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EVALUATION OF ANTIULCER ACTIVITY OF SAPONIN-RICH FRACTION OF *MORUS ALBA* LEAVES

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Keywords:

Anti-ulcer, *Morus alba*, Macerated, Methanol, Saponin

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ABSTRACT: A peptic ulcer is an excoriated area of the stomach or intestinal mucosa caused principally by the digestive action of gastric juice or small upper intestinal secretions. A peptic ulcer is a conglomerate of heterogeneous disorders that manifests as a lesion in the lining of the gastrointestinal mucosa bathed by acid and pepsin. Peptic ulcers frequently occur along the lesser curvature of the antral end of the stomach or, more rarely, in the lower end of the esophagus, where stomach juices frequently reflux. It occurs because of an imbalance between aggressive factors (gastric acid and pepsin) and defensive factors (gastric mucus, bicarbonate, prostaglandins). The white mulberry tree (*M. alba* L.) is a deciduous tree originating from Asia but currently cultivated in subtropical, tropical, and moderate environments. *M. alba* leaves contain triterpenoid and steroidal saponin (lupeol, β- Sitosterol), bioflavonoids (rutin, moracetin, quercetin-3-triglucoside and isoquercitrin), coumarins, volatile oil, alkaloids, amino acids and organic acids, rutin, quercetin. *M. alba* leaves have various pharmacological properties such as anti-cancer, Anti- dopaminergic, and Hepatoprotective activity. Antimicrobial activity, antioxidant activity. Due to the presence of several phytoconstituents in this plant have been used for ages in various traditional medicine systems to treat cough, tonify blood, constipation and diabetes.

INTRODUCTION: An ulcer is a crater-like lesion in a membrane; ulcers that develop in areas of the GIT exposed to acidic gastric juice are called peptic ulcers. 'peptic' refers to pepsin, a stomach enzyme that breaks down proteins. A peptic ulcer is defined as disruption of the mucosal integrity of the stomach and duodenum leading to a local defect or excavation due to active inflammation [9]. They may arise in the form of single or multiple lesions.

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Oxidative stress is believed to be important in initiating and aggravating peptic ulcer disease. One of the common denominators for the genesis of this disease is the involvement of free radicals. Reactive oxygen species (ROS) are generated through numerous normal metabolic processes.

These ROS are responsible for the oxidation of tissues leading to lipid peroxidation. This lipid peroxidation can cause loss of membrane fluidity, impaired ion transport and membrane integrity, and finally, loss of cellular functions and tissue damage. Various antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT) and reduced gluthathione (GSH) control accumulation of reactive oxygen species [10]. Any imbalance in the activity of these enzymes normally leads to faulty

disposal of free radicals and its accumulation leads to ulceration [11]. Several semi-synthetic and synthetic drugs including proton pump inhibitors, H2 receptors antagonists, mucosal defensive agents, and various antimicrobial agents, are available to treat ulcers. Still, clinical evaluation of these drugs has shown side effects such as nausea, abdominal pain, constipation, diarrhoea, gynaecomastia, drug interaction. Due to the occurrence of many side effects by using synthetic drugs for many diseases, medicinal plants are considered the main source of new drugs as they have fewer or no side effects. About 64% of the total global population remains dependent on traditional medicines for their health care system. Whereas about 85% of India's rural population depends on wild varieties of plants for the treatment of various diseases, they suffer from [12]. Herbal medicines are being considered with lesser adverse effects, economical, effective and relatively less toxic [13].

A variety of botanical products have been reported to possess anti-ulcer activity, because they contain various phytochemical constituents such as saponins, flavonoids, tannins, *etc*. Saponin shows antiulcer activity because they are a group of polyphenolic compounds showing free radical scavenging action and activating mucous membrane protective factors [14]. *M. alba* Linnaeus, commonly known as white mulberry belongs to family Moraceae [15]. Mulberry is a fast-growing deciduous plant that grows under various conditions *i.e*., tropical, subtropical and temperate [16].

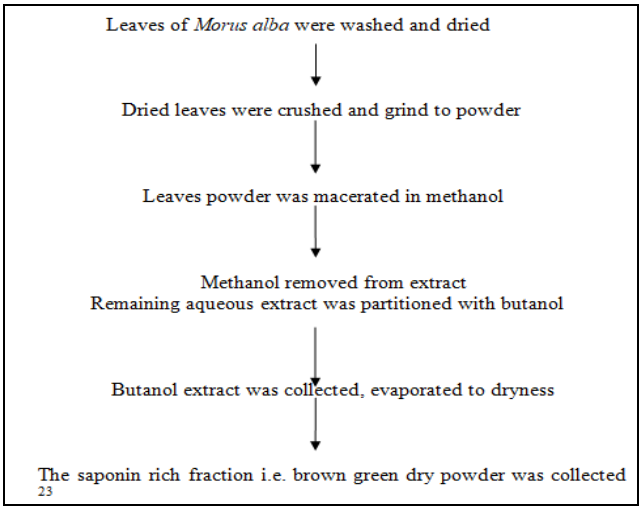
Mulberry leaves contains various phytochemicals such as alkaloids, anthocyanins, flavonoids, stilbenes, triterpenoids saponin (lupeol), steroidal saponin (β- sitosterol), coumarins and phenolic acids, anthocyanin, stilbenes [17] and glycosides, benzofuran derivatives, anthroquinones, morusimic acid, oleanolic acid [18, 19, 20, 21]. Antiulcer activity of *M. alba* leaf extract has been previously reported. The results of the present study suggest a direct protective effect of *M. alba* extracts on gastric mucosal damage and that the gastroprotective action of this plant may be due to its anti- oxidant properties [22]. Doi K *et al.* reported that l-butanol extract of mulberry leaves scavenged the DPPH radical and inhibited the oxidative modification of rabbit and human LDL. However, antiulcer activity of the saponin-rich fraction of M. alba leaves has not been previously reported.

