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Research Article

Gastro-Protective Effect of Root Extract of *Barleria buxifolia* on Aspirin-Induced Gastric Ulcer in Rats

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ABSTRACT

The key assessment of the study was evaluating the gastro-protective properties of *Barleria buxifolia* root extract (REBB) in ulcerative rats. The roots of *B. buxifolia* have properties like antioxidant, anti-inflammatory, and other health benefits. Induction of gastric ulcers was done with aspirin (150 mg/kg, b.w., p.o) for 3 days and was accompanied by treatment with REBB (200 & 400 mg/kg orally) for 15 days. Ranitidine (20 mg/kg, orally) was received as the standard treatment for 14 days. Ulcer index, percent inhibition of ulceration, lipid peroxidation (LPO), tumor necrosis factor-alpha (TNF-α) levels, and histopathological examination of the gastric mucosa were measured. Aspirin-induced stomach ulcers were seen in 100% of the groups, whereas other animal groups, aside from the control group, possessed relatively comparable inductions. Ulcer number, ulcer index (p <0.01), and LPO (p <0.05) showed a significant reduction in the 400 mg/kg and ranitidine (20 mg/kg) when compared to the aspirin-induced control group. Whereas the ulcer score (p <0.001) and TNF-α (p <0.05) showed significant reduction in the ranitidine-treated group but showed objective improvement but statistically non-significant results in 200 and 400 mg/kg REBB. Hence, *B. buxifolia* root extract effectively mitigates aspirin-induced gastric ulceration in rats, underscoring its potential as a gastro-protective agent.

INTRODUCTION

A stomach ulcer is linked to an excess of pepsin and acid production. The organs most impacted by gastrointestinal secretions are the stomach, lower oesophagus, and proximal or distal duodenum and jejunum.[1] Stress, lifestyle choices, alcohol consumption, tobacco, and excessive non-steroidal anti-inflammatory drugs (NSAIDs) use are the main causes of peptic ulcers and related issues.[2,3] These are important factors that contribute to persistent inflammation in the stomach, which may change the mucosal lining and blood flow in the target area,[4] and degenerative gastric secretions,[5,6] which contribute to the disease. Radicals are also responsible for mucosal injury since they can damage DNA, alter cell metabolism completely, and break down elements of the epithelial basement membrane.[7] Increased expression of NOS, the production of interleukin-1beta (IL-1β) and tumor necrosis factor-alpha (TNF-α), and the death of epithelial cells, which trigger NF-κβ nuclear factor stimulation, are associated with mucosal tissue damage, inflammation, and ulcer symptoms. Induction of stomach mucosal damage and inflammation with sustained inflammatory reactions delays the healing process at the ulcer area.[8]

Plants are widely recognized as a rich source of medicinal compounds dating back to prehistoric times. Natural biodiversity is essential for managing and combating illness. India is thought to be home to 8% of all species. *Barleria buxifolia* One of the major species in Barleria is linn, which is a member of the Acanthaceae family. This species is native to Sri Lanka and India.[9] The leaves, flowers, roots, stems, and seed extracts of this genus of plants contain an abundance of bioactive compounds that have demonstrated significant therapeutic potential to address various diseases and circumstances. Both leaf roots were

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traditionally used to treat inflammation, bronchitis, and cough. A previous study reported the presence of saponins, flavonoids, phenylethanoid glycosides, quinones, iridoids, and immunostimulating proteins as potent phytochemicals.[10] Peptic ulcer disease does not have a treatment; it needs to be managed throughout life and demands continued treatment through the rest of the year, and the treatment available for the disease may cause adverse consequences for long-term use. *B. buxifolia* is a potent antioxidant and anti-inflammatory agent, making it a pharmacological advantage in the present ulcer treatment landscape. These qualities have the potential to be helpful adjuvant treatments for stomach ulcers.

Traditional medicine therapy takes a personalized and holistic approach, addressing the root cause of health issues rather than just managing symptoms. Herbal medicines restore balance and immunity, promoting overall health and wellbeing. Using medicinal plants such as *B. buxifolia* may be more economical than costly pharmaceutical medications. This is especially helpful in low-income areas where access to traditional medical care may be restricted. Determining the chemical compounds that give *B. buxifolia* its ulcer-healing properties may help scientists create novel pharmaceutical medications that utilize these organic ingredients, providing new therapy options for ulcers and possibly other gastrointestinal issues.

