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ROLE OF SILYMARIN IN MANAGEMENT OF PEPTIC ULCERIN RATS

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ABSTRACT

Peptic ulcer disease which include gastricand duodenal ulcer is the most prevalent gastrointestinal disorder. Despite medical advances, the management of peptic ulcer and its complications remain a challenge, with high morbidity and death rates for the disease. Milk thistle seeds are rich in flavonolignans silybin (A and B), isosilybin (A and B), silydianin, silychristin, and isosilychristin, as well as the flavonoid, taxifolin**.**sylimarin has been demonstrated to have beneficial effects in studies of hepatoprotective and possesses antioxidant activity**.** The present investigation aimed to evaluate the anti-ulcer activity and the possible mechanismsof action ofsylimarin against ethanol induced gastric ulcers using curative models at two dosage level (50 & 100 mg/kg). The obtained results showed that, oral administration of *sylimarin*for rats in doses of 50 and 100mg /kg body weight for 7 days post induction of gastric ulcer induce a potent antiulcerogenic activity in a dose dependent manner indicated by significant decrease in ulcer area, ulcer index, ulcer number and score but increased mucus weight and PH. Sylimarin at doses 50&100 mg/kg induced a significant enhancement in the gastric GSH, CAT, SOD levels of ethanol-induced ulcer rats and reduced the elevated level of MDA. The present study, showed that sylimarin restoration of gastric histopathology aberrations and leukocyte influx signifying its potential anti-ulcer actions.

KEYWORDS: Gastric ulcer- SYLIMARIN- curative—Prophylactic – Mechanism.

2. INTRODUCTION

Ulcers are an open sore of the skin or mucus membrane characterized by sloughing of inflamed dead tissue.[1] Ulcers are lesions on the surface of the skin or a mucousmembrane characterized by a superficial loss of tissue. The peptic ulcers are erosion of lining of stomach or the duodenum.[2] Gastric ulcers (GU), characterized by pain; ulcers are common in older age group. Other symptoms may include nausea, vomiting, and weight loss. Although patients with gastric ulcers may have normal or diminished acid production, yet ulcers may occur even in complete absence of acid.[3] The principle factors causing this disease are inadequate dietetic habits, prolonged use of non-steroidal anti-inflammatory drugs, stress and infection by *Helicobacter pylori,* in addition to other factors of genetic origin.[4] Reduced Glutathione (GSH) is an important for mucosal integrity since depletion of GSH from the gastric mucosa by electrophilic compounds inducesmacroscopic mucosal ulceration.[5]

Several classes of pharmacological agents have proved effective in the control of peptic disorders.[3] These groups include antacids as aluminum hydroxide, magnesium trisilicate; acid suppressive agents[6] Anticholinergic[7] Cytoprotective,[8] (prostaglandin analogues[4] antimicrobial for eradication of H. pylori as amoxicillin, clarithromycin,[6]

The search for novel non-toxic, antiulcer preparations from medicinal plants is currently in vogue in order to obtain alternative sources of medicine for the management of gastric hyper secretion, gastro duodenal ulcers and ulcerative colitis. Different screening studies have shown that several of these plants do contain useful cyto-protective antiulcer effects.[9] Silymarin, abioflavoinoid obtained from fruits of silibummarinum or milk thistle, contains activeingredients silybin, silychristin, and silydianin. Preliminary studies reveal silymarin has antioxidantactivity and protects the cellular constituents, hence has been used as hepatoprotective agent intreatment of liver disorders.[10] Polyphenolic compounds as Flavonoides are occurred widely in fruit, vegetables, tea, cocoas and red wine.[11] Flavonoids have been found to play important roles in the non-enzymatic protection against oxidative stress.[12] It has been further pointed out that silymarin is used medicinally to treat liver disorders, including acute and chronic viral hepatitis, toxin/drug-induced hepatitis, cirrhosis and alcoholic liver diseases. It is also effective in certain cancers. Its mechanism of action includes inhibition of hepatotoxin binding to receptor sites on the hepatocyte membrane; reduction of glutathione (GSH) oxidation to enhance its level in the liver and intestine; antioxidant activity; and stimulation of ribosomal RNA polymerase and subsequent protein

synthesis, leading to enhanced hepatocyte regeneration.[13] Yoshikawa et al in their study have reported the possible role of oxidative free radicals in mediating cold restraint stress induced gastric injury in albino rats.[14]

On basis of these reports the aim of the present study was to evaluate the curative capacity of sylimarin against ethanol induced gastric ulcer in rats, and to demonstrate the possible mechanisms of actions.

