

**GASTROPROTECTIVE AND ANTIOXIDANT EFFECTS OF  
HYDROALCOHOLIC FRUIT EXTRACT OF *PITHECELLOBIUM DULCE*  
ON ETHANOL INDUCED GASTRIC ULCER IN RATS**

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**Summary**

The study was designed to evaluate the gastroprotective effect of hydroalcoholic fruit extract of *Pithecellobium dulce* (HAEPD) in the injury of rat gastric mucosa induced by absolute ethanol and as well as to elucidate the role of reactive oxygen species, lipid peroxidation and some important antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), glutathione peroxidase (GSH-Px) and myeloperoxidase (MPO) in these effects as markers of ulceration process following oral administration of HAEPD and Omeprazole in rats with absolute ethanol-induced ulcers. HAEPD (200 mg/ kg b wt) as well as Omeprazole (30 mg/kg b wt) was administered orally once a day for 30 days before ulcer induction with absolute ethanol (1 ml/ 200 g b wt). Pretreatment with HAEPD and Omeprazole inhibited the ulceration damage of absolute ethanol by 68% and 58.51% respectively. Our results also showed that gastric ulcer index was significantly reduced in rats pretreated with HAEPD as compared with ethanol-treated controls. Moreover, in rats pretreated with HAEPD, there is significant reduction of TBARS content in gastric mucosa was found as compared to those rats treated with ethanol alone. In contrast, SOD, GR, GSH-Px activities were significantly increased in gastric mucosa of HAEPD pretreated rats with respect to those treated with ethanol alone. HAEPD and Omeprazole showed decreased MPO activity when compared to ethanol treated rats as a result of gastric injuries. Hence, our results demonstrates that HAEPD pretreatment exerts gastroprotective effects in ethanol-induced gastric ulcers in rats as evidenced by stimulation of antioxidant enzymes such as SOD, CAT, GR and GSH-Px which are scavengers of ROS and therefore prevents the gastric damage induced by them.

**Key words:** Antioxidant enzymes, Ethanol, Gastroprotective effect, myeloperoxidase, Omeprazole, *Pithecellobium dulce* (Leguminosae).

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### Introduction

Antioxidants are the foremost defense system that limits the toxicity associated with free radicals primarily superoxide ( $O_2^-$ ) anion and hydroxyl radical ( $OH^\cdot$ ). Oxidative stress and free radical mediated process have been implicated in the pathogenesis of gastrointestinal disorders<sup>1</sup>. Recently non steroidal anti-inflammatory drug's (NSAID)<sup>2</sup> and reactive oxygen species (ROS) have been shown to play a critical role in gastric ulceration process. The role of ROS in the development of acute experimental gastric lesions induced by stress, ethanol and NSAID is well known<sup>3</sup>. Ethanol manifests its harmful effects either through direct generation of reactive metabolites or indirectly changing the structure and functions of cellular components and promotes enhanced oxidative damage<sup>4,5</sup>. Excessive production of  $O_2$  radical's leads to altered enzymes activity, decreased DNA repair, impaired utilization of  $O_2$ , lipid peroxidation (LPO) and protein oxidation. Some of these alterations induced by oxidative stress have been recognized to be characteristic features of necrosis and subsequently leads to organ damage<sup>6</sup>. Ethanol induced gastric mucosal injury is associated with extensive damage to mucosal capillaries and vascular permeability<sup>7,8</sup>. Mucosal capillary necrosis, vascular congestion and thrombosis in the subepithelial layer accompany disruption of gastric mucosal barrier. In addition to ethanol various other factors are also involved in the pathogenesis of injury<sup>9</sup>. Marked increase in oxidative stress in gastric ulcer is indicated by transient changes in activities of antioxidant enzymes like superoxide dismutase (SOD), catalase, glutathione peroxidase (GSH-Px), glutathione reductase (GR) etc. Glutathione is an important intracellular scavenger involved in protective mechanism against number of noxious stimuli. Sulphydryl (SH) containing compounds prevents the acute hemorrhagic erosions caused by ethanol, NSAIDs or stress in animal models<sup>10</sup>. In the same way, various antioxidant enzymes such as SOD, an important radical superoxide scavenger; GSH-Px an enzyme involved in the elimination of hydrogen peroxide and lipid hydroperoxides play an important role in cell protection<sup>11,12</sup> and cellular catalase are potent antioxidant defenses in a variety of tumor cells<sup>13</sup>, endothelial cells<sup>14</sup> and epithelial cells<sup>15</sup>. Hence these antioxidants may play a protective role in gastric mucosal damage against ethanol.