Materials and Methods

Plant Material and Authentication

B. buxifolia roots were procured from Indian Jadibooti and the specimen was authenticated by Dr. Noorunnisa Begum Foundation for Revitalisation of Local Health Traditions, Bangalore- 560064, FRLHL Acc. No 6394.

Preparation of Plant Extraction

The roots of *B. buxifolia* were thoroughly cleaned with fresh water, and then the plant roots were air-dried and ground into a rough powder. Soxhlation was used to extract the phytochemical components found in the roots using methanol. About 500 mL of methanol was used for each 100 g of *B. buxifolia* root powder. Cycles were repeated a minimum of three times to get the residues. A rotary evaporator is then used to evaporate the solvent and air-dry it for 24 hours, and the extract is collected and stored for further investigation.[11,12]

Experimental Animals

The study used wistar female rats (weighing between 150 and 200 grams) and the animals were kept acclimatized under standardized conditions. Animal care was taken in accordance with the CPCSEA. The IAEC registration number is KCP-IAEC/12/22-23/02/01/07/23.

Aspirin-induced Gastric Ulcer Method[13,14]

Five groups (n = 6) of animals were grouped.

Group I: The normal control group was given only regular saline (10 mL/kg, orally) for 18 days.

Group II: The diseased group received aspirin 150 mg/kg orally diluted with 3 mL of normal saline solution for three days, throughout which the animals were starved to induce ulceration

Group III: Standard drug group, ranitidine 20 mg/kg orally diluted with 10 mL/kg of normal saline solution for 15 days, followed by 150 mg/kg/aspirin diluted with 3 mL of normal saline for three days.

Group IV: Test drug group rats were received 200 mg/kg orally of extracted root of *B. buxifolia* for 15 days and then treated with 150 mg/kg/P.O of aspirin diluted with 3 mL of normal saline solution for 3 days.

Group V: Test drug group: Rats received 400 mg/kg/P.O of extract root of *B. buxifolia* for 15 days. Afterward, 150 mg/kg/P.O of aspirin was administered diluted with 3 mL of normal saline solution for 3 days.

Test drug dose selection

Obtained from literature survey as mentioned 200 and 400 mg/kg, b.w.[10]

After 24 hours of aspirin administration, all the animals of the groups were anesthetized using phenobarbital, and blood was collected through cardiac puncture and sacrificed. The stomach was isolated and the samples were centrifuged for five minutes at 2500 rpm and examined. After this, the stomach was taken out right away for ulcer score assessment and histology. The tissue samples were fixed for 24 hours in 10% formalin. Using a rotary microtome, tissues about 5 µm thick were sliced and placed into paraffin blocks. These parts underwent H&E treatment in compliance with standard procedures. Through microscopic analysis, the anti-ulcerogenic effects of *B. buxifolia* were investigated.

Ulcer Score, %Inhibition and Ulcer Index

After the rats were sacrificed, stomachs were opened along their wider curvature and carefully cleaned under running tap water. After that, it was put on the slide and examined for ulcers using a 10X magnification. Determination of the ulcer score was done sing the formula:

Normal colored stomach (0), red coloration (0.5), spot ulcers (1), hemorrhagic streaks (1.5), 2 = ulcers ≥ 3 but ≤ 5, and 3 = ulcers > 5

The following is the equation for the ulcer index:

UI = UN + US + UP × 10-1

Where,

UI = Ulcer index,

UN = the animal's average number of ulcers

US = Animals with an average severity score,

UP = percentage of ulcer-ridden animals.

The determination of % ulcer inhibition is as follows:

$$\% \text{ Inhibition of ulcer} = \frac{\text{Ulcer Index control} - \text{Ulcer Index test}}{\text{Ulcer index control}} \times 100$$

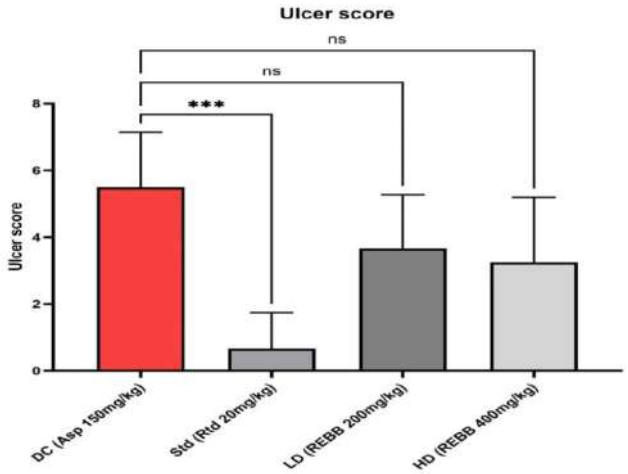
Antioxidant enzyme study lipid peroxidation (LPO) and pro-inflammatory cytokine TNF-α were assessed. The test was based on sandwich ELISA, BD OptEIA™, and bioscience. The assay procedure was briefly mentioned in reference (DOI: 10.25004/IJPSDR.2024.160108).