3. MATERIAL AND METHODS

3.1. Drugs and chemicals and solutions

Sylimarin of Milk thistle was obtained as 95% white powder form Nanjing Health Herb Bio-Tech Co., Ltd, China. Lazoprazole was obtained kindly as pure powder form 98.5% fro Medizen Company for pharmaceutical, Alexandria, Egypt. Absolute ethanoland formaldehyde solution 40% was purchased from El-Nasr pharmaceutical ChemicalCo.), Cairo, Egypt.

3.2. Animals

Swiss albino mice of both sex (25–30 g) and male Wistar rats (140–170 g) were used. Animals were maintained under standard conditions (temperature 23 ± 1.0 °C, humidity 55 ± 10%, 12 h light/12 h dark cycle) and housed in standard polypropylene cages with wire mesh top and they fed with a standard pellet diet with water *ad libitum* and were allowed to adapt to the laboratory environment for one week before experimentation.

3.3. Acute toxicity (LD_{50}) test

Acute toxicity of milk thistle *SYLIMARIN* were studied in mice following their single oral administration. Swiss albino mice in groups of six animals, received upgraded doses of 500, 1000, 155, 2000, 2500, or 3000 mg/kg for Sylimarin. Control animals received the vehicle and were kept under the same conditions. Signs of acute toxicity and number of deaths per dose within 24 h were recorded for determination of oral median lethal dose.[15]

3.4. Antiulcer activity

Thirty male Wistar rats of 160–180 g body weight were used in this study: animals wasserved for the curative model. 5 groups each of 6 animals were used. Rats of groups I served as normal control, received isotonic saline solution 0.5 ml/100 g. Group II received ethanol (absolute) 1.0 ml/200g orally once; Group III(standard) treated with lansoprazol 30 mg/kg

orally then received ethanol 1.0 ml/200g orally once. Groups IVandV and were received ethanol 1.0 ml/200g orally once then given the sylimarin in doses of50 or 100 mg/kg for, 1 hour post ulcer induction thenonce daily for 7 consecutive days.

Induction of peptic ulcer was carried out using absolute ethanol-induced ulcer method as described by[16] for the prophylactic model while the curative model was first described. In addition, assessment of gastric lesions was carried out.[17] Lesion scores were quantified by the scoring system (0–5).[18] Ulcer indices (mm) were calculated as the sum of the total length of long ulcers and petechial lesions in each group of rats divided by its number. The percent of protection was determined according to the formula:

% Protection of control ulcer = (Control UI - Test UI/Control UI) × 100

3.5. Measurement of Mucus Production

The gastric mucus of each rat was obtained by gently scraping the mucosa with a glass slide and the collected mucus was weighed by using a precision electronic balance (Sartorius, Germany)

3.6. Determination of PH

An aliquot of 1ml gastric juice was diluted with 1ml of distilled water and pH of the solution was measured using PH meter.

3.7. Biochemical investigations for SYLIMARIN

Sylimarin was administered to twogroups of animals (n = 6)(50 and 100 mg/kg). Three other groups of animals were used, two received water orally and the third received oral lansoprazole (30 mg/kg) to serve as normal control, ulcer control and standard groups respectively. All medications were administrated for 7 successive days. Peptic ulcer was induced, one hour post the last dose, by oral administration of absolute ethanol (1 ml/200 g/kg). All rats were sacrificed by an overdose of ether, the stomachs were rapidly removed, blood samples were collected and different biomarkers were measured.

Stomach homogenate (10%) was prepared in phosphate buffer (100 mM, pH 7.4), centrifuged at 10,000 g for15 min at 4 °C and used to evaluate the antioxidant enzyme activities such as Malondialdehyde; (MDA), catalase (CAT), superoxide dismutase (SOD) and GSH in rat stomach tissues using corresponding assay kits obtained from Sigma-Aldrich (St. Louis, MO,USA).

