# PROTECTIVE EFFECT OF *ARECA CATECHU* EXTRACT ON ETHANOL INDUCED GASTRIC MUCOSAL LESIONS IN RATS

**M Senthil Amudhan, V Hazeena Begum**

Department of Siddha Medicine, Faculty of Science, Tamil University, Vakaiyur, Thanjavur - 613 010, Tamilnadu, India.

**Summary**

In the present study, the antiulcerogenic activity of defatted ethanol extract of *Areca catechu* was investigated. Ethanol treatment significantly increased the levels of gastric mucosal malondialdehyde (MDA, an index of lipid peroxidation), nitric oxide (NO) and increase in the activities of myeloperoxidase (MPx, an index neutrophil infiltration) and xanthine oxidase (XO) enzymes. Pretreatment with *A. catechu* in the doses of 250mg/kg and 500mg/kg body weight, prevented the formation of gastric mucosal MDA, NO contents and activities of MPx and XO. Ethanol induction also showed a significant reduction of the gastric mucosal glutathione (GSH), sialic acid and deoxyribo nucleic acid (DNA) levels. The pretreatment with *A. catechu* maintained the levels similar to normal control level. Based on these data, the protective effects of both 250mg/kg and 500mg/kg doses of *A. catechu* on ethanol induced gastric mucosal injury may be attributed to its antioxidant effect.

**Key words:** Antiulcer, *Areca catechu,* Polyphenol, Antioxidants

Corresponding Author : Dr V. Hazeena Begum,
Professor and Head, Department of Siddha Medicine, Faculty of Science,
Tamil University, Vakaiyur, Thanjavur - 613 010, Tamilnadu, India.
E-mail: drvhazeenabegum@gmail.com

## Introduction

Ethanol induced gastric lesion is accompanied with formation of free radicals and reactive oxygen species (ROS) [1]. These radicals particularly seemed to play an important role in ulcerative and erosive lesions of the gastrointestinal tract as they attack and damage many biological molecules [2]. The stomach and upper gastrointestinal tract are the main sites of ethanol metabolism. Acute ethanol induction increases the oxidative stress, deoxyribo nucleic acid (DNA) damage; increased xanthine oxidase activity and malondialdehyde levels and decreased total glutathione (GSH) content in gastric mucosal cells [3]. Increase in the xanthine oxidase (XO) activity subsequently initiates the pro-inflammatory mediators from endothelium [4,5]. These mediators then activate neutrophils and accumulation of activated neutrophils in the gastric mucosa may be a source of free radicals [6]. Myeloperoxidase, an important enzyme of neutrophils, those activities in gastric mucosa is an indicator of infiltration of neutrophils into mucosa [7]. Nitric oxide (NO) is a crucial mediator of gastrointestinal mucosal defense but, paradoxically, it also contributes to mucosal damage [5]. NO, a uniquely diffusible messenger molecule, participates in many physiological and patho- physiological processes in the gastrointestinal tract.

Also it is generally accepted that gastric ulcer results from an imbalance between aggressive factors and the maintenance of the mucosal integrity through the endogenous defense mechanism [8]. Despite the development of anti-secretary antiulcer drugs, scientist are still in search for a drug having antiulcer and antioxidant properties to control the incidence of gastric ulceration in humans with no or minimum toxicity. Absolute ethanol method of inducing gastric lesions is rapid and convenient way of screening plant extracts for a direct cytoprotective effect on the gastric mucosa.

The Areca nut palm (*Areca catechu* L.) belongs to the family *Palmceae,* which is a commercial crop cultivated as a source of masticatory nut popularly known as *A. catechu* or betel nut or supari. Vagbhata ($4^{th}$ century AD) described its medicinal properties and its effective use against leucoderma, leprosy, cough, fits, worms, anemia and obesity. The powered nuts are used in diarrhea and urinary disorders [9]. *A. catechu* is considered useful as an external application on ulcer and in skin disorders. *A. catechu* is anti helminthes and used in veterinary medicine as a vermifuge. A paste of dry *A. catechu* powder is used as dentifrice.