Ulcer is a common global problem with increasing incidence and prevalence attributed to several factors such as stress, exposure to bacterial infection and the use of NSAIDs. Mucosal damage, an initial step in ulcer development has been known correlated with oxidative stress by ROS generation and hypersecretion of HCl through  $H^+$ ,  $K^+$ -ATPase action<sup>16</sup>. *Pithecellobium dulce* (Roxb.) Benth. (Leguminosae) commonly known as "guamachil" or "kamatsile" is a member of fabaceae family that grows up to 18m height, native of tropical America and cultivated throughout the plains of India and in the Andamans.

It is known as “Vilayatibabul” in Hindi and “Kodukkapuli” in Tamil. In traditional medicine, the leaves of *Pithecellobium dulce* used as remedy for ear ache, leprosy, peptic ulcer, tooth ache and venereal diseases and also the leaves have been reported to possess astringent, emollient, abortifacient and antidiabetic properties<sup>17</sup>. The bark of the plant is reported to be used as astringent in dysentery, febrifuge and eye inflammation<sup>18</sup>. Steroid, saponin, lipids, phospholipids, glycosides, glycolipids and polysaccharides have been reported from the seeds<sup>19</sup>. The constituents of *Pithecellobium dulce* fruits<sup>20</sup> and the anti inflammatory activity due to saponin fraction of *Pithecellobium dulce* fruits<sup>21</sup> were also studied. The antivenomous activity of polyphenols<sup>22</sup> from bark extract of *Pithecellobium dulce* and free radical scavenging properties<sup>23</sup>, antimycobacterial activity of afzelin (kaempferol-3-O-a-L-rhamnopyranoside) isolated from the alcoholic extracts of leaves of *Pithecellobium dulce* were recently reported<sup>24</sup>. Our previous study on H<sup>+</sup>,K<sup>+</sup>-ATPase inhibition and HPLC chromatogram of hydroalcoholic fruit extract of *Pithecellobium dulce* was found to contain rich quantity of phenolic compounds and revealed the presence of flavonoids – quercitrin, rutin, kaempferol, naringin, daidzein<sup>25</sup> and a preliminary study on phytochemical screening and antioxidant potential of aqueous fruit extract of *Pithecellobium dulce* were studied earlier<sup>26</sup>. Thus it is evident that the plant has great potentials in treating a number of ailments where the free radicals have been reported to be the major contributing factor. Therefore, the present study was undertaken to evaluate the antiulcerogenic effect of HAEPD in terms of its antioxidant status and myeloperoxidase activity on ethanol induced gastric mucosal damage.

## Materials and Methods

### Collection of plant material

The fleshy fruits of *Pithecellobium dulce* were collected from Kakkalur, Thiruvallur district, Chennai Tamil Nadu, India. The plant material was duly authenticated (PARC/2008/208).

### Preparation of plant extract

The fleshy fruits of *Pithecellobium dulce* were washed thoroughly and dried in shade at room temperature. The air dried materials were coarsely ground into homogenous powder and macerated with 70% ethanol for a week. The hydroalcoholic fruit extract of *Pithecellobium dulce* (HAEPD) was filtered through filter paper and the extracts were evaporated and concentrated under reduced pressure (bath temperature 50°C). The dried extract was stored in air tight container for the antiulcerogenic study.

### **Animals**

Adult male albino wistar rats, weighing 150-200g were provided by Directorate of Centre for Animal Health studies, Madhavaram, Milk Colony, Chennai. The animals were housed in groups of six in a clean polypropylene cages at room temperature in 12 hour dark/12 hour light cycles with both food (Hindustan Lever Ltd., Bombay, India) and water *ad libitum*. Animals used in the present study were housed and cared in accordance with the federal government legislation on animal care. Also the experiments were authorized by the ethical committee for animal care (290/CPCSEA/12/12/08-02).

### **Dose selection and administration route**

The dose was selected on the basis of acute toxicity study of HAEPD. Since the LD<sub>50</sub> value of HAEPD was found to be 3916.66 mg/kg b wt by arithmetic method of Karber<sup>27</sup> and so the effective dosage which can be applied for efficacy studies may be fixed as 100, 200 and 300 mg/kg b wt as the minimum, mid and maximum dose. A single dose of 200mg/kg b wt of hydro alcoholic extract (HAEPD) was used in the present study to determine the gastroprotective and antioxidant status on ethanol induced gastric mucosal damage. The above mentioned extract of *Pithecellobium dulce* in a volume of 2.0ml/ 150g b wt was prepared freshly each time and given once a day orally for 30 days. Similarly a positive control "Omeprazole" in a volume of 1ml/150g b wt (30 mg /kg b wt) was also prepared freshly each time and given once a day orally for 30 days.

### **Ulcer induction**

Ulceration was induced by gastric instillation of 1.0 ml of ethanol (60%)/200 g. All treatment was administered one hour before oral administration of ethanol and the animals were sacrificed one hour later.

