International Journal of Biological Chemistry

ISSN 1819-155X

Academic Journals Inc.

www.academicjournals.com

International Journal of Biological Chemistry 4 (2): 79-89, 2010
ISSN 1819-155X

Supplementation of Betaine Attenuates HCl-Ethanol Induced Gastric Ulcer in Rats

[1,2]B. Ganesan, [2]R. Yathavamoorthi, [3]K.H. Sabeena Farvin and [2]R. Anandan
[1]Vinayaka Missions University, Ariyanoor, Salem-636308, Tamil Nadu, India
[2]Biochemistry and Nutrition Division, Central Institute of Fisheries Technology, Matsyapuri (PO), Cochin-682029, India
[3]Section of Aquatic Lipids and Oxidation, National Institute of Food (DTU-Food), Technical University of Denmark, B. 221, Søltofts Plads, DTU, DK-2800 Kgs., Lyngby, Denmark

Abstract: The anti-ulcer effect of betaine was evaluated in rat model of HCl-ethanol induced gastric ulcer. Oral administration of HCl-ethanol caused a significant increase in the number of lesions in the gastric mucosa, volume of gastric juice, acidity and decreased activity of the pepsin. An increased level of lipid peroxidation and diminished tissue antioxidant system was also observed. Pre oral treatment with betaine (250 mg/kg/day for 21 days) counteracted all these changes induced by HCl-ethanol and maintained the rats at near normalcy. The gastro protective potential was further confirmed by histopathological studies of the gastric mucosa. The anti-ulcerogenic activity of betaine might be ascribable to its ability to neutralize the hydrochloride secreted into the stomach and/or to its free radical scavenging activity which protects the gastric mucosa against oxidative damage by decreasing lipid peroxidation and strengthening the mucosal barrier.

Key words: Betaine, ulcer, acidity, pepsin, lipid peroxidation, antioxidant

INTRODUCTION

Gastric ulcer is one of the most common diseases affecting the Gastrointestinal (GI) tract. It is a multifaceted disease with a complex pluricausal etiology that is not fully understood. The two major etiological factors for gastric ulcer are *Helicobacter pylori* infection and Nonsteroidal Anti-Inflammatory Drug (NSAID) consumption (Huang *et al*., 2002). Currently, 70% of all gastric ulcers occurring can be attributed to *H. pylori* infection. *Helicobacter pylori* weaken the protective mucous coating of the stomach and duodenum, which allows acid to penetrate to the sensitive lining beneath (Bobrzynski *et al*., 2005). There is a balance in the stomach between the aggressive digestive capabilities of acid/pepsin and the mucosal defense/mucosal turnover. Ulceration occurs when there is a disturbance of the normal equilibrium caused by either enhanced aggression or diminished mucosal resistance (Anandan *et al*., 1999). Increased acid-pepsin secretion, impaired bicarbonate neutralization and mucus secretion, enhanced contractility of gastric wall and reduced gastric mucosal blood flow represent some of the established pathogenic factors of gastric ulceration

Corresponding Author: K.H. Sabeena Farvin, Section for Aquatic Lipids and Oxidation, National Institute of Food (DTU-Food), Technical University of Denmark, B. 221, Søltofts Plads, DK-2800 Kgs, Lyngby, Denmark
Tel: + 45 45 25 25 42 Fax: + 45 45 88 47 74

(Allen and Flemstrom, 2005). Therefore, the drug treatment of peptic ulcers is targeted at either counteracting aggressive factors (acid plus pepsin, active oxidants, PAF, leukotrienes, endothelins, bile or exogenous factors including NSAIDs) or stimulating the mucosal defences (mucus, bicarbonate, normal blood flow, prostaglandins, nitric oxide) (Tepperman and Jacobson, 1994).

The modern approach to control gastric ulceration is to inhibit gastric acid secretion, to promote gastro protection, to block apoptosis and to stimulate epithelial cell proliferation for effective healing (Bandhopadhyay *et al*., 2002). Most of the antisecretory drugs such as proton pump inhibitors (omeprazole, lansoprazole, etc.) and histamine H2-receptor blocker (ranitidine, famotidine, etc.) are extensively used to control increased acid secretion and acid related disorders caused by stress, NSAID's and *H. pylori*, but there are reports of adverse effects and relapse in the long run (Martelli *et al*., 1998; Wolfe and Sachs, 2000). The search for natural sources has led to the identification of several potential drugs of herbal origin that are becoming part of the integrative health care systems of industrialized nations. Most of these natural compounds reduces the offensive factors and proved to be safe, clinically effective, better patient tolerance and relatively less expensive (Goel and Sairam, 2002).