The major biochemical components of *A. catechu* are polyphenol (20%), fat (15%), starch (20%) and alkaloids (0.5%). *A. catechu* showed potent antioxidative activity [10], inhibition of free radicals [11], reactive oxygen species [12] and anti-hyaluronidase activity [13]. *A. catechu* extracts relaxed aortic ring preparations of isolated rat aorta [14]. An antidepressant property via monoamine oxidase (MAO)-A inhibition has also been suggested for the dichloromethane fraction from *A. catechu* [15].

The polyphenols, mostly flavonols, include about 10 per cent of (+) catechin, 2.5 per cent epicatechin, 12 per cent of (+) leucocyanidin, the remaining portion being complex flavonoids in varying degrees of polymerization [16]. A series of dimeric, trimeric, and tetramertic procyanidins has been isolated from seeds of *A. catechu* [17].

*A. catechu* hydrolysable tannins which include tannic acid, showed antibacterial properties on growth of salivary and selected oral microorganisms [18]. *A. catechu* extracts exhibited strong inhibitory activities against pancreatic cholesterol esterase (pCEase), decrease in absorption of cholesterol oleate [19] and intestinal free cholesterol [20]. The arecoline in *A. catechu* showed hypoglycemic activity [21]. The crude *A. catechu* extract and polyphenols promoted wound healing in incision and dead space wound [22]. *A. catechu* possessed anti-*H. Pylori* effects [23]. The aim of this study was to investigate the protective effects of a defatted ethanol extract obtained from *A. catechu* on ethanol induced gastric ulcer in rats.

## Methods

### Preparation of Plant Extract

The mature seeds of 9-10 months old *Areca catechu* were collected from Central Plantation Crops Research Institute, Vittal, Karnataka during December 2005 and made coarse powder and refluxed using 70% ethyl alcohol in a soxhlet apparatus for 12 hours. The residue of ethanol extract was defatted using petroleum ether (a yield of 7%). The defatted ethanol extract was used for further studies.

### Animals and Experimental Design

Albino Wistar female rats (150-200 g) were fed with a standard diet and water *ad libitum*. Group1 animals were intragastrically administered each with 5mL of saline/kg. Group 2 animals were intragastrically administered each with 5mL of absolute ethanol (5 ml/ kg) by gastric intubations. Group 3 rats were intragastrically administered each with 250 mg /kg body weight of *A. catechu* extract. Group 4 rats were intragastrically administered each with 500 mg/kg body weight of *A. catechu* extract. Animal experiments were carried out following the guidelines of the animal ethics committee of the institute.

### Induction of gastric ulcer or lesions

The defatted ethanolic extract were dissolved in water and 250mg, 500mg of the water extract were given intragastrically 30minutes before administration of 5ml/kg of ethanol. After 60minutes animals were killed under anesthesia and stomach were removed, opened along curvature and rinsed with saline. The mucosa was weighed and homogenized and used for biochemical analysis.

**Biochemical analysis**

Lipid peroxidation (LPO) was assessed by the level of its byproduct-malondialdehyde (MDA) using 1,1,3,3-tetraethoxypropane as the standard [24]. Nitric Oxide (NO) level was estimated by using the method of Sastry et al. [25]. Myeloperoxidase (MPx) activity was measured in tissues by a procedure documented by Bradley et al. [26]. Xanthine oxidase (XO) activity was measured by the method of Stripe and Della Corte [27] in gastric mucosa homogenates. Gastric mucosal glutathione (GSH) was measured following the method of Sedlak and Lindsay [28]. Sialic acid was estimated following the method described by Warren [29]. The method described by Bregman [30] was used to determine the levels of nucleic acids.

**Statistical analysis of data**

Values are mean $\pm$ SD for rats in the each and statistical significant difference between mean values were determined by one way analysis of variance (ANOVA) followed by Turkey's test for multiple comparison values of $P < 0.05$ were considered to be significant.