3.8. Histopathological Study

For histological examination, stomach tissues of rats designed for Curative effect of SYLIMARIN were sectioned with 5 µm thickness, and stained with hematoxylin and eosin (H & E). This was done by a pathologist who was unaware of the histological injury treatment records.

3.9. Statistical analysis

All values were expressed as mean ± S.D. Comparisons between means were carried out using one-way ANOVA test followed by Tukey's HSD test using SPSS, version 14.

Statistical significance of differences between two means was assessed by unpaired Student's *t*-test. Differences at $p < 0.05$ were considered statistically significant.

4. RESULTS

4.1. Acute toxicity: (LD_{50} test)

Neither morbidity nor mortality was recorded in group of mice treated with any of the dose of sylimarin up to 3000mg/kgbody weight during 24 h of observation. It was suggested that oral LD_{50} of the evaluated extract were higherthan 3000 mg/kg. Since substances possessing LD_{50} higherthan 50 mg/kg are non-toxic[19], the testedextracts were considered safe.

4.2. Anti-ulceractivity

Ethanol induced ulcers in rats showed severe damage and extensive hemorrhagicnecrosis of gastric mucosa. The standard group treated with lansoprazole showed less injury with significantreduced areas of gastric ulcer formation compared to ulcer control group. Curative effects of the tested doses (50 and 100mg/kg. b.wt) ofsylimarin of Milk thistle on absolute alcohol induced gastric ulcer in rats is shown in table 1&2 and fig 1 -2.

Oral administration of Silymarin in doses of 50 and 100 mg/kg body weight for 7 days before induction of gastric ulcer induced a marked antiulcerogenic activity in a dose dependent manner indicated by significant decreased in ulcer area, ulcer index, ulcer number and score but increased mucus weight and PH. The prophylactic antiulcerogenic activity of Silymarin (100 mg/kg) were more potent compare to lansoprazole (30 mg/kg) while the lower dose of Silymarin was significantly ($P<0.05$) less effective than either the higher one or the lansoprazole (Fig1&2). Sylimarin was found to be effective antiulcerogenic agent at different doses either when used as gastroprotective therapy or when used as curative remedy.Oral

administration of SYLIMARIN reduced the severity of gastric damage in a dose dependent manner (Tables 1&2 and Figure 2-3). Sylimarin in a dose of 100 mg/kg was as effective as lansoprazole (30 mg/kg) in reducing all parameters of peptic ulcer in both models (prophylactic and curative).

Results of antioxidant activity of silymarinwas studied in rats using ethanol induced gastric ulcer model are presented in (table 2). Oral administration of ethanol significantly ($P<0.05$) decreased reduced glutathione content (GSH), catlase (CAT), superoxide dismutase (SOD), while it elevated malondialdehyde (MDA) as a lipid peroxidation marker in stomach tissue as compared to control group. The obtained data showed elevation of reduced glutathione (GSH), catlase (CAT), superoxide dismutase (SOD) activities significantly ($P<0.05$), while it decreased malondialdehyde (MDA) activities significantly ($P<0.05$) in rats treated via oral route with EGCG (Table 2), and those treated with Lansoprazole as compared to ethanol induced rats or control group administered saline. Ethanol administration decreased significantly glutathione (GSH) by 86.06, catalase (CAT) by 84.82 %, superoxide dismutase (SOD) by 76.84 %, and increased malonaldehyde (MDA) by 284.8 % as compared to healthy group. Oral administration of EGCG in dose 50 mg/kg body weight or sylimarin significantly ameliorate glutathione (GSH) level, as well as catlase (CAT), superoxide dismutase (SOD), and malonaldehyde (MDA) activities to be similar to that of control healthy rats.