Betaine, also known as trimethylglycine or glycine betaine, is a quaternary amine, closely related to the amino acid, glycine. It is found in microorganisms, plants and animals and is a significant component of many foods, including wheat, shellfish, spinach and sugar beets (Zeisel *et al*., 2003). Betaine was reported to be non-perturbing to cellular metabolism, highly compatible with enzyme functions and stabilizes cellular metabolic function under different kinds of stress in various organisms and animal tissues (Lammers *et al*., 2005). It has been shown to acts as an organic osmolyte in a large variety of life forms, including microbes, plants and marine invertebrates (Wright *et al*., 1992). Studies have shown that betaine protects against hyperhomocysteinemia and myocardial infarction in experimental animals (Schwahn *et al*., 2003; Ganesan *et al*., 2007, 2008, 2009). Experimental evidences have shown that betaine protects chick intestinal cells from coccidia infection, alleviates symptoms and improves performance (Fetterer *et al*., 2003). It has also shown to decrease endoplasmic reticulum stress and liver injury in alcohol-fed mice (Ji and Kaplowitz, 2003). Though, the beneficial properties of betaine are promising, its anti ulcerogenic effect is yet to be an unexplored area.

The purpose of the present study was to assess the protective effect of betaine on HCl-ethanol induced gastric ulcer condition with respect to changes in the volume of gastric juice, acid out put, peptic activity, number of lesions in the gastric mucosa, lipid peroxidation, tissue antioxidant status and histopathology in Wistar rats.

MATERIALS AND METHODS

Chemicals

Epinephrine, betaine, 1, 1, 3, 3-tetra ethoxypropane malondialdehyde bis (diethyl acetal), reduced glutathione (GSH), 5, 5'-dithiobis (2-nitrobenzoic acid) (DTNB), N-phenyl-*p*-phenylenediamine and 1-chloro-2, 4,dinitrobenzene (CDNB) were purchased from M/s. Sigma Chemical Company, St. Louis. MO, USA. All the other chemicals used were of analytical grade.

Animals

Wistar strain male albino rats, weighing 150-180 g, were selected for the study. The animals were housed individually in polyurethane cages under hygienic and standard

Table 1: Composition of the diet

Ingredients	Composition (g/100 g diet)
Carbohydrate (Nitrogen free)	56.2
Crude protein	22.0
Ash	7.5
Crude oil	4.2
Crude fibre	3.0
Glucose	2.5
Vitamins	1.8
Sand silica	1.4
Calcium	0.8
Phosphorus	0.6

environmental conditions (28±2°C, humidity 60-70%, 12 h light/dark cycle). The animals were allowed a standard diet [Sai Feeds, Bangalore, India] and water *ad libitum*. The diet provided metabolizable energy of 3600 kcal (Table 1). The experiment was carried out as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India and approved by the Institutional Animal Ethics Committee (IAEC).

Experimental Protocol

The experimental animals were divided into 4 groups of six rats each. Rats in group I (normal control) received only the standard diet. In group II, normal rats were treated with betaine (250 mg/kg/day, p.o.) for 21 days. In group III, ulcer was induced by oral administration of 1.5 mL of 0.15 M HCl in 70% v/v ethanol (Hara and Okabe, 1985) after 21 days of feeding with standard diet. Group IV was pretreated with betaine (250 mg/kg/day, p.o., for 21 days) before the induction of ulcer as described for group III.

At the end of the experiment, all 4 groups underwent surgery according to the method of Takeuchi *et al.* (1976) and gastric juice was collected for 4 h. The rats were then sacrificed by using chloroform and the stomach was removed after the esophagus had been clamped. The gastric juice collected was centrifuged and the volume was noted. The stomach was inflated with normal saline and then examined for the number of lesions. Number of lesions was recognized and scored by gross inspection. The investigator evaluating mucosal lesions was blinded to the treatment. The total acidity was determined by titration with 0.02N NaOH with phenolphthalein as indicator.

The mucosal tissue scraped from the stomach was used for the determination of peptic activity (Anson, 1938), lipid peroxides, reduced glutathione, glutathione peroxidase, glutathione-s-tranferase, catalase and superoxide dismutase.

Biochemical Assays

Lipid Peroxidation (LPO) was assayed by the method of Ohkawa *et al.* (1979) in which the malonaldehyde (MDA) released served as the index of LPO. 1, 1, 3, 3-tetra ethoxypropane malondialdehyde bis (diethyl acetal) was used as standard. The level of lipid peroxides was expressed as nmoles of MDA formed mg^{-1} protein.

Reduced Glutathione (GSH) was determined by the method of Ellman and Archs (1959). The method is based on the reaction of reduced glutathione with 5, 5-dithiobis (2-nitrobenzoic acid) to give a yellow-colored compound that has absorbance at 412 nm. The amount of glutathione was expressed as μmol g^{-1} wet tissue.

Glutathione Peroxidase (GPx) [EC 1.11.1.9] activity was measured by the method of Paglia and Valentine (1967). The enzyme activity was expressed as nmoles of GSH oxidized/min/mg protein. Glutathione-*S*-Transferase (GST) (EC 2.5.1.18) activity was determined by the method

of Habig *et al.* (1974). GST activity was expressed as μmol 1-chloro-2, 4,dinitrobenzene (CDNB) conjugate formed/min/mg protein.