### **Ulcer protective study**

The rats were divided into six groups of six animals each. The animals were kept fasting for 12 hours prior to the experiment but water was permitted *ad libitum*.

Group I – Normal control rats.

Group II – Animals treated with 1 ml ethanol (60%)/200 g orally on 30<sup>th</sup> day.

Group III – Animals treated with 2 ml HAEPD (200 mg/kg b wt) orally for 30 days.

Group IV – Animals treated with 1 ml ethanol (60%)/200 g + Pretreatment with 2 ml HAEPD (200mg/ kg b wt) orally for 30 days.

Group V – Animals treated with 1 ml of Omeprazole (30 mg /kg b wt) orally for 30 days.

Group VI – Animals treated with 1 ml of ethanol (60%)/200 g + Pretreatment with 1.0ml Omeprazole (30 mg/kg b wt) orally for 30 days.

### **Ulceration Index**

One hour after absolute ethanol ulcer induction, the animals were sacrificed by cervical dislocation. Briefly, after collecting gastric juice the stomach were removed and opened along the greater curvature and rinsed with 0.1mol/L ice-cold PBS. The stomach was then examined under microscope (100 x) to observe erosions and made scores as 1-5: 1 small round hemorrhagic erosion, 2 -haemorrhagic erosion <1mm, 3 - Hemorrhagic erosion of 2-3mm and 5 - haemorrhagic erosion > 4mm. The score was multiplied by 2 when the width of the erosion is larger than 1mm<sup>28</sup>.

Ulcer index (UI) and % inhibition were calculated thus,

$$UI = \frac{\text{Total ulcer score}}{\text{No of animals Ulcerated}}$$

$$\% \text{ of inhibition} = \frac{\text{UI of ethanol treated group} - \text{UI of pretreated groups}}{\text{UI of ethanol treated}} \times 100$$

### **Biochemical investigation of stomach tissues**

After the macroscopic analyses, thiobarbituric acid reactive substances (TBARS), glutathione peroxidase (GSH-Px), glutathione reductase (GR), superoxide dismutase (SOD), catalase (CAT), and myeloperoxidase (MPO) enzyme activities in the rat's stomach tissues were measured. The gastric mucosa was scrapped from the antral portion of the stomach with a scrapper and stored at 4°C for biochemical estimation. The scrapped gastric mucosa was subjected to prepare the mucosal homogenate (pH 7.2). The homogenate was then centrifuged at 3000 rpm for 10 minutes and the supernatant was used for antioxidant status on ethanol induced gastric mucosal damage.

**Biochemical estimations****Determination of TBARS content**

The extent of LPO was determined by analyzing the levels of TBARS in the gastric mucosa were measured according to the method described by Ohkawa et al., 1979<sup>29</sup> with minor modification. To 0.5 ml of tissue homogenate, 1.5 ml of 20% acetic acid, 0.2 ml of SDS and 1.5 ml of TBA were added. The mixture was made up to 4 ml with distilled water and heated for 1 hour at 95°C. After cooling, 4.0 ml of butanol – pyridine mixture was added and shaken well and centrifuged at 4000 rpm for 10 min. The organic layer was taken and its absorbance was read at 532 nm and the results were expressed as n mol/g protein.

**Determination of SOD activity**

Superoxide dismutase activity was measured by Kakkar et al., 1984<sup>30</sup> based on the inhibition of the formation of nicotinamide adenine dinucleotide, phenazine methosulfate and amino blue tetrazolium formazan. 0.5 ml of tissue homogenate was mixed with 0.4 ml of ethanol and chloroform mixture and centrifuged. To the supernatant, assay mixture (sodium pyrophosphate buffer (0.025 M, pH 8.3), phenazine methosulphate, nitroblue tetrazolium and reduced nicotinamide adenine dinucleotide (NADH)) was added and incubated at 30°C for 90s. The reaction was arrested by the addition of glacial acetic acid and mixed with n - butanol. The intensity of the colour developed in butanol was measured at 560 nm. SOD activity was measured by the degree of inhibition of this reaction and is expressed as millimole/min/mg protein.

**Determination of CAT activity**

CAT was assayed colorimetrically at 620 nm as described by the method of Sinha 1972<sup>31</sup>. The reaction mixture of 1.5 ml contained 1.0 ml of 0.01 M, pH 7.0 phosphate buffer, 0.1 ml of tissue homogenate (supernatant) and 0.4 ml of 2.0 M H<sub>2</sub>O<sub>2</sub>. The reaction was stopped by the addition of 2.0 ml of dichromate – acetic acid reagent (5% potassium dichromate and glacial acetic acid mixture in the ratio of 1:3). Results are expressed as millimole/min/mg tissue.

