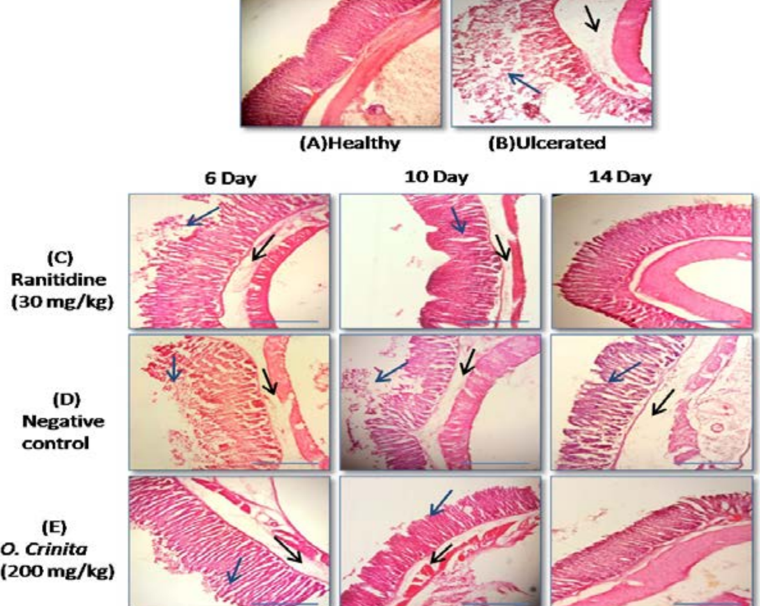

Figure 5. Effect of methanolic leave extract of *O. crinita* on histology of gastric epithelium in ethanol induced peptic ulcer damage in swiss albino mice. Healthy mice (A) showing normal stomach's architecture or morphology of the stomach. Stomach of ulcerated (B) mice showing several mucosal injury (blue arrow) edema with leucocytes and inflammation of sub mucosal layer (black arrow). (C) represents ranitidine (30mg/kg) treated mice in 6, 10 and 14 day respectively. (D) represents negative mice in 6, 10 and 14 days respectively. (E) represents *O. crinita* treated mice in 6, 10 and 14 days. Scale bar: 100µm.

Scanning electron microscopy of healthy group depicted intact epithelial cells with perfect arrangement. Ulcerated groups showed damaged epithelial cells along with erosion and damaged gastric pits. Treatment with *O. crinita* (200 mg/kg) revealed a normal epithelium with slight erosion. However, ranitidine (30 mg/kg) treated showed intact epithelium like normal group (Fig 6).