Catalase (CAT) [EC 1.11.1.6] activity was assayed according to the method of Takahara *et al.* (1960). The enzyme activity was expressed as nmol of H_2O_2 decomposed/min/mg protein. Superoxide Dismutase (SOD) (EC.1.15.1.1) activity was determined according to the method of Misra and Fridovich (1972) based on the oxidation of epinephrine-adrenochrome transition by the enzyme. One unit of SOD activity is calculated as the amount of protein required to give 50% inhibition of epinephrine autoxidation.

Histological Studies

The gastric mucosal tissues from control and experimental groups were fixed in 10% buffered formalin (100 mL 37-40% formaldehyde, 4 g sodium phosphate monobasic and 6.5 g of sodium phosphate dibasic in 900 mL of distilled water; pH 7.0) for 24 h (Raghuramulu *et al.*, 1983). The fixative was removed by washing through running tap water overnight. After dehydration through ascending grades of alcohols, the tissues were cleaned in methyl benzoate, embedded in paraffin wax. Sections were cut into 3-5 μ thickness and stained with haematoxylin and eosin. After dehydration and cleaning, the sections were mounted and observed under light microscope. The sections obtained were checked and confirmed by a pathologist.

Statistical Analysis

Results are expressed as Mean±SD. Multiple comparisons of the significant ANOVA were performed by Duncan's multiple comparison test. A p-value <0.05 was considered as statistically significant. All data were analyzed with the aid of statistical package program SPSS 10.0 for Windows (Kirkpatrick and Feeney, 2003).

RESULTS

No significance difference was observed in the level of body weight, total feed consumption and feed efficiency ratio in control and experimental groups of rats (Table 2). The group III rats were apparently dull in food intake and fluid consumption after the oral administration of 1.5 mL of 0.15 M HCl in 70% v/v ethanol. However, the behavior of the animals orally administered with betaine was comparable to that of normal controls.

Significant ($p<0.05$) increase in the number of lesions was observed in the gastric mucosa of group III ulcer-induced rats as compared to group I control animals (Table 3). This was paralleled by a significant ($p<0.05$) rise in the volume of gastric juice, acid out put and a significant ($p<0.05$) reduction in peptic activity. Oral pretreatment with betaine (250 mg/kg/day, p.o., for 21 days) significantly ($p<0.05$) reduced the number of HCl/ethanol-

Table 2: Levels of body weight, accumulated weight gain, total feed consumption and feed efficiency ratio in control and experimental groups of rats

Growth parameters	Control (group I)	Betaine (A) (group II)	Ulcer (B) (group III)	Betaine+Ulcer (A+B) (group IV)
Initial body weight (g)	157.00±11.3[a]	160.00±11.5[a]	163.00±11.7[a]	159.00±11.4[a]
Accumulated weight gain (g)	250.00±18.0[a]	252.00±18.1[a]	257.00±18.5[a]	254.00±18.2[a]
Total feed consumption	345.00±24.8[a]	350.00±25.2[a]	355.00±25.5[a]	352.00±25.3[a]
Feed efficiency (g gain/g feed)	0.72±0.05[a]	0.72±0.05[a]	0.72±0.05[a]	0.720±0.05[a]

A: Betaine, 250 mg/kg b.wt./day, p.o., for 21 days; B: Ulcer was induced by oral administration of 1.5 mL of 0.15 M HCl in 70% v/v ethanol. Results are Mean±SD for 6 animals. Values that have superscript letter (a) is not significantly ($p<0.05$) differ with each other

Table 3: Number of lesions, volume of gastric juice, acid output and pepsin activity of the gastric mucosa of control and experimental groups of rats

Parameters	Control (group I)	Betaine (A) (group II)	Ulcer (B) (group III)	Betaine+Ulcer (A+B) (group IV)
Number of lesions	-	-	10.55±0.76^{a}	1.50±0.11^{b}
Volume of gastric juice	1.35±0.10^{a,c}	1.20±0.08^{a}	3.26±0.24^{b}	1.45±0.10^{c}
Acid output	146.00±10.65^{a}	126.00±9.19^{b}	300.00±21.90^{c}	160.00±11.68^{a}
Pepsin	696.00±50.80^{a}	676.00±49.34^{a}	520.00±37.96^{b}	660.00±48.18^{a}

A: Betaine, 250 mg/kg b.wt./day, p.o., for 21 days; B: Ulcer was induced by oral administration of 1.5 mL of 0.15 M HCl in 70% v/v ethanol. Values expressed : Volume of gastric juice, mL/4 h; Acid out put, µEq/4 h; pepsin, µmol tyrosine liberated/4 h. Results are Mean±SD of six animals. Values that have a different superscript letter (a, b, c) differ significantly ($p<0.05$) with each other

