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Gastroprotective role of bioflavonoid silymarin in animal model of acute cold-restraint stress induced gastric ulceration

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Abstract: *Introduction:* Imbalance between the aggressive and defensive factors and presence of acid- pepsin is supposed to play a major role in aetiopathogenesis of peptic ulcer disease. Recently, role of oxidative free radicals have been implicated in mediating cold-restraint stress, H.pylori, NSAID induced gastric injury. *Objective:* The present study was undertaken to evaluate the possible role of antioxidant bioflavonoid silymarin in acute cold- restraint stress model of gastric ulceration. *Methods:* Silymarin 50mg/kg was administered orally for 5 days to albino rats (n=6) on fifth day animals were subjected to cold restraint stress. After 3 hours animals were sacrificed and their stomachs were studied for adherent mucin content, and ulcer index. The results were compared with those of control (n =6) and omeprazole (n = 6) treated groups. *Results and conclusion:* Silymarin administration decreased the ulcer index, increased the adherent mucin content and reduced ulceration compared to control group ($P<0.05$). The results were parallel to standard drug omeprazole ($P<0.01$). Silymarin protects gastric mucosa from ulceration and increases adherent mucin. Therefore we conclude that antiulcer effect of silymarin may be mediated through its antioxidant activity.

Key words: Silymarin, cold -restraint stress, antioxidant, gastroprotection.

Introduction

Peptic ulcer is a chronic disease which impairs the quality of life and is associated with increased morbidity and mortality. Imbalance between gastroduodenal mucosal defenses and counter-vailing aggressive forces are supposed to play important role in causation of peptic ulcer, recently oxidative free radicals have been implicated in mediating NSAID, H. Pylori, ethanol, and cold restraint stress induced gastric injury [1-4].

The extracts of plants have been tested for antiulcer activity since ancient times. Silymarin, a bioflavoinoid obtained from fruits of silibum marinum or MILK THISTLE, contains active ingredients silybin, silychristin, and silydianin. Preliminary studies reveal silymarin has antioxi-dant activity and protects the cellular constituents, hence has been used as hepatoprotective agent in treatment of liver disorders [5]. 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 [6]. On basis of these reports the present study was undertaken to evaluate the possible role of silymarin, an antioxidant and a bioflavonoid, in animal models of cold -restraint stress induced gastric injury.

Material and Methods

Eighteen albino rats of Wistar strain of either sex weighing 150-200 g were divided into three groups of six each. The control group (Group I) received distilled water 1ml daily for 5 days. Group II received Silymarin 50mg/ kg in 0.01% $NaHCO_3$, once daily orally for 5 days. Group III received omeprazole 3.6 mg/ kg orally in propylene glycol daily for 5 days as given in Table No1 [7].

Table-1: Drugs and doses administered in various groups

Groups	Drugs	Dose (Oral x 5days)	Vehicle (solvent)
I	Distilled water (Control)	1 ml	–
II	Silymarin	50 mg/kg	0.01% $NaHCO_3$*
III	Omeprazole	3.6 mg/kg	Propylene glycol*

Note: Therapeutic equivalent doses of the drugs in animals were calculated using Paget and Bernes Table [7].
*Vehicle treated groups showed no effects on stomachs

Six additional animals were taken for estimation of normal adherent mucin. Vehicles NaHCO3 and Propylene glycol treated groups presented with no effects on stomachs. On fifth day, after drug administration the animals were subjected to cold -restraint stress by the method of Vincent et al [8]. At the end of three hours animals were sacrificed, stomachs were isolated and washed in normal saline. Each of the stomach was studied for ulceration and mucosal damage, adherent mucin and ulcer index was calculated.

a) Ulcer index was calculated by method of Dipak Das etal [9].
   0 = No pathology, 1 = Pin point ulcers (1-2 mm), 2 = Medium ulcers (3-4 mm), 4 = Large ulcers (5-6 mm), 8 = large ulcers (> 6 mm) size .Mean ulcer index was calculated in each group. The results were analyzed by Student t-test. [10].
b) Adherent mucin was estimated by using method of Corne et al [11]. Alcian blue a histological dye, which stains acidic mucins was used for quantitative estimation of adherent mucin.

After sacrificing the animals, stomachs were removed and everted, they were soaked for 2 hours in 0.1% alcian blue 8GX dye, dissolved in 0.16M sucrose buffered with 0.05 M sodium acetate adjusted to pH 5.8 with hydrochloric acid. Uncomplexed dye was removed by two successive washes at fifteen and forty five minutes in 0.25 M sucrose. Dye complexed with mucus was diluted by immersion in 10 ml aliquots of 0.5 m $MgCl_2$ for two hours. The resulting blue solutions were shaken briefly with equals volumes of diethyl ether and the optical density of aqueous phase was measured at 605 nm. The mean absorbance was calculated for each group, results were analyzed by converting to percentage of mucin content [11].