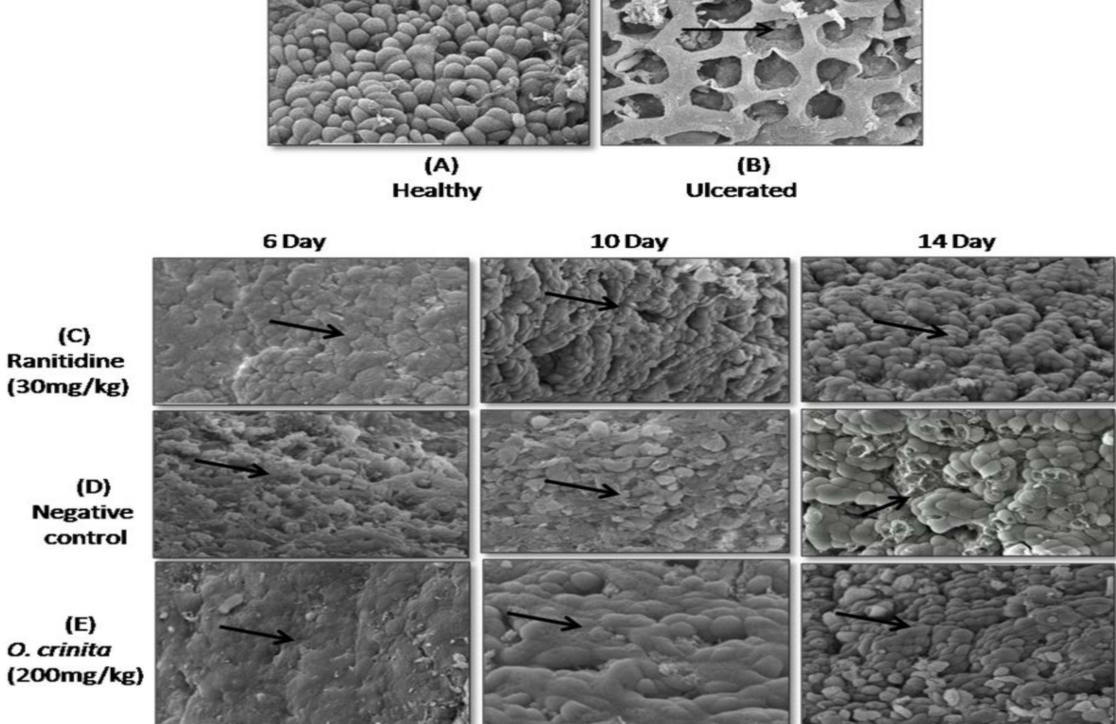

Figure 6. Scanning electron micrographs of (A) Healthy normal mice showing uniform epithelium with no damage B) Ulcerated mice showing damaged epithelium with several erosions. C) Ranitidine (30mg/kg) treated mice in 6, 10 and 14 days, respectively, showing time dependent regeneration to normal architecture of epithelium. D) Negative control (treated with saline only) mice in 6, 10 and 14 days. (E) *O. crinita* (200 mg/kg) treated mice in 6, 10 and 14 days showing recovered normal structural features of the epithelium. (Magnification: 20kv x 500, 50 µm)

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4. Discussion

Acute toxicity study revealed that the crude extract of the plant *O. crinita* is safe in mice at a limit dose of 2000 mg/kg bw. The doses employed in sub-acute toxicity study were selected on the basis of LD_{50} calculated in the acute toxicity study.

Sub-acute toxicity study at low dose (150 mg/kg bw) revealed a significant increase in Hb and RBC. This suggests that the plant extract may include phytochemicals that induce erythropoietin synthesis or secretion in animal's stem cells. This observation agrees with the report of Oyedemi *et al.* (2011), who reported that *Afzelia africana* extract increases RBC levels when treated the diabetic wistar rats. However, Hb, RBC and WBC levels were significantly declined when the concentration of plant extract was increased to 600 mg/kg bw. This observation agrees with the several scientists who reported a reduction in the blood parameters (RBCs, WBCs, and hemoglobin) in animals exposed to different plant extracts (Sule *et al*., 2012; Ladokun *et al*., 2015). Platelets play a major role in blood clotting and our present study does not show any significant alteration in platelets level, which suggests that the plant extract may have positive response in blood vessel and homeostasis.

The levels of three enzymes (ALT, AST, and ALP) in the blood serum are frequently employed as clinical biochemical indicators for liver disease (Yun *et al*., 2018; Bencheikh *et al*., 2019). Among these enzymes, AST level showed significant increase at higher dose (600 mg/kg bw) of *O. crinita*, indicating occurrence of cellular damage in liver. Similar kind of results was also recorded by Obakiro *et al.* (2021) where AST level was increased on administration of higher doses of *Entada abyssinica* to Wistar albino rats, and they suggested the hepatoprotective effect of the extract at lower doses. Elevation in AST activity is related to the number of affected hepatocytes and does not reflect the severity or reversibility of the lesion on a pathological basis (Wang *et al*., 2019). In the present study, no significant changes were recorded in both ALT and ALP levels, which indicates that the plant extract has negligible effects on liver function at lower doses(150 mg/kg bw and 300 mg/kg bw). Bilirubin (total and direct) act as an essential indicator to assess liver excretory function and hemolytic anaemia (Saidu *et al*., 2007). The present study showed that *O. crinita* increased total bilirubin levels of the mice administered with 600mg/kg body weight; however, direct bilirubin levels remained in normal range. The observation suggests that the extract may be responsible for haemolytic anaemia. This kind of result was also reported by Njinga *et al.* (2020), where extract of *Hibiscus sabdariffa* administration increased the total bilirubin levels in wistar rats. The end products of protein metabolism are creatinine and urea, and a rise in their blood levels suggests the extent of renal damage (Gowda *et al*., 2010). As an outcome, a rise in these indicators implies that extracts administrated at 600 mg/kg bw have an adverse effect on the renal tissue's integrity and function.

The phytochemical analysis of methanolic leaf extract of *O. crinita* demonstrated the presence of alkaloids, steroids, glycosides, flavonoids, saponins, tannins, and terpenoids. In a previous Study, Jain *et al.* (2016), reported that secondary plant metabolites specifically, alkaloids, flavonoids and terpenoids have a major impact on peptic ulcer treatment. However, flavonoids are claimed to be the most important secondary metabolites that are employed as an antiulcer agent and protect the gastric mucosa through multiple mechanisms. These mechanisms includes: free radical scavenging, increase in mucosal prostaglandin content, improvement in gastric tissue microcirculation and cytoprotection (Mota *et al*., 2009; Serafim *et al*., 2020). Flavonoids maintains gastric cytoprotective effects through modulating prostaglandins (PG's) and nitric oxide synthase (NOS) pathways, which serves to maintain stomach mucosal integrity, mediates gastric blood flow, inhibits gastric acid secretion and accelerates mucosal healing (Zhang *et al*., 2020).