Table 4: Levels of Lipid Peroxides (LPO) and reduced Glutathione (GSH) and activities of Glutathione Peroxidase (GPx), Glutathione-*S*-Transferase (GST), Catalase (CAT), and Superoxide Dismutase (SOD) in the mucosal tissue of control and experimental groups of rats

Parameters	Control (group I)	Betaine (A) (group II)	Ulcer (B) (group III)	Betaine+Ulcer (A+B) (group IV)
LPO	1.08±0.07^{a}	0.92±0.06^{b}	2.50±0.18^{c}	1.15±0.08^{a}
GSH	4.95±0.36^{a}	4.85±0.35^{a}	2.05±0.14^{b}	4.75±0.34^{a}
GPx	180.00±13.14^{a}	198.00±14.45^{b}	105.00±7.66^{c}	171.00±12.48^{a}
GST	5.26±0.38^{a}	5.02±0.36^{a}	3.08±0.22^{b}	4.90±0.35^{a}
CAT	4.15±0.30^{a}	4.00±0.29^{a}	1.50±0.10^{b}	3.90±0.28^{a}
SOD	5.92±0.43^{a}	6.16±0.45^{a}	2.50±0.18^{b}	5.50±0.40^{c}

A: Betaine, 250 mg/kg b.wt./day, p.o., for 21 days; B: Ulcer was induced by oral administration of 1.5 mL of 0.15 M HCl in 70% v/v ethanol. Values expressed: LPO, nmol malondialdehyde released/mg protein; GSH, nmol g^{-1} wet tissue; GPx, nmol GSH oxidized min^{-1} mg^{-1} protein; GST, µmol 1-chloro-2,4-dinitrobenzene conjugate formed min^{-1} mg^{-1} protein; CAT, nmol H_2O_2 decomposed min^{-1} mg^{-1} protein; SOD, one unit of the SOD activity is the amount of protein required to give 50% inhibition of epinephrine autoxidation. Results are mean±SD for six animals. Values that have a different superscript letter (a, b, c) differ significantly ($p<0.05$) with each other

induced lesions in the gastric mucosa and maintained the volume and acidity of the gastric juice at near normalcy in group IV rats compared to that of group III ulcer induced animals (Table 3). Also, it restored the peptic activity towards near normal level.

Administration of HCl/ethanol caused significant ($p<0.05$) elevation in the level of lipid peroxides in the gastric mucosa of group III ulcer-induced rats as compared to that of group I normal control animals (Table 4). This was paralleled by the significant reduction in the level of GSH and the activities of GPx, GST, CAT and SOD. Prior oral administration of betaine significantly ($p<0.05$) prevented all these alterations and maintained the rats at near normal status. The normal rats receiving betaine alone did not show any significant change when compared with the normal rats, showing that it doesn't have any adverse effect per se.

The examination of gastric mucosal tissue showed sharply defined ulcer crater at the site of exposure to HCl-ethanol almost reaching the submucosal layer and deep alteration of glandular epithelium in group III rats compared to group I normal rats (Fig. 1a-d). Damaged mucosal epithelium, leukocyte infiltration and ulcerated area covered with inflammatory exudates were also observed in group III HCl-ethanol induced rats (Fig. 1c). Prior oral administration of betaine showed absence of ulcer crater, clearance of necrosis and maintenance of mucosal layers along with normal glands even after the exposure of HCl-ethanol in group IV rats (Fig. 1d).

In histopathological investigations, cell necrosis was clearly visible (Fig. 2c) in ulcerated group III rats compared to normal controls. Cell membranes were found broken. But, the histology of group IV, betaine treated animals (Fig. 2d) was similar to that of normal behavior of the cell (Fig. 2a).

DISCUSSION

Gastric ulcer refers to the group of ulcerative disorders of upper gastrointestinal tract involving principally the most proximal portion of the duodenum and the stomach, which

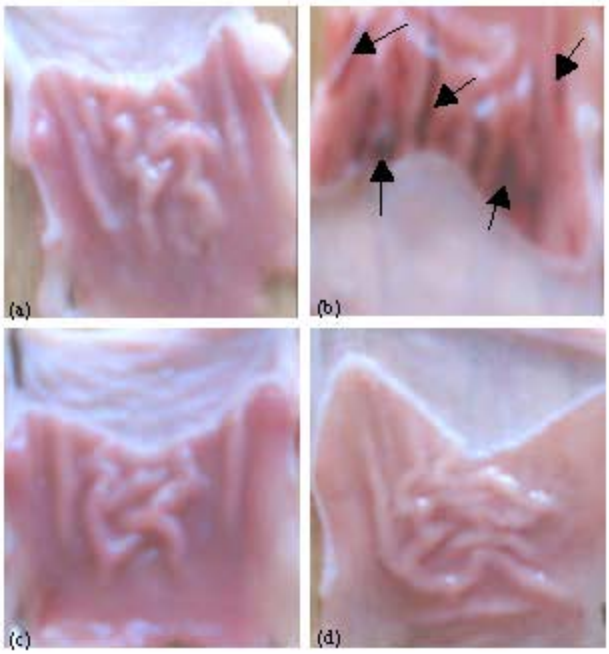

Fig. 1: Stomach of (a) normal control rats (group I) (b) Betaine treated rats (group II) (c) control rats + ulcer induced by HCl-Ethanol (group III) showing the gastric mucosal damage and (d) Betaine treated rats + ulcer induced by HCl-Ethanol (group IV)