MATERIAL AND METHODS:

Plant Material: The leaves of *Morus alba* were collected in the month of October from Punjab Agriculture University Ludhiana in 2016, India and authenticated by Dr. Sunita Garg, Chief Scientist, Raw Material Herbarium and Museum (RHMD), CSIR-NISCAIR (National Institute of Science Communication and Information Resources), New Delhi, India (Voucher specimen No. NISCAIR/RHMD/Consult/2017/3072-21). A voucher specimen was deposited in the herbarium of the institute. The leaves were cleaned, washed, dried in the shade and coarsely powdered. The powdered sample was kept in a light-protected and air-tightened container (Plate 1).

PLATE 1: MORUS ALBA LEAVES (WHITE MULBERRY)

Extraction Procedure:



Preliminary Phytochemical Analysis: Preliminary phytochemical screening of SFMA (Saponin rich fraction of *M. alba*) leaves revealed the presence of saponins only.

Experimental Animals: Active adult Wistar albino rats of either sex were selected randomly for the study. The rats were obtained from the animal house of the Department of Pharmacology, G.H.G Khalsa College of Pharmacy, Gurusar Sadhar, Ludhiana. Rats of 12–16 weeks, weighing 160–200 g, were used for the study. Each rat was housed in a plastic box cage under standard conditions at 19–25°C and was kept under 12/12 h light/dark cycle. The rats were allowed free access to standard pellet feed and water *ad libitum*. The protocol was approved by Animal Ethical Committee (GHG/04/2017) on 11/01/2017. Animals were randomly assigned to different groups, each consisting of six rats. Animals fasted 24hrs before the experiment. Vehicle, test and standard drug were administered to the animals after 1hr animals were anesthetized. Pyloric ligation was performed. After 4hrs of pyloric ligation, animals were sacrificed under anesthesia and stomach was cut, removed from the animal's body. The stomach was opened along the greater curvature, and gastric content was collected for the estimation of various pharmacological and biochemical parameters. The experimental results data was significantly analyzed by one-way Anova followed by Tukey's multiple ranges test using Graph Pad Prism version 5.0 Software. The p-value <0.05 was considered to be significantly significant.

Statistical Analysis: All the results were expressed as mean ± standard error mean (SEM). The experimental results and biochemical estimations data was statistically analyzed by one-way ANOVA followed by Tukey's multiple range tests using Graph pad Prism version 5.0 software. The p-value < 0.05 was considered to be statistically significant.

RESULTS: The results of anti-ulcer activity of saponin-rich fraction of *Morus alba* leaves is presented in **Table 2.**

Pharmacological Estimation:

Effect of SFMA Leaves on Gastric Volume in Pyloric Ligation-Induced Ulcers: Gastric volume of the vehicle control group was found to be 1.14 ± 0.16 ml, which was significantly increased in rats after pyloric ligation to 5.33 ± 0.19 ml ($p<0.001$). After administration of SFMA leaves at the dose of 250 mg/kg and 500 mg/kg, gastric volume was significantly reduced in a dose-dependent manner to 2.50 ± 0.11 ml ($p<0.01$) and 2.16 ± 0.11 ml ($p<0.001$) respectively as compared to disease control. The administration of ranitidine (50 mg/kg) also decreased the gastric volume to 1.81 ± 0.13 ml ($p<0.001$) concerning disease control. The effect of SFMA leaves (500 mg/kg) was comparable to that of ranitidine (50 mg/kg) **Fig. 1.**

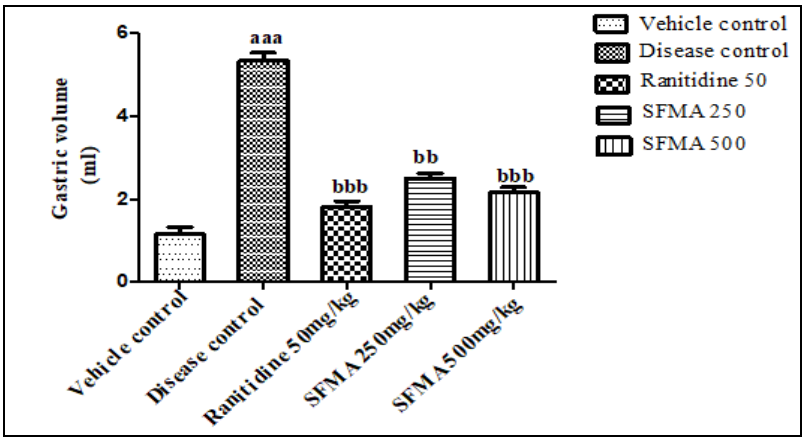


FIG. 1: EFFECT OF SFMA LEAVES ON GASTRIC VOLUME IN PYLORIC LIGATION-INDUCED ULCERS. Each value represents the mean of 5 animals ± standard error of the mean. Statistical analysis was determined using one-way ANOVA test followed by Tukey's multiple comparisons test.aaa, bbb represent ($p<0.001$) and bb represents ($p<0.01$).'a' significantly different from vehicle control group, 'b' significantly different from disease control group.