**Determination of myeloperoxidase activity**

Myeloperoxidase activity was measured according to the modified method of Bradley et al., 1982<sup>32</sup>. The homogenized samples were frozen and thawed for three times and centrifuged at 1500 g for 10 min at 4°C. 100µl of the homogenized supernatant was added to 1.9 ml of 10 mmol/L phosphate buffer (pH 6.0) and 1.0 ml of 1.5 mmol/L O – dianisidine hydrochloride containing 0.0005% (wt/vol) hydrogen peroxide. The absorbance was measured at 450 nm on a UV spectrophotometer. Myeloperoxidase activity in gastric tissues was expressed as µmoles/min/mg tissue.

**Determination of GSH-Px activity**

GSH-Px activity was measured by the method of Rotruck et al., 1973<sup>33</sup>. The reaction mixture contained 0.2 ml of 0.4 M, Tris - HCl buffer, pH 7.0, 0.1 ml of 10 mM sodium azide, 0.2 ml of tissue homogenate (homogenized the tissue in 0.4 M Tris - HCl buffer, pH 7.0), 0.2 ml glutathione, 0.1 ml of 0.2 mM hydrogenperoxide. Incubated the contents at room temperature for 10 min. The reaction was arrested by the addition of 0.4 ml of 10% TCA and centrifuged. Supernatant was assayed for glutathione content by using Ellmans reagent (19.8 mg of 5, 5' - dithiobisnitro benzoic acid (DTNB) in 100 ml of 0.1% sodium nitrate). A molar extinction coefficient of  $6.22 \times 10^3$   $\mu\text{mol}$  was used to determine the activity of GSH-Px. The enzyme activity was expressed as international units of enzymatic activity/g of protein. International units are expressed as  $\mu\text{moles}$  of hydroperoxides transformed/min/ml of enzyme.

**Determination of glutathione reductase**

GR was determined by the method of Ellman1959<sup>34</sup>. 1.0 ml of supernatant was treated with 0.5 ml of Ellmans reagent (19.8 mg of 5, 5' - dithiobisnitro benzoic acid (DTNB) in 100 ml of 0.1% sodium nitrate) and 3.0 ml of phosphate buffer (0.2 M, pH 8.0). The absorbance was read at 412 nm. Glutathione reductase activity was expressed as  $\mu\text{moles}/\text{min}/\text{mg}$  tissue.

**Estimation of protein**

The protein content in the gastric tissue was estimated by the method of Lowry et al., 1951<sup>35</sup>. The tissue sample and the standards (1.0 mg/ml Bovine serum albumin in double distilled water) in different tubes were treated with 5.0 ml of reagent mixture (48% sodium potassium tartarate, 2% copper sulphate and 3% sodium carbonate in 0.1 N sodium hydroxide; added in a ratio of 1:1:48 by volume). Then Folin phenol reagent (1:2) was added to the reaction mixture and allowed to stand for 30 min at room temperature. The optical density was read at 710 nm using water as reagent blank.

**Histopathological evaluation**

Stomach was excised and rinsed with ice cold solution 0.9% sodium chloride to remove blood, debris of adhering tissues and assessed the ulcer score. The central part of the damaged or ulcerated tissue was cut into half along the long diameter. The tissues were then fixed immediately in 10% neutral buffered formaldehyde for 24 hours. The fixative was removed by washing through running tap water overnight, after dehydration through a graded series of alcohols; the tissues were cleaned in methyl benzoate, embedded in paraffin wax. Sections were cut into  $5\mu\text{M}$  thickness and stained with hematoxylin and eosin. After dehydration and cleaning, the sections were mounted and observed under light microscope for details.

**Chemicals**

All reagents used for determinations of TBARS, GSH-Px, GR, SOD, CAT and myeloperoxidase enzyme activities were purchased from Sigma–Aldrich, India, Bangalore, Omeprazole (commercially obtained from Dr. Reddy’s Laboratory Pvt. Ltd., Chennai). Other reagents of analytical grade were obtained from normal commercial sources.