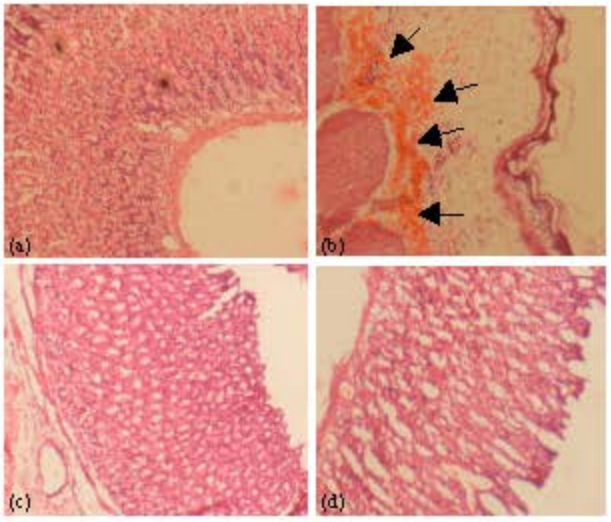

Fig. 2: Histopathology of the gastric mucosa of (a) normal control rats (group I) (b) Betaine treated rats (group II) (c) control rats + ulcer induced by HCl-Ethanol (group III) showing cell necrosis and (d) Betaine treated rats + ulcer induced by HCl-Ethanol (group IV)

have in common the participation of acid/pepsin (Borrelli and Izzo, 2000). In the present study, we have assessed the protective effects of betaine on gastric ulcer in HCl-ethanol induced gastric ulcer models. Experimental peptic ulcer may be assessed on the basis of the number of gastric mucosal lesions. The present study observed a significant ($p<0.05$) increase in the number of lesions in the gastric mucosa, acidity and volume of gastric juice, paralleled by a significant ($p<0.05$) decline in peptic activity in group III ulcer-induced rats as compared to that of group I control animals. These findings concur with an earlier reported study (Anandan *et al*., 1999). Increased production of hydrochloric acid in the ulcerated condition might be a consequence of increased permeability of the mucosa, which is important process in the development of ulcer (Bardhan *et al*., 1994). Reports by Bhatnagar *et al.* (2005) supported the findings that non-steroidal anti inflammatory drug induction is characterized by increased gastric acidity. In the present study, the prior oral administration of betaine (250 mg/kg/day, p.o., for 21 days) resulted in significant ($p<0.05$) reduction in the number of mucosal lesions, volume of gastric juice and acid output in group IV animals as compared to group III ulcer induced rats. Peptic activity was also maintained at near normal level. It probably did so by the neutralization of hydrochloric acid excessively secreted into the stomach, the neutralization of the acid secretion in the stomach has been reported to accelerate ulcer healing (Santhosh *et al*., 2007). Reports by Kettunen *et al.* (2001) have shown that the dietary betaine accumulates in the liver and intestinal tissue and stabilizes the intestinal epithelial structure in healthy and coccidia-infected broiler chicks by its membrane stabilizing properties.

Oxidative stress is known to play an important role in the pathogenesis of gastric mucosal injury. EtOH-induced gastric lesions are thought to arise as a result of direct damage of gastric mucosal cells, resulting in the development of free radicals and hyper oxidation of lipid (Jainu and Devi, 2006). The oxidation of polyunsaturated fatty acids in biological membranes may cause impairment of membrane function, decrease in membrane fluidity, inactivation of membrane receptors and enzymes, increase of non-specific permeability to ions and disruption of membrane structure (Hernandez-Munoz *et al*., 2000). In the present study, a significant rise ($p<0.05$) was noted in the level of lipid peroxides in the gastric mucosa of group III HCl-ethanol induced ulcer rats as compared to that group I normal control animals reflecting the oxidative deterioration of gastric mucosal membranes (Table 4). This is in corroboration with an earlier investigation (Koc *et al*., 2008), which suggested that the high vulnerability gastric mucosa to peroxidative damage is mainly due to a decline in the level of free radicals scavengers. Prior oral administration of betaine (250 mg/kg/day, p.o., for 21 days) resulted in significant ($p<0.05$) reduction in the level of lipid peroxidation in the gastric mucosa of group IV rats compared to group III ulcer induced rats (Table 4). It probably did so by counteracting the HCl-ethanol-generated free radicals by its antioxidant property. A previous report also reveals that betaine supplementation could protect the structural and functional integrity of the cell membrane by counteracting reactive oxygen species-mediated lipid peroxidation and protein carbonyl function (Balkan *et al*., 2004). Betaine is highly lipotropic and, when administered exogenously, it can readily pass across the membrane lipid bilayer. This ability of betaine to diffuse into intracellular compartments aids the capabilities of this natural product as an antioxidant (Kanbak *et al*., 2001). Reports by Balkan *et al.* (2004) indicated that betaine supplementation was effective in prevention of lipopolysaccharide-induced necrotic damage in liver by inhibiting kupffer cell activation and behaving as an antioxidant.