Statistical Analysis

The data were assessed as mean ± SEM (n = 6 rats). Graph Pad Prism version 9, one-way ANOVA, was used for data analysis, followed by Tukey's test to determine the significance of the group differences. The value of p <0.05 was considered statistically significant.

Result and Discussion

The majority of the time, the cause for ulcer is unknown. It is widely acknowledged that the endogenous immune mechanism's protection of integrity of the mucosa and aggressive forces' imbalance causes Ulcer. In order to restore equilibrium, several medicinal substances, such as plant extracts, are employed to suppress the stomach acid's release or strengthen the mucosal defenses by increasing the production of mucus, Prostaglandin synthesis disruption or surface epithelial cell stabilization.[15] As a histamine receptor antagonist, ranitidine inhibits the H2 receptor, lowering the amount of gastrin and acetylcholine secreted by parietal cells.[16] Comparing the Standard group to the aspirin-treated group, the Standard group's ulcer score decreased significantly. Whereas the 200 and 400 mg/kg when compared with the disease group was found to be non-significant (Fig. 1). The ulcer number was drastically reduced in 400 mg/kg REBB treated group in contrast with that of the disease control and the ranitidine treated group decreased significantly in ulcer number when compared disease control group (Fig. 2). The ulcer index is a visible indicator of gastric erosion or injury used to evaluate the degree of ulceration.[17] similarly, in this



##Asp, Aspirin, Rtd, Ranitidine, REBB, Extract of roots of *B. buxifolia*

Fig. 1: Ulcer score, values are expressed as Mean ± SEM (n = 6), ***p < 0.001 compared with disease control, aspirin, statistically non-significant ns p>0.05 with the REBB while compared with DC (Asp.)

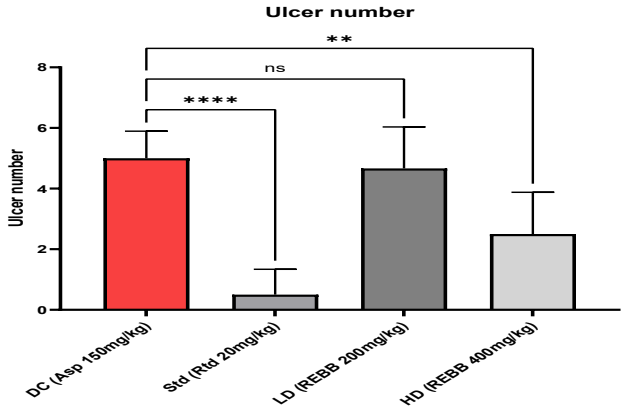


Fig. 2: Ulcer number, values are expressed as Mean ± SEM (n = 6), **p < 0.01, ***p < 0.001 compared with disease control, aspirin, Statistically non-significant ns p > 0.05 with the REBB 200 mg/kg while compared with DC (Asp.)

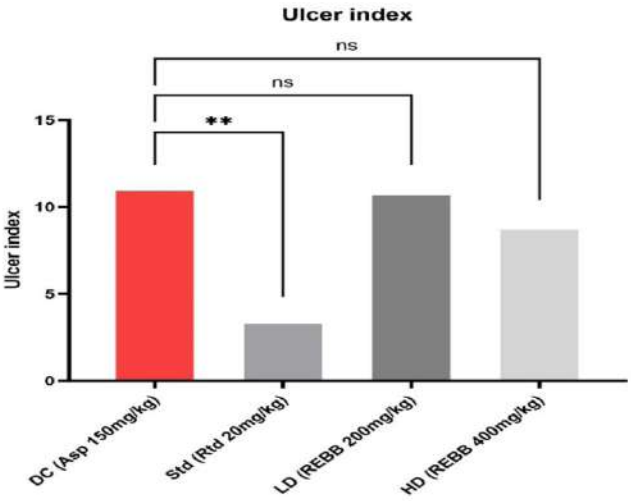


Fig. 3: Ulcer index, values are expressed as Mean ± SEM (n = 6), **p < 0.01 compared with disease control, aspirin, statistically non-significant ns p > 0.05 with the REBB while compared with DC (Asp.)