Stomach of control rats revealed normal histological structure of the gastric layers with normal mucosal epithelium and gastric glands (Fig. 3a). While examination of stomach of ethanol administrated rats showed marked histological alterations. The upper mucosal layer was severely necrosed and tangling with detachment of the epithelial linings. Marked necrosis of the gastric glands lining cells along the whole mucosal layer was prominent with marked congestion (Fig. 3b). The submucosa was severely edematous, congested and showed heavy inflammatory cells infiltration (Fig. 3c). Concerning the examination of stomach of Lansoprazole administrated rats revealed moderate degree of protection against the action of alcohol, few focal areas of mucosal epithelial desquamation were noticed leaving few minute foci that do not cover by the epithelial cells and vacuolar degeneration of the gastric glands epithelial linings (Fig. 3d). The use of silymarin at 50 and 100 mg as a prophylaxis revealed; variable degree of protection of the gastric mucosa against the action of alcohol which was dose dependent. Focal detachment of the lining epithelium was observed at 50 mg dose with necrotic changes of the gastric glands (Fig. 4a). While at 100 mg dose of silymarin, good

protection was noticed; most of the epithelial layer appeared near to normal with minimal necrotic changes (Fig. 4b) and slight vacuolation of the gastric gland linings.

DISCUSSION

This study examined the gastric ulcer healing potential of SYLIMARIN using rat ethanol-induced gastric lesion model. Rat model that used ethanol to induce gastric ulcers has been widely used forevaluation of gastro protective as well as curative capacity activity. Ethanol induced ulcers are due to superficial damage to mucosal lining epithelial cells.[20]. Gastro-protective studies showed that ethanol could injure the epithelium of stomach and disrupt the vascular endothelium.[21-22] Ethanol may increase the permeability of the vessels and develop edema in sub-mucosal layer of the stomach as well as epithelial lifting.[23] Mucosal blood flow is an importantfactor in the damage induced by ethanol and could be modulated by prostaglandins.[24] The ethanolinduced ulcers are predominant in the glandular part of stomach. It was reported that, ethanol stimulates the formation of mast cell secretary products25 and reactive oxygen species resulting in the damage of rat gastric mucosa.[26]

The obtained results showed that LD50 of sylimarin was higher than 3 g/kg.. Since substances possessing LD50 higher than 50 mg/kg are non-toxic19 (Buck et al., 1976.

Ethanol induced ulcers in rats showed severe damage and extensive hemorrhagic necrosis of gastric mucosa. The standard group treated with lansoprazole showed less injury with significant reduced areas of gastric ulcer formation compared to ulcer control group. Oral administration of silymarin in doses of 50 and 100 mg /kg body weight for 7 days either before (prophylactic)or after induction of gastric ulcer (curative) induced a marked antiulcerogenic activity in a dose dependent manner indicated by significant decreased in ulcer area, ulcer index, ulcer number and score but increased mucus weight and PH. The antiulcergenic activity of Silymarin (100 mg/kg) were more potent compare to lansoprazole (30 mg/kg) while the lower dose of Silymarin was significantly ($P< 0.05$) less effective than either the higher one or the lansoprazole. Oral administration of silymarin for 7 days in doses of 50 and 100 mg/kg body weight either before or after induction of ulcerative colitis induced marked antiulcerative colitis activity in a dose dependent manner indicated by significant decreases in ulcer area, ulcer number score and ulcer index. The antiulcerative effect of silymarin (100 mg/kg) were more potent compared to lansoprazole (30 mg/kg) while the

lower dose of silymarin was significantly (P<□0.05) less effective than either the higher one or the lansoprazole.

The curative and prophylactic capacity of silymarin against either ethanol induced gastric ulcer or acetic acid induced ulcerative colitis approved at the present study was consistent with.[14] They evaluated the gastric protection of silymarin, a lipoxygenase inhibitor, in rats. In addition[27], reported that, silymarin modulates inflammation by suppresses cellular inflammation. They reported that transcriptional profiling, metabolomics, and signaling studies were performed in human liver and T cell lines. Cellular stress and metabolic pathways were modulated within 4 h of silymarin treatment: activation of Activating Transcription Factor 4 (ATF-4) and adenosine monophosphate protein kinase (AMPK) and inhibition of mammalian target of rapamycin (mTOR) signaling, the latter being associated with induction of DNA-damage-inducible transcript 4 (DDIT4). Metabolomics analyses revealed silymarin suppression of glycolytic, tricarboxylic acid (TCA) cycle, and amino acid metabolism. Anti-inflammatory effects arose with prolonged (i.e. 24 h) silymarin exposure, with suppression of multiple pro-inflammatory mRNAs and signaling pathways including nuclear factor kappa B (NF-κB) and forkhead box O (FOXO).