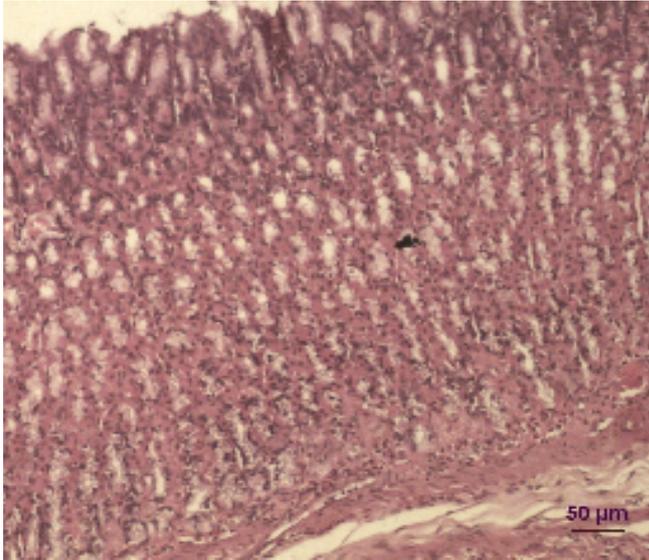
**Statistical Analysis**

Data of enzymatic activity and ulceration score were subjected to the statistical evaluation done by analysis of variance (ANOVA) coupled by Dunnett multiple ranges test with the presence of negative (healthy group) and positive (HAEPD and Omeprazole) controls by using SPSS 11.0 software. Differences among the groups were attained using the LSD option and significance was declared at  $P < 0.05$  and  $P < 0.01$ . The ethanol group was compared to the control (healthy) group. The treated groups were compared to the ethanol group.

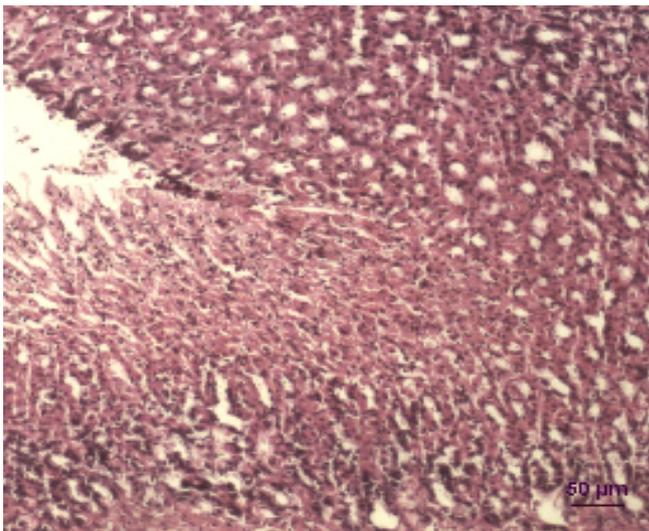
**Results and Discussion**

The oxygen derived free radicals play a key role in tissue damage during pathogenesis of various disorders of the digestive tract caused by physical, chemical and psychological factors that lead to gastric ulceration in human and experimental animals<sup>36</sup>. Oral administration of ethanol in rats causes severe gastric mucosal damage by disruption its barrier and provokes rapid, strong microvascular events in mucosal capillaries<sup>37, 38</sup>. The deleterious effect of ethanol on gastric mucosa is consequences of enhanced lipid peroxidation, decreased GSH levels and excessive generation of free radicals such as hydroxyl ethyl radical, superoxide radical, hydroxyl radical, peroxy radical and hydrogen peroxide<sup>39</sup>. Salim<sup>40</sup> and Brzozowski et al<sup>41</sup> have demonstrated that ethanol induces mucosal damage and impairs healing of lesions.

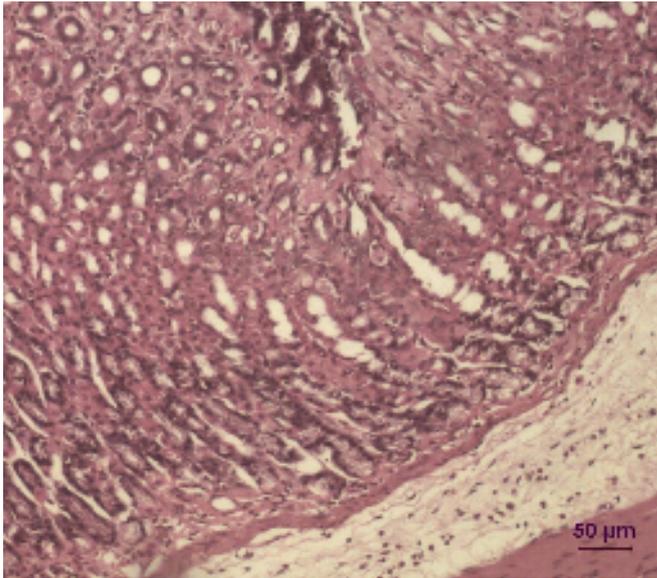
In the microscopic observation, normal arrangement of gastric cells was found in control rats (**Figure 1**). Ethanol induced rats showed an ulcer crater indicating gastric lesion with damaged mucosal epithelium and acute inflammation in the stomach (**Figure 2**). In comparison, maintenance of muscularis mucosa and a reduced size of ulcer crater were observed in HAEPD pretreated rats (**Figure 3**) and Omeprazole pretreated rats (**Figure 4**). The histological studies of HAEPD and Omeprazole alone treated rats showed normal arrangement of gastric mucosa as that of control rats.