Tissue antioxidant system plays a fundamental role in cellular defense against reactive free radicals and other oxidant species. Glutathione exerts its antioxidant function by reaction

with superoxide radicals, peroxy radicals and singlet oxygen followed by the formation of oxidized glutathione and other disulfides (Farvin *et al*., 2004). In the present study, significant ($p<0.05$) decline observed in the levels of GSH, GSH dependent antioxidant enzymes (GPx and GST) and antiperoxidative enzymes (SOD and CAT) in group III ulcer induced rats compared to control animals (Table 4) which is accordance with earlier reported studies (Anandan *et al*., 2003; Prabha *et al*., 2003; Raghavendran *et al*., 2004), indicated that the tissue antioxidant status was being operated at diminished level in HCl-ethanol induced ulceration. These enzymes are responsible for the destruction of peroxides and have a specific role in protecting the gastric mucosal tissue against oxidative damage (Ito *et al*., 1992). This reduction might have resulted from the oxidation of GSH to HCl-ethanol induced generation of free radicals which intern results in decreased activity of GSH dependent antioxidant enzymes. Reduction in the activities of the antiperoxidative enzymes might be due to the increased generation of reactive oxygen radicals, such as superoxide and hydrogen peroxide, which in turn leads to the inactivation of these enzyme activities. These depletion of antioxidant system further enhances the susceptibility of the gastric mucosal cells to oxygen metabolites and acid mediated cell damage. Hiraishi *et al*. (1994) have reported that exogenous GSH protects cultured gastric mucosal cells from oxidant-induced damage.

The animals fed with betaine (250 mg/kg/day, p.o., for 21 days) maintained the level of GSH and the activities of antioxidant and antiperoxidative enzymes to near normal in group IV rats indicating the antioxidant nature of betaine in HCl-ethanol induced oxidative stress condition. Betaine is involved in the synthesis of methionine, which serves as a major supplier of cellular cysteine via transsulfuration pathway for the synthesis of reduced glutathione (Kim *et al*., 1998). Our earlier experimental studies indicated that betaine could preserve cellular and subcellular membranes from free radical mediated oxidative damage by its antioxidant activity (Ganesan *et al*., 2009). The ability of betaine to maintain the mucosal antioxidant status at higher rate demonstrates its possible preventive efficacy in inhibiting free radical mediated ulcerogenesis. It may be possible that by blocking oxidative damage through lipid peroxidation, betaine prevents loss of membrane permeability and dysfunction of cellular proteins, leading to survival of the functionally active cells which was conformed by the histological evidence.

In conclusion, the present observations indicate that oral administration of betaine prevents HCl-ethanol induced gastric ulcer in rats. The overall anti-ulcerogenic activity of betaine is probably related to its ability to neutralize the hydrochloride secreted into the stomach and/or to its antioxidant nature by which it maintain the level of GSH and the activities of the mucosal antioxidant enzymes to near normal status. Thus, protecting the gastric mucosa against oxidative damage by decreasing lipid peroxidation and strengthening the mucosal barrier.

REFERENCES

Allen, A. and G. Flemstrom, 2005. Gastroduodenal mucus bicarbonate barrier: Protection against acid and pepsin. Am. J. Physiol. Cell Physiol., 288: 1-19.

Anandan, R., P.G.V. Nair and S. Mathew, 2003. Anti-ulcerogenic effect of chitin and chitosan on mucosal antioxidant defence system in HCl-ethanol induced ulcer in rats. J. Pharm. Pharmacol., 56: 265-269.

Anandan, R., R.D. Rekha, N. Saravanan and T. Devaki, 1999. Protective effects of picrorhiza kurroa against HCl/ethanol-induced ulcer. Fitoterapia, 70: 498-501.

Anson, M.L., 1938. The estimation of pepsin, trypsin, papain and cathepsin with hemoglobin. J. Gen. Physiol., 22: 79-89.

Balkan, J., S. Oztezcan, M. Kucuk, U. Cevikbas, N. Kocak-Toker and M. Uysal, 2004. The effect of betaine treatment on triglyceride levels and oxidative stress in the liver of ethanol-treated guinea pigs. Exp. Toxicol. Pathol., 55: 505-509.