Silymarin is a polyphenolic flavonoid EXTRACTED from milk thistle that has anti-inflammatory, cytoprotective, and anticarcinogenic activitied. silymarin, may involve suppression of NF-κB, a nuclear transcription factor, which regulates the expression of various genes involved in inflammation, cytoprotection, and carcinogenesis. They reported that; the effect of silymarin on NF-κB activation induced by various inflammatory agents. Silymarin blocked TNF-induced activation of NF-κB in a dose- and time-dependent manner. These effectsc were mediated via inhibition of phosphorylation and degradation of IκBα, an inhibitor of NF-κB and it blocked the translocation of p65 to the nucleus without affecting its ability to bind to the DNA. NF-κB-dependent reporter gene transcription was also suppressed by silymarin. Also, also sylimarin inhibits NF-κB activation induced by phorbol ester, LPS, okadaic acid, and ceramide, whereas H2O2-induced NF-κB activation was not significantly affected.[28]

Possible mechanisms of action (biochemical investigations)

Ethanol-induced gastric ulcers that arise as a result of direct damage of gastric mucosal cells, resulting in the development of free radicals and hyperoxidation of lipid.[29] A significant

decrease in the mucosal levels of non-protein sulfhydryl compounds was demonstrated in ethanol-induced gastric damage.[30] These endogenous compounds are important for maintaining the integrity of the gastric mucosa and mediating the protective effects of prostaglandins against gastric mucosal injury.[20]

In the present study ethanol-induced gastric ulcer model, gastric MDA content increased (22.6 nmol/g tissue) accompanied by decreased gastric GSH content (0.41 mg/g tissue), compared to the normal rats (MDA,7.5 nmol/mg tissue, GSH, 2.4 U/g tissue, 2). Silymarin at doses 50 and 100 mg/kg produced a significant reduction in the gastric MDA content of absolute ethanol-induced ulcer rats. In this respect, many evidences report that silymarin is powerful antioxidant. It acts as a free radical scavenger and inhibits lipid peroxidation. It protects from oxidative stress by decreasing the levels of reduced glutathione.[31] Silymarin maintains the normal membrane fluidity by directly interacting with cell membrane components, thereby preventing alteration in the content of lipid fraction.[32]

Glutathione is an important constituent of the intracellular protective mechanism against a number of noxious stimuli, including oxidative stress. Intracellular glutathione also seems to be responsible for protecting gastric cell against ethanol induced injuries. The excessive generation of oxygen radicals in the extracellular space and depletion of glutathione in conjunction with the inhibition of glutathione peroxidase activity are responsible for oxidative tissue damage of the gastric mucosa after the administration of ethanol, as suggested by various studies 33. In the present study, reduction of glutathione concentrations were observed in the control groups, whereas the rats that were treated with the silymarin showed a significant increase in the glutathione level, suggesting that silymarin prevent the depletion of non-protein sulfhydryl groups caused by ethanol treatment .

Silymarin is a polyphenolic flavonoid EXTRACTED from milk thistle that has anti-inflammatory, cytoprotective, and anticarcinogenic activitied. silymarin, may involve suppression of NF-κB, a nuclear transcription factor, which regulates the expression of various genes involved in inflammation, cytoprotection, and carcinogenesis. They reported that; the effect of silymarin on NF-κB activation induced by various inflammatory agents. Silymarin blocked TNF-induced activation of NF-κB in a dose- and time-dependent manner. These effectsc were mediated via inhibition of phosphorylation and degradation of IκBα, an inhibitor of NF-κB and it blocked the translocation of p65 to the nucleus without affecting its ability to bind to the DNA. NF-κB-dependent reporter gene transcription was also suppressed

by silymarin. Also, also sylimarin inhibits NF-κB activation induced by phorbol ester, LPS, okadaic acid, and ceramide, whereas H2O2-induced NF-κB activation was not significantly affected28 [Sunil, et al., (1999)].