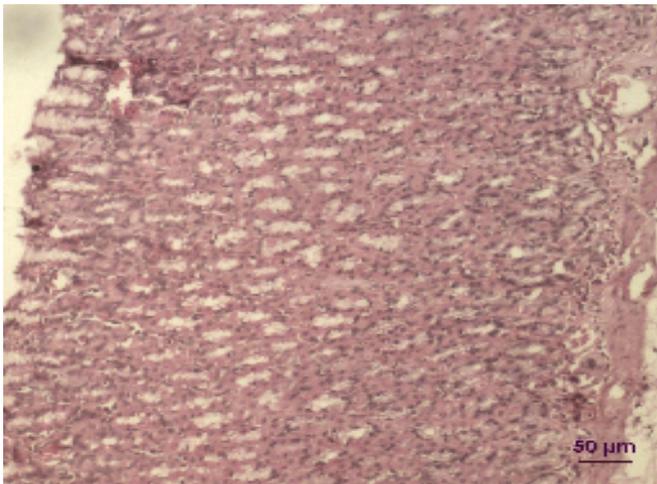
**Figure 1.** Histological examination of stomach of control rats showed normal arrangement of mucosal layer, gastric cells and no hemorrhage. HE 200x



**Figure 2.** Histological examination of stomach of ethanol induced ulcer rats (Group II) showed the degeneration, hemorrhage and oedematous appearance of the gastric mucosal tissue. HE 200x



**Figure 3.** The Group IV rats pretreated with HAEPD showed mild mononuclear cell infiltration in the lamina propria but significantly inhibited the gastric lesions formation and sub-mucosal oedema compared to Group II animals induced by ethanol. After pretreatment with HAEPD, ethanol showed a significant mucosal thickness as compared to ethanol treated group. HE 200x



**Figure 4.** The Group VI rats pretreated with Omeprazole showed normal arrangement of mucosal layer and gastric cells as that of control rats. This group significantly inhibited the gastric lesions formation and sub-mucosal oedema compared to Group II animals induced by ethanol. After pretreatment with Omeprazole, ethanol showed a significant mucosal thickness as compared to ethanol treated group. HE 200x

In order to explore the effects of antioxidant defenses on the ulceration process in all gastric tissues, the antioxidant levels SOD, CAT, GR, GSH-Px and myeloperoxidase activity were evaluated. Many reports have demonstrated that most of the gastric mucosal injury can be reduced by pretreatment with scavengers of reactive oxygen species<sup>42</sup>. In the present study HAEPD inhibited the increase in area of gastric mucosal lesions when compared with ethanol induced ulceration in rats (**Table 1**). These protective effects were observed at oral doses of 200mg/kg b wt (Group IV) and recovery of 68% was observed in 30 days. Thus the results shows that HAEPD significantly protective against gastric damage caused by ethanol. The gastroprotetive effect of 200mg/kg b wt of HAEPD was stronger than that of Omeprazole which is an H<sub>2</sub> receptor blocker.

**Table1.** Effects of Omeprazole and a single dose of hydroalcoholic fruit extract of *Pithecellobium dulce* (HAEPD) on healing of gastric ulcers produced by absolute ethanol in rats.

Experimental groups	Ulcer index(UI)	Lesion area (mm <sup>2</sup> )	% Inhibition
Control (Healthy)	-	-	-
Ethanol	0.53±0.007	2.25±0.029	34%
HAEPD	-	-	-
Pretreated HAEPD + Ethanol	0.164±0.001	0.516±0.006*	68%
Omeprazole	-	-	-
Pretreated Omeprazole + Ethanol	0.331±0.007	0.431± 0.009*	58.51%

Results are mean ± SEM (n=6) of three measurements. The ethanol group was compared to the control (healthy) group. Treated group was compared to the ethanol group. \* Significant at p<0.05.

Enzymatic and non enzymatic defense mechanism plays an important role against the toxicity and tissue damage of ROS<sup>32</sup>. The enzymatic and non-enzymatic antioxidant defenses include superoxide dismutase, glutathione peroxidase, catalase, glutathione reductase,  $\beta$  - tocopherol, vitamin C,  $\beta$  - carotene and vitamin A<sup>43</sup>. These antioxidants also play an important role in the prevention of gastric damage.

Oxidative injury induced by ethanol can be monitored in experimental animals by detecting lipid peroxidative product (TBARS). Ethanol administration results in excessive generation of free radicals such as hydroxyl ethyl radical, superoxide radical (O<sub>2</sub>-), hydroxyl radical (OH), peroxy radical and hydrogenperoxide<sup>44</sup>. All these radicals formed from the ethanol-mediated process have a great potential to react rapidly with lipids and turns into LPO<sup>45</sup>. LPO can result in membrane disorganization and subsequently decreases the membrane fluidity<sup>46</sup>. The level of lipid peroxide was reduced (**Table 2**) in the gastric tissue on oral administration of ethanol with the extract at a dose of 200mg/kg b wt for 30 days to the ulcerated group of rats compared to the ethanol induced ulcerated control.