Effect of SFMA leaves on Total Acidity in Pyloric Ligation-Induced Ulcers: Total acidity is the total amount of acid held in solution or the sum of the quantities of both the ionized and the un-

ionized portions of actual acid. Total acidity (mEq/l) in vehicle control group was found to be 23.39±1.92 mEq/l which was significantly increased in rats after pyloric ligation to 67.1 ± 1.93 mEq/l ($p<0.001$). After administration of SFMA leaves at the dose of 250 mg/kg and 500 mg/kg, total acidity was significantly decreased in a dose-dependent manner to 46.74±1.87 mEq/l ($p<0.01$) and 37.19±1.65 mEq/l ($p<0.001$) respectively as compared to disease control. The administration of ranitidine (50 mg/kg) also decreased the total acidity to 25.79±1.95 mEq/l ($p<0.001$) with respect to disease control **Fig. 2.**

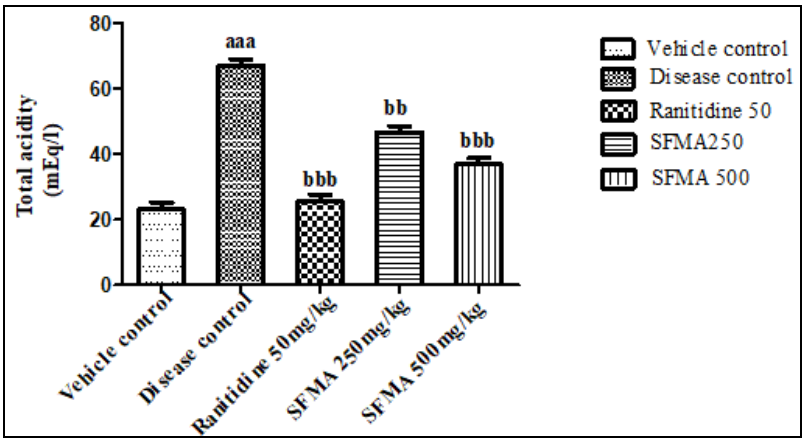


FIG. 2: EFFECT OF SFMA LEAVES ON TOTAL ACIDITY IN PYLORIC LIGATION-INDUCED ULCERS: Each value represents the mean of 5 animals ± standard error of the mean. Statistical analysis was determined using one-way ANOVA test followed by Tukey's multiple comparisons test. aaa, bbb represents($p<0.001$) and bb represents ($p<0.01$). 'a' significantly different from vehicle control group, 'b' significantly different from disease control group.

Effect of SFMA Leaves on Free Acidity in Pyloric Ligation-Induced Ulcers: Free acidity (mEq/l) in vehicle control group was found to be 8.47 ± 0.09 mEq/l which was significantly increased to 40.16 ± 1.33 mEq/l ($p<0.001$) after pyloric ligation in rats. After administration of SFMA leaves at the dose of 250 mg/kg and 500 mg/kg, free acidity was significantly decreased in a dose-dependent manner to 26.31 ± 1.00 mEq/l ($p<0.01$) and 14.96 ± 0.98 mEq/l ($p<0.001$) respectively as compared to disease control. The administration of ranitidine (50 mg/kg) also significantly decreased the free acidity to 9.63 ± 0.99 mEq/l ($p<0.001$) with respect to disease control. Effect of SFMA leaves (500 mg/kg) was comparable to that of ranitidine (50 mg/kg) **Fig. 3.**

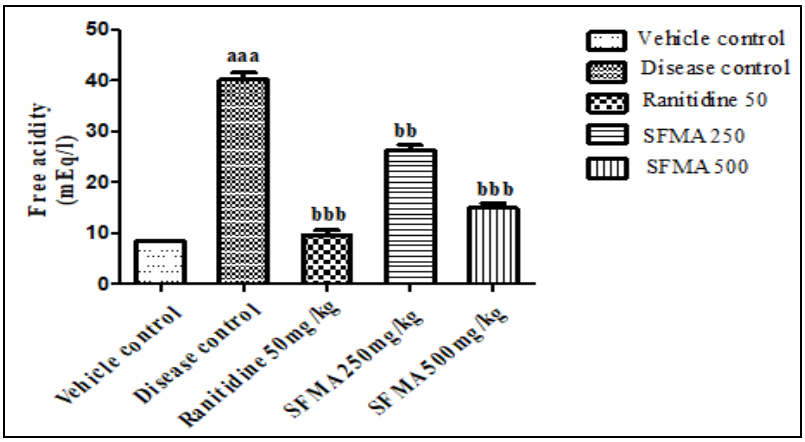


FIG. 3: EFFECT OF SFMA LEAVES ON FREE ACIDITY ON GASTRIC JUICE IN PYLORIC LIGATION-INDUCED ULCERS. Each value represents the mean of 5 animals ± standard error of the mean. Statistical analysis was determined using one-way ANOVA test followed by Tukey's multiple comparisons test. aaa, bbb, represents ($p<0.001$) and bb represents ($p<0.01$). 'a' significantly different from vehicle control group, 'b' significantly different from disease control group.