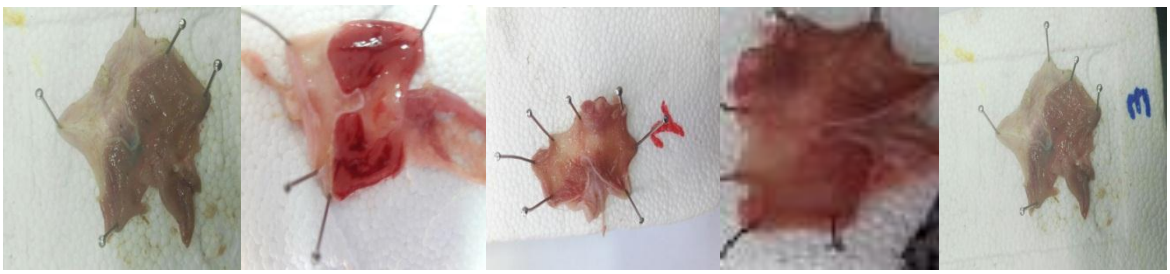
Histopathology

The present study, showed that silymarin restoration of gastric histopathology aberrations and leukocyte influx signifying its potential anti-ulcer actions. These observations are in concert with previous studies.[34-35] Reported that, revocation of neutrophil infiltration has been noticed as a crucial anti-inflammatory mechanism by which effective anti-ulcer agents protect against gastric ulcer lesions.[36] These suitable actions are likely mediated via the observed silymarin inhibition of TNF-α and oxidative stress since they provoke the expression of several adhesion molecules, including ICAM-1, that enhance leukocyte invasion to injured gastric mucosa proved in the present study and previously by other investigators.[28]

Table (1): Curative capacity of silymarin against ethanol- induced gastric ulcer in rats Mean ±SE.

Treatment	Dose mg/kg body weight	Ulcer score	Number of ulcers	Ulcer area (mm^2)	Ulcer index	Prote-ction %	Mucus weight	PH
Control Healthy	0.0	0.0	0.0	0.0	0.0	100	0.58 ±0.04a	3.2 ±0.31c
Control Positive	Saline	4.67 ±0.23a	13.2±1.12a	24.12± 1.21	17.7 ±1.56a	0.0	0.23 ±0.01d	3.2 ±0.30c
Lansoprazole	30	1.57 ±0.02b	2.01±0.25c	2.74±0.21c	2.8 ±0.15b	84.2	0.48± 0.04b	5.4 ±0.41a
silymarin	50	2.6 ±0.22b	3.41±0.43b	4.57±0.25b	3.1 ±0.12b	82.5	0.40 ±0.03c	4.1 ±0.21b
	100	1.05 ±0.11c	1.7±0.37c	2.1±0.11c	1.2 ±0.01c	90.1	0.46± 0.02b	5.2 ±0.31a

Values within a column with different letters are significantly different (P<□0.05).



A B C D E

Fig. 1: Effect of *silymarin* on ethanol-induced gastric ulcer in rats. A, Control group; B, Ethanol group 1 mL/200g b.wt.; C, Lansoprazole 30 mg/kg b.wt.; D, Silymarin 50 mg/kg b. wt.; E, Silymarin 100 mg/kg b.wt.