Bandhopadhyay, U., K. Biswas, R. Chatterjee, D. Bandyopadhyay and I. Chattopadhyay *et al*., 2002. Gastroprotective effect of neem (*Azadiracta indica*) bark extract: Possible involvement of H+K+ATPase inhibition and scavenging of hydroxyl radical. Life Sci., 71: 2845-2865.

Bardhan, K.D., J. Ahlberg, W.S. Hislop, C. Lindholmer and R.G. Long *et al*., 1994. Rapid healing of gastric ulcers with lansoprazole. Aliment. Pharmacol. Ther., 8: 215-220.

Bhatnagar, M., S.S. Sisodia and R. Bhatnagar, 2005. Antiulcer and antioxidant activity of Asparagus racemosus WILLD and Withania somnifera DUNAL in rats. Ann. N. Y. Acad. Sci., 1056: 261-278.

Bobrzynski, A., P.C. Konturek, S.J. Konturek, M. Plonka, W. Bielanski and D. Karcz, 2005. *Helicobacter pylori* and nonsteroidal anti-inflammatory drugs in perforations and bleeding of peptic ulcers. Med. Sci. Monitor, 11: 132-135.

Borrelli, F. and A.A. Izzo, 2000. The plant kingdom as a source of anti-ulcer remedies. Phytother. Res., 14: 581-591.

Ellman, G.L. and Archs, 1959. Tissue sulfhydril groups. Biochem. Biophys., 82: 70-77.

Farvin, K.H.S., R. Anandan, S.H. Kumar, K.S. Shiny, T.V. Sankar and T.K. Thankappan, 2004. Effect of squalene on tissue defense system in isoproterenol-induced myocardial infarction in rats. Pharmacol. Res., 50: 231-236.

Fetterer, R.H., P.C. Augustine, P.C. Allen and R.C. Barfield, 2003. The effect of dietary betaine on intestinal and plasma levels of betaine in uninfected and coccidia-infected broiler chicks. Parasitol. Res., 90: 343-348.

Ganesan, B., R. Rajesh, R. Anandan and N. Dhandapani, 2007. Protective effect of betaine on changes in the levels of protein, glycoproteins and amino acids in isoprenaline-induced myocardial infarction in rats. Afr. J. Biochem. Res., 1: 117-123.

Ganesan, B., R. Anandan and R. Rajesh, 2008. Protective effect of betaine on changes in lipid profile, lipoproteins and fatty acid composition in experimentally induced myocardial infarction in wistar rats. Int. J. Biomed. Pharmaceutical Sci., 2: 65-69.

Ganesan, B., S. Buddhan, R. Anandan, R. Sivakumar and R. Anbinezhilan, 2009. Antioxidant defense of betaine against isoprenaline-induced myocardial infarction in rats. Mol. Biol. Rep. (In Press). 10.1007/s11033-009-9508-4

Goel, R.K. and K. Sairam, 2002. Antiulcer drugs from indigenous sources with emphasis on *Musa sapientum, Tamrabhasna, Asparagus racemosus* and *Zingiber officinale*. Ind. J. Pharmacol., 34: 100-110.

Habig, W.H., M.J. Pabst and W.B. Jakoby, 1974. Glutathione-S-transferases: The first enzymatic step in mercapturic acid formation. J. Biol. Chem., 249: 7130-7139.

Hara, N. and S. Okabe, 1985. Effects of gefarnate on acute gastric lesions in rats. Nippon Yakurigaku Zasshi., 85: 443-446.

Hernandez, R. Munoz, C. Montel Ruiz and Vazquez O. Martinez, 2000. Gastric mucosal cell proliferation in ethanol-induced chronic mucosal injury is related to oxidative stress and lipid peroxidation in rats. Lab. Invest., 80: 1161-1169.

Hiraishi, H., A. Terano, S. Ota, H. Mutoh and T. Sugimoto *et al*., 1994. Protection of cultured rat gastric cells against oxidant induced damage by exogenous glutathione. Gastroenterology, 106: 1199-1207.

Huang, J., S. Sridhar and R. Hunt, 2002. Role of *Helicobacter pylori* infection and non-steroidal anti-inflammatory drugs in peptic-ulcer disease: A meta-analysis. Lancet, 359: 14-22.

Ito, M., D. Shii, T. Segami, R. Kojima and Y. Suzuki, 1992. Preventive actions of N-(3-aminopropionyl)-L-histidinato zinc (Z-103) through increases in the activities of oxygen-derived free radical scavenging enzymes in the gastric mucosa on ethanol-induced gastric mucosal damage in rats. Jap. J. Pharmacol., 59: 267-274.

Jainu, M. and C.S. Devi, 2006. Antiulcerogenic and ulcer healing effects of *Solanum nigrum* (L.) on experimental ulcer models: Possible mechanism for the inhibition of acid formation. J. Ethnopharmacol., 104: 156-163.

Ji, C. and N. Kaplowitz, 2003. Betaine decreases hyperhomocysteinemia, endoplasmic reticulum stress and liver injury in alcohol-fed mice. Gastroenterology, 124: 1488-1499.