Effect of SFMA Leaves on Ulcer Index in Pyloric Ligation-Induced Ulcers: Ulcer index in the vehicle control group was found to be 0.00±0.00 which was significantly increased after

pyloric ligation to 7.38 ± 0.20 ($p<0.001$). After administration of SFMA leaves at the dose of 250 mg/kg and 500 mg/kg, the ulcer index was significantly reduced in a dose-dependent manner to 1.76±0.34 ($p<0.01$) and 1.60±0.26 ($p<0.001$), respectively as compared to disease control. The administration of ranitidine (50 mg/kg) also significantly decreased the ulcer index to 1.12±0.19 ($p<0.001$) concerning disease control. The effect of SFMA leaves (500 mg/kg) was comparable to that of ranitidine (50 mg/kg) **Fig. 4.**

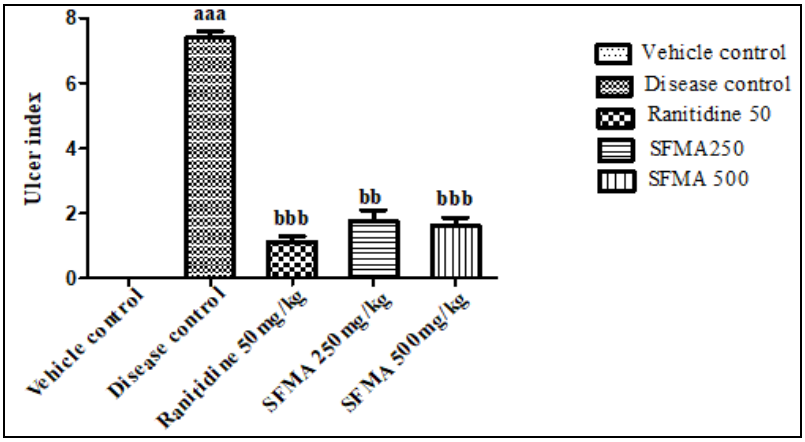


FIG. 4: EFFECT OF SFMA LEAVES ON ULCER INDEX IN PYLORIC LIGATION-INDUCED ULCERS. Each value represents the mean of 5 animals ± standard error of the mean. Statistical analysis was determined using one-way ANOVA test followed by Tukey's multiple comparisons test. aaa, bbb represents ($p<0.001$) and bb represents ($p<0.01$). 'a' significantly different from vehicle control group, 'b' significantly different from disease control group.

Effect of SFMA Leaves on pH of Gastric Acid in Pyloric Ligation-Induced Ulcers: The pH of gastric acid of vehicle control group was found to be 6.17 ± 0.19, significantly decreased in rats after pyloric ligation to 1.52 ± 0.19 ($p<0.001$). After administration of SFMA leaves at the dose of 250 mg/kg and 500 mg/kg, pH of gastric acid was significantly increased in a dose-dependent manner to 4.25 ± 0.13 ($p<0.01$) and 5.36 ± 0.16 ($p<0.001$) respectively as compared to disease control. The administration of ranitidine (50 mg/kg) also increased the pH of gastric acid to 5.84 ± 0.19 ($p<0.001$) concerning disease control. The effect of SFMA leaves (500 mg/kg) was comparable to that of ranitidine (50 mg/kg) **Fig. 5.**

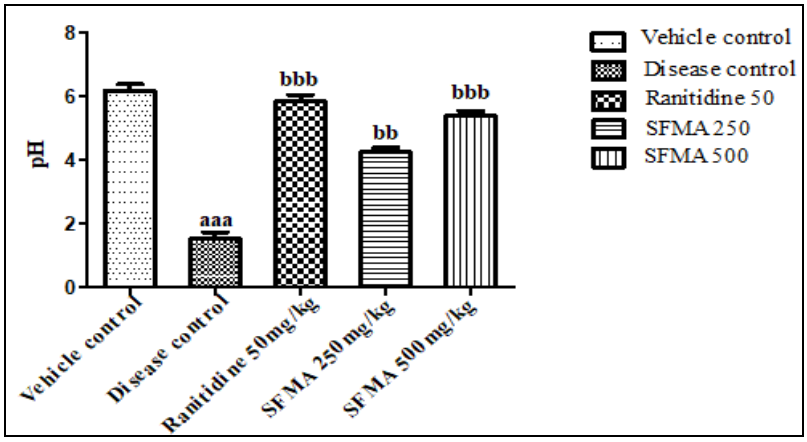


FIG. 5: EFFECT OF SFMA LEAVES ON PH OF GASTRIC ACID IN PYLORIC LIGATION-INDUCED ULCERS. Each value represents the mean of 5 animals ± standard error of the mean. Statistical analysis was determined using a one-way ANOVA test followed by Tukey's multiple comparisons test. aaa, bbb, ($p<0.001$) and bb represents ($p<0.01$). 'a' significantly different from vehicle control group, 'b' significantly different from disease control group.

Effect of SFMA Leaves on Ulcer Protection in Pyloric Ligation-Induced Ulcers: The ulcer protection in the vehicle control group was 100.00 %, significantly higher than the disease control group 00.00 % ($p<0.001$). After administration of SFMA leaves at the dose of 250 mg/kg and 500

mg/kg, the percentage protection of ulcer was significantly increased in a dose-dependent manner to 76.15 % ($p<0.01$) and 78.31 % ($p<0.001$), respectively as compared to disease control. The administration of ranitidine (50 mg/kg) also increased the percentage protection of ulcers to 84.81 % ($p<0.001$) concerning disease control. The effect of SFMA leaves (500 mg/kg) was comparable to that of ranitidine (50 mg/kg) **Fig. 6.**