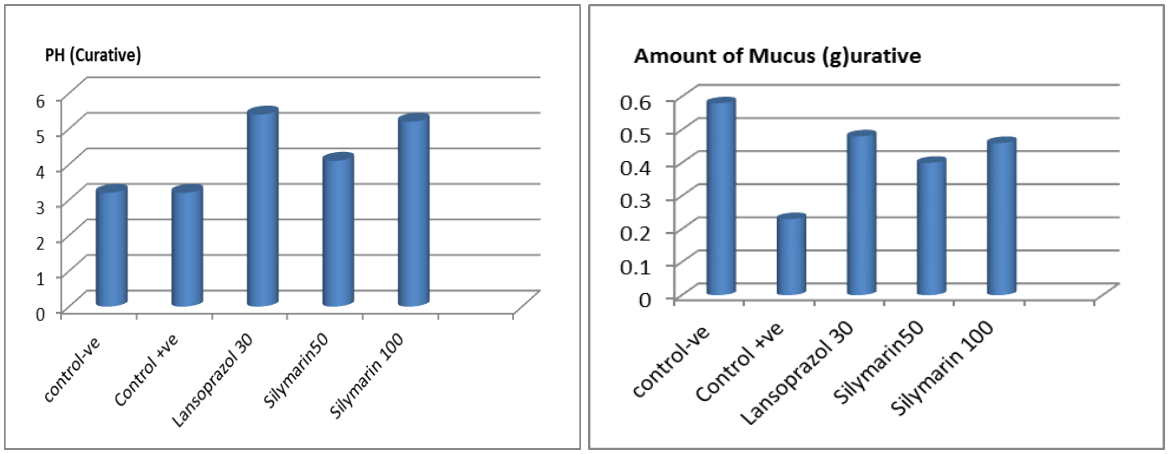


Figure 2: Effect of Curative of Sylimarin on PH and amount of mucus against absolute alcohol-induced ulcer in rats.

Table 2: Effect of *silymarin* on the content of reduced glutathione (GSH), catalase (CAT), superoxide dismutase (SOD) and malondialdehyde (MDA), in stomach tissue of rats. (n=5).

Group	Dose (mg/kg b.wt.)	GSH (mg/g)	CAT (U/g)	SOD (U/g)	MDA (nml/g)
Control Healthy	0.0	2.4 ± 0.41a	0.95± 0.01a	138.4 ± 3.57a	7.5 ± 1.136d
Control Positive	Ethanol	0.41 ±0.1d	0. 22± 0.02d	34.65 ±7.13c	22.6.±2.33a
Lansoprazole	Ethanol +30	2.02± 0.18a	0.9± 0.026a	142. 5± 6.83a	8.2±0.897c
silymarin	Ethanol +50	1.3± 0.3c	0.41± 0.08c	68.25± 6.82b	12.2± 1.11b
	Ethanol +100	2.01±0.12a	0.88 ± 0.088a	140.25±12.41a	8.5±0.74 c

Values within a column with different letters are significantly different (P≤0.05)