Kanbak, G., F. Akyuz and M. Inal, 2001. Preventive effect of betaine on ethanol-induced membrane lipid composition and membrane ATPases. Arch. Toxicol., 75: 59-61.

Kettunen, H., K. Tiihonena, S. Peuranena, M.T. Saarinena and J.C. Remusb, 2001. Dietary betaine accumulates in the liver and intestinal tissue and stabilizes the intestinal epithelial structure in healthy and coccidia-infected broiler chicks. Comp. Biochem. Physiol., 130: 759-769.

Kim, S.K., Y.C. Kim and Y.C. Kim, 1998. Effects of singly administered betaine on hepatotoxicity of chloroform in mice. Food Chem. Toxicol., 36: 655-661.

Kirkpatrick, L.A. and B.C. Feeney, 2003. A Simple Guide to SPSS for Windows for Versions 8.0, 9.0, 10.0 and 11.0. 4th Edn., Wadsworth Thomson Learning, Australia, ISBN-10: 0534580866, pp: 150.

Koc, M., H. Imik and F. Odabasoglu, 2008. Gastroprotective and anti-oxidative properties of ascorbic acid on indomethacin-induced gastric injuries in rats. Biological Trace Element Res., 126: 222-236.

Lammers, P.E., J.A. Beck, S. Chu and S.A. Kempson, 2005. Hypertonic upregulation of betaine transport in renal cells is blocked by a proteasome inhibitor. Cell Biochem. Function, 23: 315-324.

Martelli, A., F. Mattioli, E. Mereto, G. Brambilla-Campart, D. Sini, G. Bergamaschi and G. Brambilla, 1998. Evaluation of omeprazole genotoxicity in a battery of *in vitro* and *in vivo* assays. Toxicology, 30: 29-41.

Misra, H.P. and I. Fridovich, 1972. The role of superoxide anion in the auto-oxidation of epinephrine and a simple assay for superoxide dismutase. J. Biol. Chem., 247: 3170-3175.

Ohkawa, H., N. Onishi and K. Yagi, 1979. Assay for lipid peroxides in animal tissue by thiobabituric acid reaction. Anal. Biochem., 95: 351-358.

Paglia, D.E. and W.N. Valentine, 1967. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J. Lab. Clin. Methods, 2: 158-169.

Prabha, T., M.D. Babu, S. Priyambada, V.K. Agrawal and R.K. Goel, 2003. Evoluation of *Pongamia pinnata* root extract on gastric ulcers and mucosal offensive and defensive factors in rats. Ind. J. Exp. Biol., 41: 304-310.

Raghavendran, H.R., A. Sathivel and T. Devaki, 2004. Efficacy of brown seaweed hot water extract against HCl-ethanol induced gastric mucosal injury in rats. Arch. Pharm. Res., 27: 449-453.

Raghuramulu, N., K.N. Madhavan and S. Kalyansundhram, 1983. A Manual of Laboratory Techniques. NIN, ICMR, Silver Prints, Hyderabad, India.

Santhosh, S., R. Anandan, T.K. Sini and P.T. Mathew, 2007. Protective effect of glucosamine against ibuprofen-induced peptic ulcer in rats. J. Gastroenterol. Hepatol., 22: 949-953.

Schwahn, B.C., Z. Chen, M.D. Laryea, U. Wendel and S. Lussier-Cacan *et al*., 2003. Homocysteine-betaine interactions in a murine model of 5,10-methylenetetrahydrofolate reductase deficiency. FASEB J., 17: 512-514.

Takahara, S., B.H. Hamilton, J.V. Nell, T.Y. Ogubra and E.T. Nishimura, 1960. Hypocatalasemia: A new genetic carrier state. J. Clin. Invest., 39: 610-619.

Takeuchi, K., S. Okabe and K. Takagi, 1976. A new model of stress ulcer in the rat withpylorus ligation and its pathogenesis. Am. J. Dig. Dis., 21: 782-788.

Tepperman, B.L. and E.D. Jacobson, 1994. Circulatory Factors in Gastric Mucosal Defense and Repair. In: Physiology of the Gastrointestinal Tract, Johnson, L.R. (Ed.). Raven Press, New York, pp: 1331-1352.

Wolfe, M.M. and G. Sachs, 2000. Acid suppression: Optimizing therapy for gastroduodenal ulcer healing, gastrooesophageal disease and stress-related erosive syndrome. Gastroenterology, 118: S9-S31.

Wright, S.H., T.M. Wunz and A.L. Silva, 1992. Betaine transport in the gill of a marine mussel, *Mytilus californianus*. Am. J. Physiol., 263: R226-R232.

Zeisel, S.H., M.H. Mar, J.C. Howe and J.M. Holden, 2003. Concentrations of choline containing compounds and betaine in common foods. J. Nutr., 133: 1302-1307.