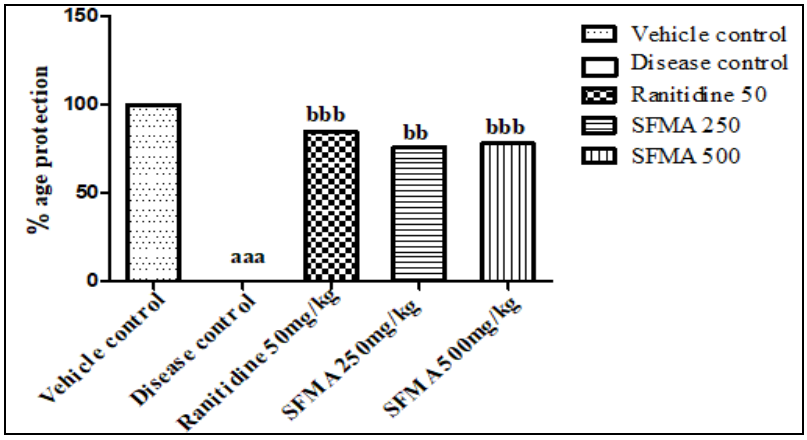


FIG. 6: EFFECT OF SFMA LEAVES ON ULCER PROTECTION IN PYLORIC LIGATION-INDUCED ULCERS. Each value represents the mean of 5 animals ± standard error of the mean. Statistical analysis was determined using a one-way ANOVA test followed by Tukey's multiple comparisons test. aaa, bbb represents ($p<0.001$) and bb represents ($p<0.01$) 'a' significantly different from vehicle control group, 'b' significantly different from disease control group.

Biochemical Estimations:

Effect of SFMA Leaves on Lipid Peroxidation (LPO) in Pyloric Ligation- Induced Ulcers: The lipid peroxidation (nMoles/mg protein) in vehicle control group was found to be 23.91±0.14 nMoles/mg protein ($p<0.001$) which was significantly increased after pyloric ligation to 47.34±1.22 nMoles/mg protein ($p<0.001$). After administration of SFMA leaves at the dose 250 and 500 mg/kg lipid peroxidation was reduced in a dose dependent manner to 31.09 ± 1.28nMoles/mg protein ($p<0.01$) and 27.35 ± 1.25 n Moles/mg protein ($p<0.001$) respectively as compared to disease control. The administration of ranitidine (50 mg/kg) also significantly reduced the lipid peroxidation to 22.71 ± 1.19 nMoles/mg protein ($p<0.001$) with respect todisease control. Effect of SFMA leaves (500 mg/kg) was comparable to that of ranitidine (50 mg/kg) **Fig. 7.**

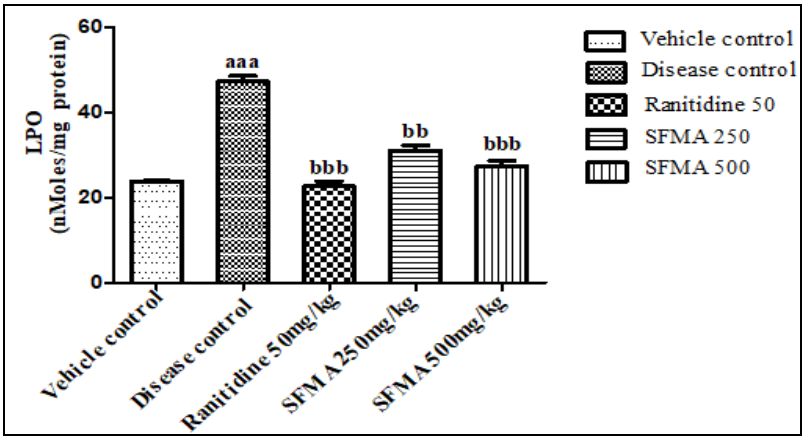


FIG. 7: EFFECT OF SFMA LEAVES ON LIPID PEROXIDATION IN PYLORIC LIGATION-INDUCED ULCERS. Each value represents the mean of 5 animals ± standard error of the mean. Statistical analysis was determined using one-way ANOVA test followed by Tukey's multiple comparisons test. aaa, bbb represents ($p<0.001$) and bb represents ($p<0.01$). 'a' significantly different from vehicle control group, 'b' significantly different from disease control group.

Effect of SFMA leaves on Superoxide Dismutase (SOD) in Pyloric Ligation-Induced Ulcers: The activity of superoxide dismutase (U/mg protein) in vehicle control group was found to be 4.47 ± 0.09

U/mg protein which was significantly reduced after pyloric ligation to 2.06 ± 0.19 U/mg protein ($p<0.001$). After administration of SFMA leaves at the dose 250 and 500 mg/kg, the SOD activity was significantly increased in a dose-dependent manner to 3.31 ± 0.13 U/mg protein ($p<0.01$) and 3.76 ± 0.09 U/mg protein ($p<0.001$) respectively as compared to disease control. The administration of ranitidine (50 mg/kg) also significantly increased the SOD activity to 4.29 ± 0.14 U/mg protein ($p<0.001$) concerning disease control. The effect of SFMA leaves (500 mg/kg) was comparable to that of ranitidine (50 mg/kg) **Fig. 8.**