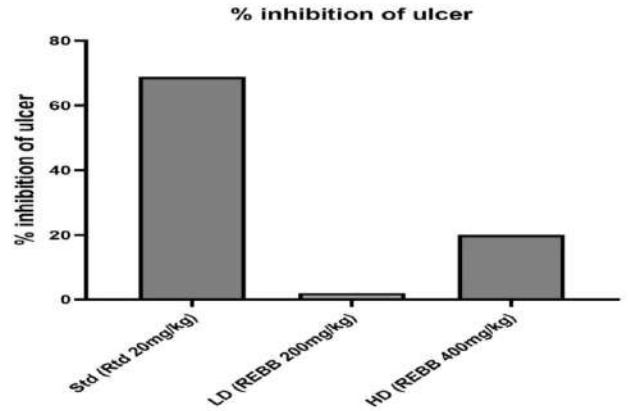


Fig. 4: % Inhibition of ulcer, values are expressed as Mean ± SEM (n = 6), REBB 400 mg/kg indicated that herb was efficacious but they had lesser efficacy then the ranitidine.

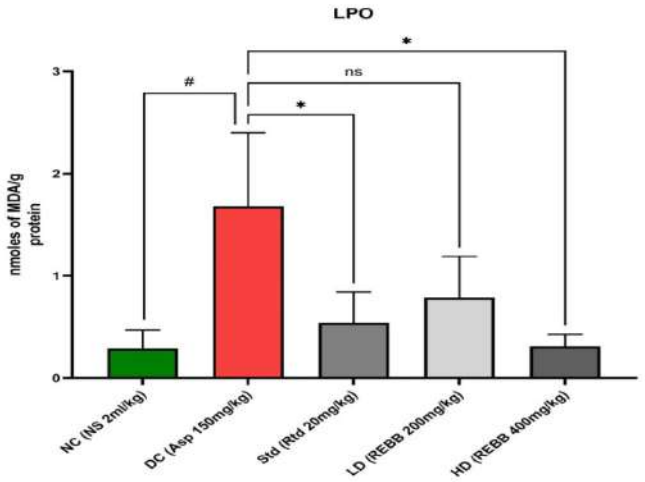


Fig. 5: Antioxidant enzyme, LPO: Values are expressed as Mean ± SEM (n = 6), #$p < 0.05$ compared with Normal saline, *$p < 0.05$ compared with Disease control, Aspirin, Statistically non-significant ns $p > 0.05$ with the REBB 200 mg/kg while compared with DC (Asp.).

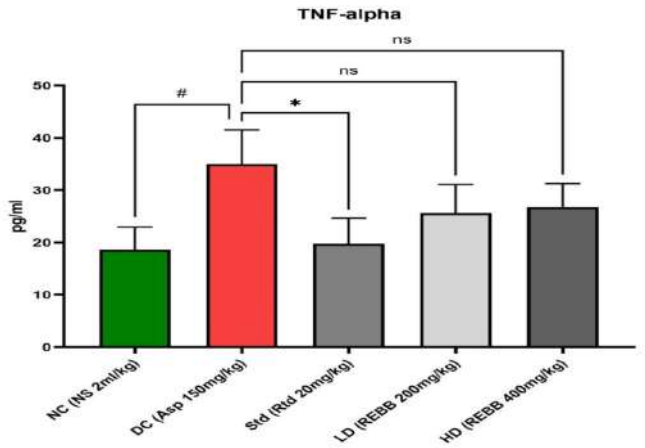


Fig. 6: Pro-inflammatory cytokine, TNF-alpha: Values are expressed as Mean ± SEM (n = 6), #$p < 0.05$ compared with normal saline, *$p < 0.05$ compared with disease control, aspirin

current study, it was found to have reduced significantly in ulcer index in the ranitidine group as well as 400 mg/kg REBB treated group in comparison with the disease group (Fig. 3). Fig. 4, shows that the standard group as well as 400 mg/kg REBB treated group demonstrated a substantial rise in the percentage of ulcer inhibition when compared to Aspirin treated group. ROS and RNS also take part in gastric ulcer formation.[18] This harmful effect is prevented by antioxidants comprising both enzymatic and non-enzymatic antioxidants.[19] Fig. 5, shows that the antioxidant level (LPO) in the aspirin-treated group increased drastically whereas the 400 mg/kg showed a significant reduction and the 200 mg/kg showed a non-significant reduction when compared to the disease control. A significant increase in plasma TNF-alpha was found in the disease group compared to the negative or normal group. A significant reduction noticed in the Ranitidine