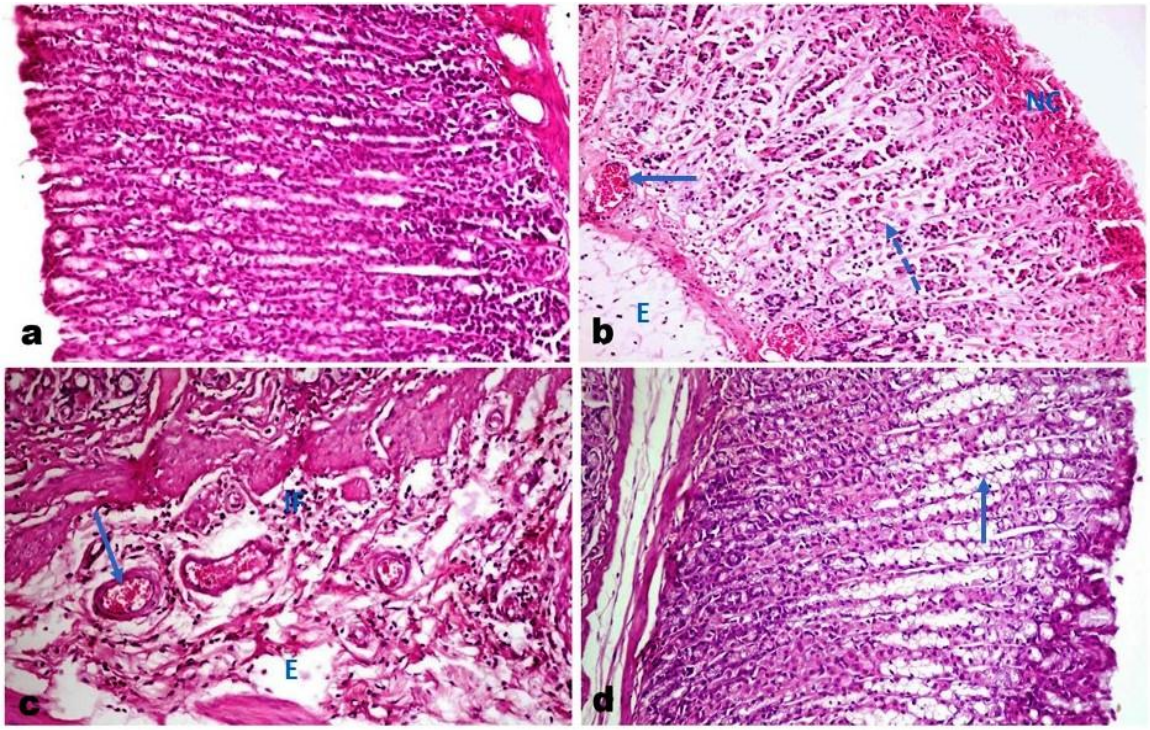


Figure 3: Stomach of (a) control rat, (b and c) stomach of alcohol administrated rat showing necrosis (NC) of the upper mucosal layer, congestion of the mucosal blood vessels (arrow) and necrosis of the gastric glands (dashed arrow) as well as severe submucosal congestion (arrow), edema (E) and inflammatory cells infiltration (IF). (d) Stomach of Lansoprazole administered rat showing few focal areas of mucosal epithelial desquamation and vacuolation of the glands linings (arrow). (H&E, X200).

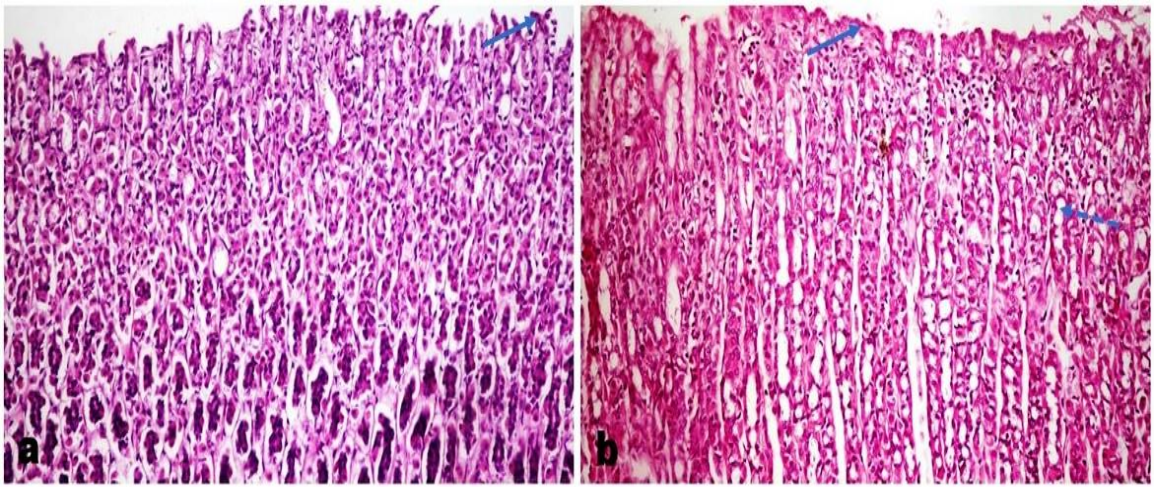


Figure 4:(a-b)Stomach of silymarin administered rats, (a) at 50mg dose showing focal detachment of the lining epithelium with necrotic changes of the gastric glands. (b) at 100mg dose showing good protection of the gastric epithelium (arrow) with mild vacuolation of the glandular epithelium (dashed arrow). (H&E, X200).

CONCLUSION

Sylimarin induced potent anti-ulcerogenic andantioxidant activities in a dose dependent manner. The antisecretory activity appears to be mainly related to the suppression of inhibition of TNF-α and oxidative stress since they provoke the expression of several adhesion molecules in gastric including MDA, SOD, GSH and CT. Inhibition of theinflammatory mediator TNF-α may be one of the possible mechanisms of action of sylimarin.

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