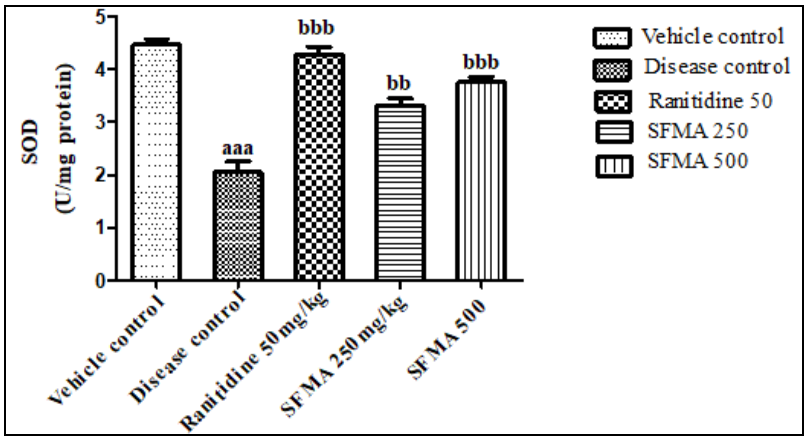


FIG. 8: EFFECT OF SFMA LEAVES ON SUPEROXIDE DISMUTASE ACTIVITY IN PYLORIC LIGATION-INDUCED ULCERS. Each value represents the mean of 5 animals ± standard error of the mean. Statistical analysis was determined using one-way ANOVA test followed by Tukey's multiple comparisons test. aaa, bbb represents ($p<0.001$). 'a' significantly different from vehicle control group, 'b' significantly different from disease control group.

Effect of SFMA leaves on Reduced Glutathione (GSH) in Pyloric Ligation- Induced Ulcer: The level of reduced glutathione (µmol/mg protein) in vehicle control group was found to be 1.91 ± 0.12 µmol/mg protein ($p<0.001$) which was significantly reduced after pyloric ligation to 0.45 ± 0.11 µmol/mg protein ($p<0.001$). After the administration of SFMA leaves at the dose 250 and 500 mg/kg the level of GSH was increased in a dose-dependent manner to 1.47 ± 0.12µmol/mg protein ($p<0.01$) and 1.62 ± 0.14 µmol/mg protein ($p<0.001$) respectively as compared to disease control. The administration of ranitidine (50 mg/kg) also significantly increased the level of GSH to 1.80 ± 0.07 µmol/mg protein ($p<0.001$) with respect to disease control. Effect of SFMA leaves (500 mg/kg) was comparable to that of ranitidine (50 mg/kg) **Fig. 9.**

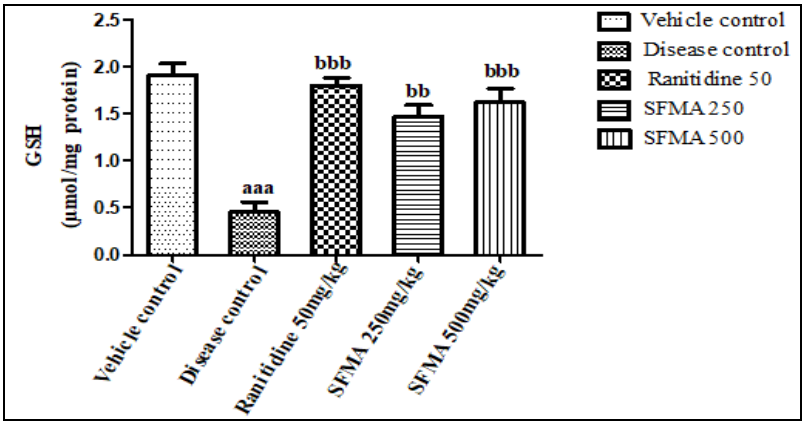


FIG. 9: EFFECT OF SFMA LEAVES ON LEVEL OF REDUCED GLUTATHIONE (GSH) IN PYLORIC LIGATION-INDUCED ULCERS. Each value represents the mean of 5 animals ± standard error of the mean. Statistical analysis was determined using one- way ANOVA test followed by Tukey's multiple comparisons test. aaa, bbb represents ($p<0.001$) and bb represents ($p<0.01$). 'a' significantly different from vehicle control group, 'b' significantly different from disease control group.

Effect of SFMA leaves on Catalase in Pylorus Ligation-Induced Ulcers: The activity of Catalase (U/mg protein) in vehicle control group was found to be 36.83 ± 0.83 U/mg protein which was

significantly reduced after pyloric ligation to 16.74 ±0.06 U/mg protein ($p<0.001$). After administration of SFMA leaves at the dose 250 and 500 mg/kg, the catalase activity was significantly increased in a dose-dependent manner to 24.17 ± 0.05 U/mg protein ($p<0.01$) and 31.15 ± 0.96 U/mg protein ($p<0.001$) respectively as compared to disease control. The administration of ranitidine (50 mg/kg) also significantly increased the catalase activity to 33.50 ± 0.99 U/mg protein ($p<0.001$) with respect to disease control. The effect of SFMA leaves (500 mg/kg) was comparable to that of ranitidine (50 mg/kg) **Fig. 10.**

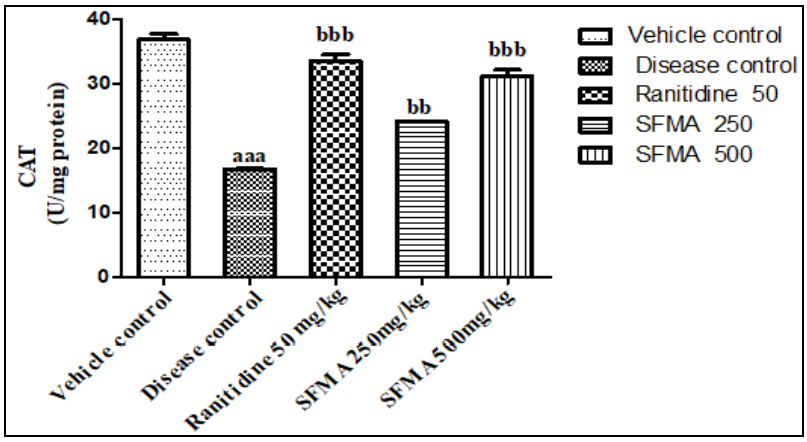


FIG. 10: EFFECT OF SFMA LEAVES ON CATALASE ACTIVITY IN PYLORIC LIGATION-INDUCED ULCERS. Each value represents the mean of 5 animals ± standard error of the mean. Statistical analysis was determined using one-way ANOVA test followed by Tukey's multiple comparisons test. aaa, bbb ($p<0.001$) and bb represents ($p<0.01$), 'a' significantly different from vehicle control group, 'b' significantly different from disease control group.