**Table2.** Thiobarbituric acid reactive substances (TBARS), GR (Glutathione reductase) and glutathione peroxidase (GSH-Px) in gastric mucosal damage induced by ethanol, pretreated with hydroalcoholic fruit extract of *Pithecellobium dulce* (HAEPD) and Omeprazole.

Experimental groups	TBARS (nmol/g of protein)	GSH-Px (UI/g of protein)	GR ( $\mu$ mol/min/mg tissue)
Control (Healthy)	0.063 $\pm$ 0.02	454.15 $\pm$ 1.39	27.96 $\pm$ 0.62
Ethanol	0.571 $\pm$ 0.007	185.77 $\pm$ 0.95**	47.65 $\pm$ 0.54**
HAEPD	0.057 $\pm$ 0.0003	434.18 $\pm$ 0.89*	32.72 $\pm$ 0.884**
Pretreated HAEPD + Ethanol	0.076 $\pm$ 0.0004	395.9 $\pm$ 0.6369*	26.42 $\pm$ 0.54**
Omeprazole	0.063 $\pm$ 0.0004	427.73 $\pm$ 1.86*	27.95 $\pm$ 0.35**
Pretreated Omeprazole + Ethanol	0.095 $\pm$ 0.001	374.41 $\pm$ 0.65*	31.97 $\pm$ 0.68**

Results are mean  $\pm$  SEM (n=6) of three measurements. The ethanol group was compared to the control (healthy) group. Treated group was compared to the ethanol group. \* Significant at p<0.05; \*\* Significant at p<0.01.

SOD is considered as the first line of defense against the deleterious effects of oxygen radicals in the cells and it scavenges ROS by catalyzing the dismutation of superoxide to  $H_2O_2$ <sup>47</sup>. There is evidence to indicate that ethanol significantly depresses SOD activities<sup>45</sup>. It has been reported that ethanol inhibited SOD and thus superoxide radicals could not convert to  $H_2O_2$  (**Table 3**). The inhibition of SOD activity may result in an increased flux of superoxide in cellular compartments which may be the reason for the increased lipid peroxidative indices in our study.

Catalase acts as a protective antioxidant against the deleterious effects of LPO. CAT a highly reactive enzyme that reacts with  $H_2O_2$  to form water and molecular oxygen. It can also form methanol, ethanol, formic acid and phenols by donating hydrogen. In the present study we established that the plant extract and Omeprazole decreased CAT activity (**Table 3**), which had been increased by ethanol. Co administration of HAEPD with ethanol significantly modulates the antioxidant status in tissues, suggesting the enhancing effect of HAEPD on cellular antioxidant defenses. The antioxidant mechanism of HAEPD may include the scavenging of  $O_2^-$ ,  $OH^-$ , peroxynitrite and peroxy radical<sup>48</sup> and decreasing the activities of cytochrome 450, particularly CYP2E1<sup>49</sup>. *In vitro* studies have shown that HAEPD effectively scavenges the free radicals compared to AEPD (aqueous fruit extract of *Pithecellobium dulce*) and the respective standards<sup>25</sup>.

GSH is a powerful nucleophilic antioxidant plays pleiotropic roles, including maintaining cells in a reduced state, serving as an electron donor for certain antioxidative enzymes (glutathione peroxidase) and critical for cellular protection such as detoxification of ROS, conjugation and excretion of toxic molecules and control of inflammatory cytokines<sup>50</sup>. Depletion of GR in tissues leads to impairment of the cellular defenses against ROS and may result in peroxidative injury. Thus in our findings the levels of GR were significantly decreased when compared to ethanol treated rats (Table 2) and our findings are consistent with other published reports which showed GR concentration is decreased during ethanol ingestion<sup>51,52</sup>. GSH-Px is an important enzyme which plays a key role in the elimination of  $H_2O_2$  and lipid hydroperoxides in gastric mucosal cells and is also essential for maintaining a constant ratio of reduced glutathione to oxidized glutathione in the cell<sup>12</sup>. In contrast with SOD and GSH-Px activities, which were significantly increased in rats treated with HAEPD (Table 2 and 3), CAT activity was not significantly modified by treatment with ethanol or HAEPD (Table 3). This finding seems to be due to the fact that GSH-Px plays a much greater role than CAT in the removal of low steady state concentration of  $H_2O_2$ . Therefore, it is known that GSH-Px is the main antioxidant enzyme to remove  $H_2O_2$  and CAT shows a lower affinity for ROS. In this context, our result is in concordance with that reported by Billi et al<sup>53</sup> and Kanter et al<sup>54</sup>.