Ethanol intake predominantly damages the glandular portion of the stomach since it penetrates rapidly in to the gastric mucosa and leads to gastric lesion such as extensive submucosal edema, hemorrhage, desquamation of epithelial cells and infiltration of inflammatory cells (Mousa *et al*., 2019; Ciciliato *et al*., 2022; Sadek, 2022). The repeated oral dose study for ethanol-induced ulcer model showed a significant reduction in ulcer index ($p< 0.05$) in 6, 10 and 14 days treatment with *O. crinita* at dose 200mg/kg. Besides this, the percentage of ulcer healing significantly increased in a time dependent manner from 6 days to 14 days treatment. However, ranitidine (30mg/kg bw) showed a strong healing effect in ethanol induced peptic ulcer. The obtained result indicates that the ulcer healing efficacy of the plant extract is somehow similar with the reference drug (Abebaw *et al*., 2017). Further, the extract's antiulcer activity is mostly owing to its anti-secretory properties, in addition to cytoprotective effects or plant mucosal tissue regeneration due to the presence of active photochemical (Mekonnen *et al.,* 2020). Protein which supplies nutritional factor, increases the resistance of the stomach wall against the combined attack of pepsin and hydrochloric acid in ulcerated condition (Qin *et al*., 2018). In the present study, repeated treatment of ulcerated mice with *O. crinita* (200 mg/kg bw) showed significant time dependent increase in the protein level in 6, 10 and 14 days. Previously, a significant increase in total protein content upon treatment with Schiff base derived dibromo compound was reported in rats with acute superficial hemorrhagic mucosal lesions (Saremi *et al*., 2019). One of the essential criteria to determine the status of the mucosal resistance/barrier is the state of mucus secretion in the stomach. The increase in carbohydrate content in *O. crinita* treated and ranitidine treated groups over that of the negative ulcerated group appears to be due to stimulation of mucus secretion by the phytoproducts and drugs. Increased mucus secretion by gastric mucosa can inhibit gastric ulceration by preventing back-diffusion of H^+ ions and by buffering of the acid gastric juice (Ribeiro *et al*., 2016). Hereby, it is suggested that the antiulcer activity of *O. crinita* observed in the present study could be due to restoration of the mucosal barrier system as observed in the histopathological study. Similarly, Gopinathan and Nija, (2014), also found that the elevating total carbohydrate content significantly increased the antiulcer efficacy by preserving the mucosal barrier system.

Gastric mucosa plays an important role in defensive mechanism. It maintains the structural integrity of stomach and protects the gastric wall from the aggressive and

noxious agents by producing mucous-bicarbonate barrier (Sidahmed *et al*., 2019). Saleh *et al*. (2016) reported that Gastric motility changes are crucial in the development and prevention of experimental gastric lesions. Results of microscopic analysis revealed that the plant extract at the dose of 200 mg/kg bw showed significant reduction in necrotic lesions than the negative control group and healing ability is comparable to that of the positive control group. This suggests that the extract shows healing action by reducing the stomach motility. These findings are found to be consistent with earlier work carried out by Halabi *et al*. (2014), where new schiff based derived complex showed flattering of mucosal folds by decreasing the gastric motility in ethanol induced gastric ulcer. Histological study further confirmed that treatment with *O. crinita* (200 mg/kg) restore the damage gastric mucosa with a clear reduction of sub mucosal edema, leukocyte infiltration, and with the epithelium lining being protected almost to the appearance of the normal control group. This suggests that extract might stimulate the mucus secretion which enables to heal the damage mucus layer. Similar observation has been reported by Takayama *et al*. (2011) and Halabi *et al*. (2014). In the current study, scanning electron microscopy of gastric mucosa of ethanol induced ulcerated group exhibited damaged epithelial cells, erosions with widened gastric pits. On the contrary, Scanning electron micrographs of *O. crinita* (200 mg/kg bw) 14 day treated groups showed an intact epithelium with negligible erosion; thus, the present finding has also been supported by histological and surface topographical studies to establish the ulcer healing potential of the plant *O. crinita*.

5. Conclusions

The findings from this study showed that methanolic crude extract of *O. crinita* exerts antiulcer activity via gastric mucin secretion, which could be possibly attributed to phytochemicals present in the plant extract. Further research is required to isolate the active elements involved for the anti-ulcer efficacy and to determine the specific mechanism of action in gastric ulcer healing.

Acknowledgement

The authors would like to thank the University Grant of Commission (UGC, New Delhi) for providing financial support (NEHU NON- NET Fellowship to SR). Department of Zoology and Sophisticated Analytical Instruments Facility (SAIF), North-Eastern Hill University, Shillong is also acknowledged for providing infrastructural facilities.

Author contribution

BR designed the work, corrected and finalized the manuscript. SR carried out the experiment, wrote the manuscript and prepared the final draft. All authors read and approved the final manuscript.

Funding:

This study did not receive any external funding.

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Ethical statement

The experimental study was carried out by in compliance with the ethical guidelines issued by the committee for the purpose of control and supervision on 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 Uniersity, Shillong, Meghalaya, India on 28th