Macroscopic Evaluation: Macroscopic changes in various treatment groups after pyloric ligation-induced peptic ulcer in rats are shown in **Fig. 11-15**. The images of the stomachs of the groups *viz.*, vehicle control, disease control, standard and treatment groups SFMA leaves 250mg/kg and 500mg/kg in pyloric ligation- induced ulcers.

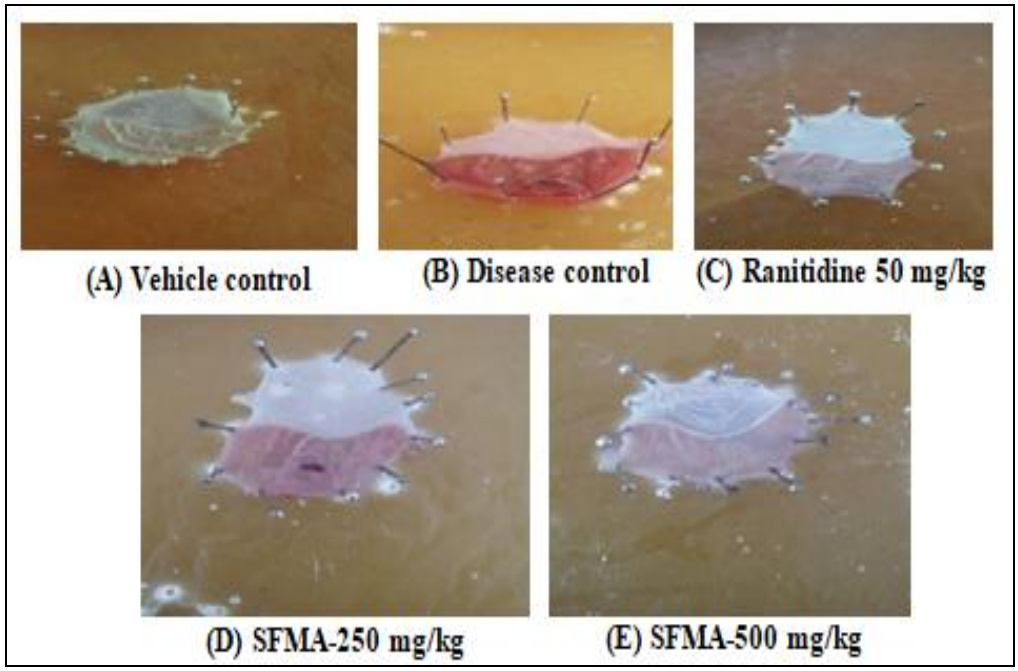


FIG. 11-15: MACROSCOPIC VIEW OF PYLORIC LIGATION-INDUCED ULCERS IN RATS. (a) vehicle control group showing normal mucosal layer, (b) disease control group showing red color of mucosal layer haemorrhagic streaks, perforation, spot ulcer (c) pre-treatment with ranitidine (50 mg/kg) showing no signs of ulcer of mucosal layer (d) showing minimum signs of ulcer of mucosal layer in rats pre-treated with SFMA leaves 250 mg/kg and (e) showing no signs of ulcer of mucosal layer in rats pre-treated with SFMA leaves 500 mg/kg.

TABLE 1: PERCENTAGE YIELD OF SAPONIN RICH FRACTION OF *M. ALBA* LEAVES

Extract	Yield	Colour	Solubility
1000g	5.3%	Brown green	Butanol

TABLE 2: RESULTS OF ANTI-ULCER ACTIVITY OF SAPONIN RICH FRACTION OF *MORUS ALBA* LEAVES

Group	Name	Treatment	Pylorus ligation
1	Vehicle control	0.5% w/v CMC	-
2	Disease control	0.5% w/v CMC	After 24 hours of fasting in rats
3	SFMA leaves dose- I	250 mg/kg, p.o. in 0.5% w/v CMC	After 24hrs of fasting and after 1hrs of administration of SFMA leaves dose- I in rats
4	SFMA leaves dose- II	500mg/kg, p.o. in 0.5% w/v CMC	After 24hrs of fasting and after 1hrs of administration of SFMA leaves dose-II in rats
5	Standard	Ranitidine 50mg/kg, p.o. in 0.5% w/v CMC	After 24hrs of fasting and after 1hrs of administration of Standard drug in rats

All the results were expressed as mean ± standard error mean (SEM). The data of experimental results and biochemical estimations was statistically analyzed by one-way ANOVA followed by Tukey's multiple range tests using Graph pad prism version 5.0 software. The p-value < 0.05 was considered to be statistically significant.