**Table 3.** Effect of single dose of hydroalcoholic fruit extract of *Pithecellobium dulce* (HAEPD) and Omeprazole on levels of Superoxide dismutase (SOD), Catalase (CAT) enzymes in rats of ethanol-induced gastric tissues.

Experimental groups	SOD (mmol/min/mg protein)	CAT (mmol/min/mg tissue)
Control (Healthy)	125.17±0.77	81.47±0.28
Ethanol	92.07±0.97**	127.23±0.52**
HAEPD	123.53±0.90**	75.70±0.89**
Pretreated HAEPD + Ethanol	131.07±0.33**	78.41±0.43**
Omeprazole	121.57±0.82**	73.55±0.22**
Pretreated Omeprazole Ethanol	134.43±0.82**	92.19±1.15*

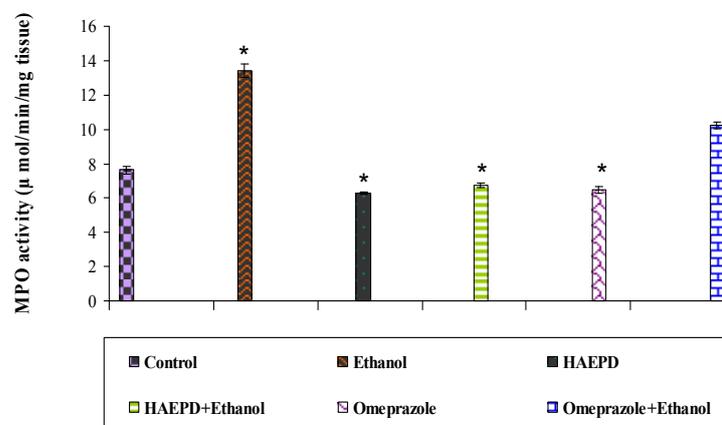
Results are mean ± SEM (n=6) of three measurements. The ethanol group was compared to the control (healthy) group. Treated group was compared to the ethanol group.\* Significant at p<0.05; \*\* Significant at p<0.01.

However, the results revealed an increase in GSH-Px activity due to the pretreatment with the plant extract and this enhancement indicates that the antiulcerogenic effect of HAEPD may appear through glutathione metabolism.

Tissue myeloperoxidase activity is a sensitive and specific marker for acute inflammation due to neutrophil infiltration in various gastric injuries<sup>55,56,57,58,59,60</sup>. As shown in **Figure 5**, the MPO activity in ethanol-administrated rat stomach tissues increases in comparison with that occurring in the tissues of healthy rats (P<0.05). The increase in enzyme activity level may be associated with increase in the levels of neutrophil infiltration and H<sub>2</sub>O<sub>2</sub> in the gastric damaged tissues administered with ethanol. In our study the pretreated HAEPD (Group IV) and pretreated Omeprazole

(Group VI) showed a tendency to counteract the increase in MPO concentration caused by ethanol. However *in vitro* study of HAEPD significantly decreased MPO activity and may be related to its gastro-protective ability.

**Figure 5.** Effects of hydroalcoholic fruit extract of *Pithecellobium dulce* (HAEPD) and Omeprazole on changes in the activity of myeloperoxidase (MPO) in ethanol-induced gastric tissues of rats.



Results are mean  $\pm$  SEM of six measurements. The ethanol group was compared to the healthy group. HAEPD and Omeprazole treated groups were compared to the ethanol group. \*Significant at  $p < 0.05$ .

### **Conclusion**

Many researches have proved that antioxidants may play an important role not only by protecting against gastric mucosal injury, but also by inhibiting progression of gastric ulcer. In conclusion, this experiment showed that ethanol successfully induced ulcers in rat stomachs while the experimental drugs (HAEPD and Omeprazole) reduced them. The levels of the antioxidant enzymes (SOD, Catalase, GR and GSH-Px) and MPO were adversely affected by ulcer induction. However HAEPD and Omeprazole alleviated the adverse effects on these enzymes by their gastro-protective effect mediated by endogenous scavenger of ROS. Our results suggest that gastro-protective effect HAEPD may be related to its positive effects on the antioxidant system and MPO activity of rats affected by ethanol- induced gastric ulcers. These results provide an additional support for the popular use of this plant as an antiulcer remedy in the Indian traditional medicine. So we suggest that natural antioxidant and gastro-protective agents in *Pithecellobium dulce* may be effective as plant gastro-protector and thus may have some obvious therapeutic implications. A further detailed study on various other parameters of mucosal defensive factors could elucidate their exact mechanism of actions and their usefulness in the treatment of gastric ulcer.

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