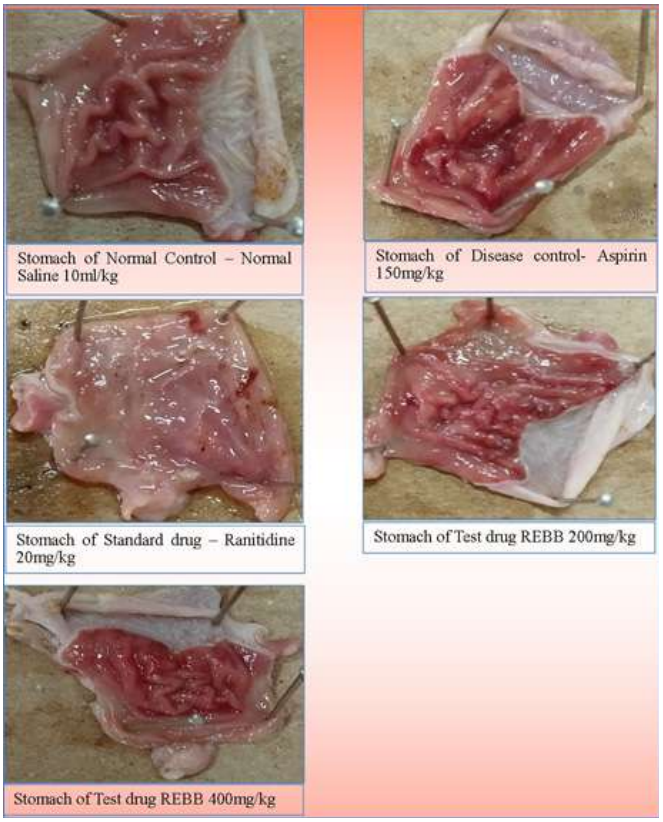


Fig. 7: Gross appearances of stomach

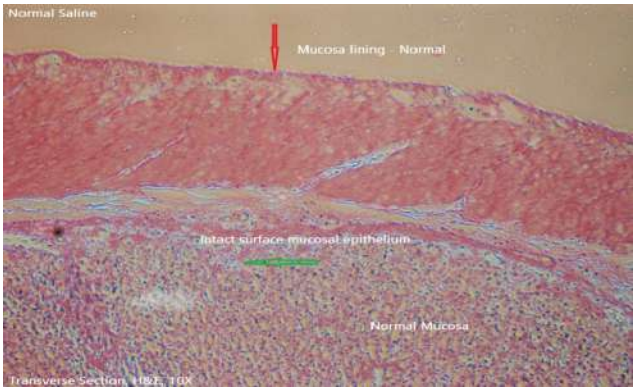


Fig. 8: Stomach – normal control

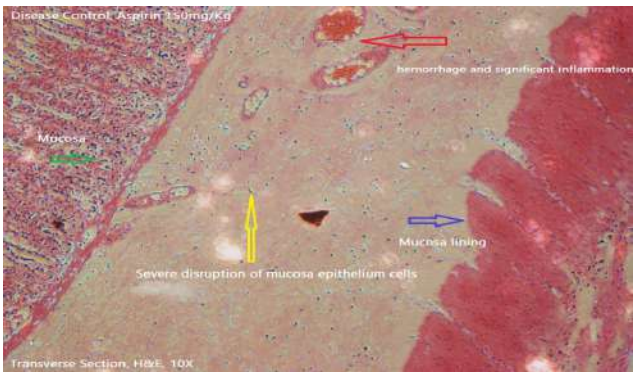


Fig. 9: Stomach – disease control – aspirin 150 mg/kg

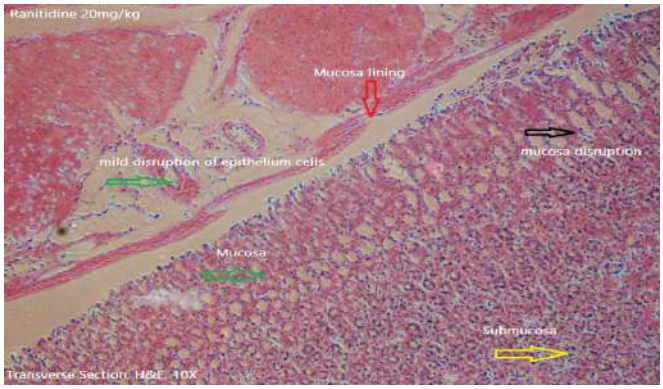


Fig. 10: Stomach – standard drug – ranitidine 20 mg/kg

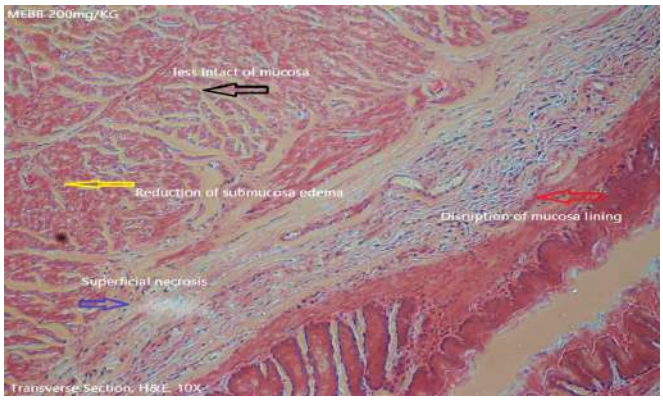


Fig. 11: Stomach – REBB test drug – 200 mg/kg

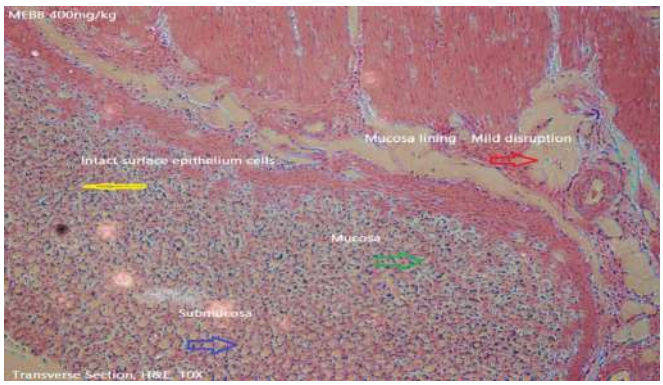


Fig. 12: Stomach – REBB test drug – 400 mg/kg

group. But TNF-α levels in both 400 and 200 mg/kg showed non-significant reduction when compared with the disease group (Fig. 6). Histopathological study and pictogram (Fig. 7) show the methanolic root extracts of *B. buxifolia* at the dose 400 mg/kg body weight moderately enhanced the mucosa structural integrity, thus reducing the formation of lesions of the mucosa, intact in surface epithelium cells, and less infiltration of inflammatory cells.

Histopathological Assessment

In the histopathological evaluation, the normal control group (Fig. 8) shows normal architecture, normal mucosa lining, and intact surface mucosal epithelium. The disease control (Fig. 9) due to the treatment with aspirin in, shows severe disruption of the epithelium (yellow arrow) and there is the occurrence of hemorrhage with significant inflammation (red arrow). The mucosal lining was completely disrupted (blue arrow). In ranitidine-treated animals (Fig. 10), there was mild disruption of epithelium cells (green arrow), and the presence of normal mucosal lining was also observed. Whereas in the REBB 200 mg/kg treated group (Fig. 11), it is found that there is less intact mucosa (black arrow), and the presence of reduction in the sub-mucosa edema (yellow arrow), and shows the disruption of the mucosa lining (red arrow). Whereas in the REBB 400 mg/kg treated group (Fig 12) shows a mild disruption of the mucosal lining (red arrow), intact surface epithelium cells (yellow arrow), and also it determines the normal mucosal lining.

Conclusion

The findings of the study assessing the anti-ulcer properties of *B. buxifolia* roots extracted with methanol in rat models of aspirin-induced ulcers have demonstrated that REBB 400 mg/kg showed significant anti-ulcer properties, inhibiting % of ulcer lesions, ulcer index, and score. Reduction in LPO in plasma indicates the inhibition of oxidative stress, TNF-alpha remains unchanged statistically, and histology assessment shows reduced mucosal lining disruption, intact surface epithelium cells and sub-mucosa lining, and reduction of infiltration of inflammatory cells.

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