Results

The control group presented with features of ulceration. On gross examination serosal surface of stomach showed marked induration, dilated blood vessels, ecchymosis and hemorrhagic sites. Mucosal surface presented with features of severe degree of hyperemia, congestion and large number of pin point ulcers of varying sizes with central clots. There were no features of perforation in any of the stomach. The ulcer Index was very high (35±1.5, Table No2). Microscopic features were suggestive of acute gastric ulceration with depithelialization, neutrophil infiltration, edema and hemorrhage. The adherent mucin content was reduced in control group (87.5%) in comparison to normal rats (100 %, Table No3).

Table-2: Ulcer index and % injury in cold-restraint stress induced gastric ulcer in albino rats

Sr. No	Group	Mean ulcer index	% injury
1	Control (Distilled water)	35.0±4.5 [a]	100
2	Silymarin	7.16± 0.7 [a]	20
3	Omeprazole	3.0±1.4[b]	9

Note: Mean ulcer index values are (Mean ±SEM), Significance: a = P<0.05, b = P< 0.01

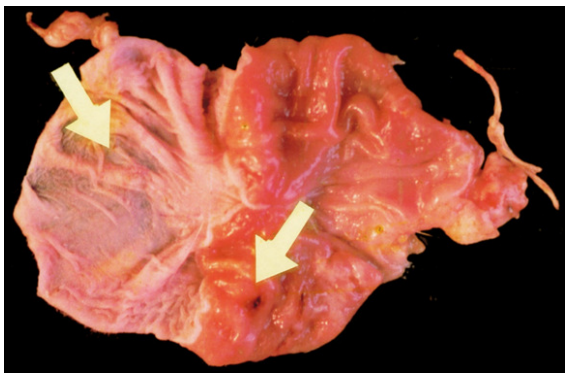
Table-3: Effect of various drugs on adherent mucin content in cold-restraint stress rats

Sr. No	Groups	Mean absorbance	Mucin %
1	Control	0.14	87.5
2	Silymarin	0.15	90.4
3	Omeprazole	0.17	106
4	Normal rats	0.16	100

Animal pretreated with silymarin showed few signs of mucosal injury, but percentage of damage was less compared to control group. Serosal surface revealed very few dilated blood vessels and petechial hemorrhages (FigureNo1). Mucosal surface revealed few ulcers of varying sizes; correspondingly ulcer index was also reduced (9.6±1.7, P<0.05, Table No2). Increase in mucin content was observed in comparison to control rats (90.4 %, Table No3). The features were suggestive of anti-ulcer activity of silymarin. Animals pretreated with omeprazole maintained near normal pattern. Serosal surface looked amber colored with few signs of dilated blood vessels and hemorrhagic suffusions. Mucosal surface retained the normal rughae pattern with minimal signs of mucosal injury. The ulcer index was markedly reduced (3.0±1.4, P<0.01, Table No2).

Concentration of adherent mucin was (106 %) slightly more than normal rats (Table No3). Thus animals treated with standard drug omeprazole showed anti-ulcer activity.

Figure-1: Gross Photograph showing minimal ulceration in silymarin treated group



The present study thus demonstrated that silymarin provides gastroprotective effect in animal model of cold- restraint stress ulcer. The results were parallel to animal treated with standard drug omeprazole.

Discussion

The results showed that silymarin reduced the mean ulcer index and percentage of injury significantly and also increased the adherent mucin content in cold -restraint stress induced gastric ulcer in comparison to control group. The mechanism of development of stress ulcers by oxidative free radicals has been proposed by DAS et al [12]. Since, silymarin provided gastroprotection, we assume silymarin by its anti-oxidant activity protected gastric mucosa from injury. The possible mechanism might be silymarin being lipid soluble diffuses into the biological membrane, as acute stress depletes glutathione levels, pretreatment with silymarin replenishes glutathione and superoxide dismutase (SOD) levels [13]. The peroxyl and alkoxyl radicals generated during oxidative stress are scavenged thus preventing peroxidation mediated injury [13]. Thus, it can be proposed that silymarin protects gastric mucosa against stress injury by its antioxidant and scavenging activity.

The protective role of silymarin in gastric ulcers has also been demonstrated using different models of gastric ulcer. Alarcon de la lastra C etal demonstrated the protective effect of silymarin in cold-restaint stress induced ulcers in rats [13]. Karmeli et al in their study have proposed that stress causes depletion of alpha tocopherol and gastric peroxidase enzyme while supplementation of these antioxidants prevents ulceration [14]. Salim A.S. in a double blind clinical trial has reported the gastroprotective effect of antioxidant Dimethyl Sulfoxide (DMSO) and allopurinol in patients with refractory peptic ulcer disease [15].

Conclusion

It can thus be concluded that acute stress generates reactive oxygen species, causing gastric injury by various mechanisms. While pretreatment with a suitable antioxidant such as silymarin provides gastroprotection. Therefore, antioxidant like silymarin might prove to be useful therapeutic drug in treatment of recurrent refractory peptic ulcer in near future. Further experimental and clinical studies are needed for confirmation.

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