DISCUSSIONS: Peptic ulcer disease is one of the most common disruptions of the mucosal integrity of the stomach (a gastric ulcer) and small intestine (a duodenal ulcer). It has generally been accepted that cause of gastric ulcers are multi factorial and appear to be due to an imbalance among the aggressive factors (such as acid/ pepsin, bile, alcohol, tobacco and caffeine, *H. pylori* infection, NSAIDs, stresses) and mucosal defensive mechanisms (including mucus secretion, bicarbonate production, mucosal blood flow, cellular repair mechanism, prostaglandin E and growth factor). Peptic ulcer treatment using synthetic drugs (such as H_2 blockers, proton pump inhibitors and NSAIDs) results in adverse effects, relapses and drug interactions [24]. Therefore, herbal medicines containing active chemical components is now a day being considered as the main source of new drugs and appropriate alternatives for treatment of peptic ulcer [25].

Preliminary phytochemical screening of SFMA (Saponin-rich fraction of *M. alba*) leaves revealed the presence of saponins only. The present study was carried out to study the effect of saponin-rich fraction of *M. alba* leaves in rat model of peptic ulcer using pyloric ligation.

Accumulation of gastric content and high acidic pH, lead to auto digestion of the gastric mucosa and breakdown of the gastric mucosal barrier cause ulcer [26] which was determined by calculating UI (ulcer index). UI in disease-control rats was greater than that of vehicle-control rats. After 4hrs of pyloric ligation, PU was assessed in the disease and vehicle control groups. In our study, in the disease control rats, there was the presence of bleeding ulcers, erosions, red coloration of the mucosa, mucosal oedema, haemorrhages, perforation, and tissue necrosis [27]. After administration of SFMA leaves, UI came down which may be due to its ulcer protective effect. In the similar study reported by [28] shows that *M. alba* leaves reduce ulcer index in indomethacin-induced ulcer in rats. Also, another study confirms gross gastric lesion evaluation done by M. alba leaves against ethanol-induced gastric ulcer in rats.

In the present study, it could be noticed that pyloric ligation decreased the pH of gastric juice in the disease control group compared to the vehicle control group. This was also confirmed by the determination of gastric free and total acidity, which was also significantly increased in the disease control group as compared to vehicle control. Treatment with both the doses of SFMA leaves increased gastric juice's pH and reduced the free and total acidity due to its antisecretory effect. Similarly in previous study lupeol and β- sitosterol saponin from petroleum ether extract and chloroform extract of *Spathodea falcate* shows anti-ulcerogenic affect against indomethacin and ethanol induced gastric ulcer in rats [29].

Pyloric ligation leads to an increase in gastric volume due to the accumulation of gastric acid in the stomach [30]. After administration of both doses of SFMA leaves, gastric volume was significantly decreased. This may be due to the antisecretory effect of *M. alba* leaves on acid in the stomach. Pylorus-ligation also increased lipid peroxidation and decreased Superoxide dismutase, catalase and

reduced glutathione, thus leading to oxidative stress. Oxidative damage of the gastric mucosal cell membrane is caused by reactive oxygen species (ROS). This ROS is the major contributing factor of pyloric ligation-induced gastric ulceration. Increased production of ROS can cause a decrease in the membrane permeability, activities of enzymes and receptors and activities of cells [31, 32]. Results of the present study also indicate similar alterations in the antioxidant status after pylorus ligation. Lipid peroxidation is a free radical-mediated process. It involves forming and propagating lipid radicals, oxygen uptake, and rearranging double bonds in unsaturated lipids, which eventually destroys membrane lipids (increased TBARS generation led to oxidative stress causing ulceration) [33]. In the present study, after 4hrs of pyloric ligation caused to increase the level of lipid peroxidation (TBARS level/ enhanced MDA level). However, a significant decrease in lipid- peroxidation was observed by pre-treatment with both the doses 250mg/kg and 500mg/kg of SFMA leaves, suggesting its protective effect.

Preventive anti-oxidant such as superoxide dismutase (SOD), catalase (CAT) is the first defence against reactive oxygen species [34]. In the current study, after pyloric ligation, the level of SOD and CAT were found to be decrease in the disease control group as compared to the vehicle control group. However, administration of both the doses 250mg/kg and 500mg/kg of SFMA enhance the level of SOD and CAT in pylorus-ligated rats, suggesting its efficacy in preventing free radical-induced damage.

The function of GSH in an oxidation-reduction process is to act as a reductant, resulting in the formation of glutathione disulphide (GSSG). In the first few hours of oxidative stress, free radicals damage leads to the consumption of GSH, directing decreased GSH levels and decreased levels of GSH represent its increased consumption by the cells as a consequence of ROS generation; therefore, it is considered a marker of short term oxidative stress [35, 36]. In the present study, the level of GSH was decreased in the stomach of pylorus-ligated rats due to oxidative stress. In contrast, treatment with both doses of SFMA leaves significantly helped to restore the level of GSH, subsequently either restoring the biogenesis of GSH or decreasing the oxidative stress. In the present study, saponin-rich fraction of *M. alba* leaves shows antiulcer effect. The cytoprotective effect of *Morus alba* may be attributed due to the presence of saponin. Thus saponin rich fraction of *Morus alba* leaves possess protection against gastric mucosal damage attributed to the free radical scavenging activity of saponin.

CONCLUSION: From the above observation, it can be concluded that pre-treatment with both the doses (250mg/kg and 500mg/kg) of SFMA leaves cause a significant reduction in UI of the stomach along with a significant reduction in acid secretory parameters like total acidity, free acidity and volume of gastric fluid indicating the inhibition of aggressive factors. There was an increase in the pH of the gastric fluid, indicating the cytoprotective effect of *M. alba* leaves. Therefore, SFMA ameliorated the ulcer formation as it decreased the gastric mucosal damage and had anti- secretory effect on acid.

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