## Results

Table 1 shows the increased levels of malondialdehyde and nitric oxide and the increased activities of myeloperoxidase and xanthine oxidase in gastric mucosa of ethanol induced rats. Pretreatment with *A. catechu* extract at 250mg and 500mg doses reduced the levels of MDA and NO and decreased the activities of MPx and XO enzymes.

Table 1. Effect of *A. catechu* on gastric mucosal malondialdehyde (MDA), nitric oxide (NO), xanthione oxidase (XO) and myeloperoxidase (MPO).

| | **MDA** | **NO** | **MPx** | **XO** |
|---|---|---|---|---|
| Control | 8.03 ± 0.48 | 2.14 ± 0.15 | 4.81 ± 0.25 | 0.59 ± 0.04 |
| Ethanol | 11.12 ± 0.52[a*] | 2.96 ± 0.14[a*] | 8.56 ± 0.32[a*] | 0.82 ± 0.07[a*] |
| *A. catechu* (250 mg/kg) | 5.67 ± 0.47[b*] | 2.10 ± 0.13[b*] | 3.25 ± 0.28[b*] | 0.65 ± 0.07[b*] |
| *A. catechu* (500 mg/kg) | 6.27 ± 042[b*] | 2.17 ± 0.17[b*] | 3.21 ± 0.22[b*] | 0.54 ± 0.03[b*] |

Each value is expressed as mean ± SD for six rats in each group. Units: MDA-nmol/g tissue; NO-nmol/g tissue; MPx-µmol/min/mg tissue and XO-µg of uric acid formed/min/mg tissue. Superscript letters represent $p<0.05$ (Tukey's test). [a]As compared with control, [b]As compared with Ethanol. [*]$p<0.001$.

The contents of reduced glutathione, sialic acid and DNA were found to be significantly decreased in ethanol induced gastric ulcer. Pretreatment with *A. catechu* extract at 250mg and 500mg doses, prevented the ethanol induced decrease in gastric mucosal GSH, sialic acid and DNA contents (Table 2).

Table 2. Effect of *A. catechu* on gastric mucosal gulthathione (GSH), sialic acid and DNA.

| | GSH | Sialic Acid | DNA |
|---|---|---|---|
| Control | 3.96 ± 0.22 | 5.45 ± 0.24 | 0.78 ± 0.03 |
| Ethanol | 2.02 ± 0.17 [a*] | 3.82 ± 0.26 [a*] | 0.54 ± 0.04 [a*] |
| *A. catechu* (250 mg/kg) | 3.69 ± 0.19 [b*] | 5.19 ± 0.31 [b*] | 0.71 ± 0.03 [b*] |
| *A. catechu* (500 mg/kg) | 4.14 ± 0.18 [b*] | 5.89 ± 0.28 [b*] | 0.74 ± 0.05 [b*] |

Each value is expressed as mean ± SD for six rats in each group. Units: GSH-nmol/mg tissue; sialic acid-mg/g tissue and DNA-mg/g tissue. Superscript letters represent $p<0.05$ (Tukey's test). [a]As compared with control, [b]As compared with Ethanol. [*]$p<0.001$.

## Discussion

Ethanol-induced gastric injury is associated with the significant production of free radicals [31] leading to increased lipid peroxidation, which causes damage to cell and cell membranes [32]. In the present study the level of MDA was significantly increased in ethanol induced gastric mucosal injury. Pretreatment with *A. catechu* extract attenuated ethanol-induced gastric mucosal injury, and significantly inhibited the gastric mucosal malondialdehyde level, which is an index of lipid peroxidation. The reduced level of MDA suggested the antioxidative activity of *A. catechu* extract mainly through the presence of flavonoids and procyanidins [11]. Many reports have demonstrated that most injury of gastric mucosa can be reduced by pretreatment with scavengers of reactive oxygen species [32]. Plant flavonoids and procyanidins have been shown to scavenge radicals in a dose dependent manner and therefore are viewed as promising therapeutic drugs for free radical pathologies [33].

Nitric oxide reacts with free radicals, thereby producing the highly damaging peroxynitrite. In this present study, the nitric oxide content was significantly increased in ethanol induced ulcer rats. Pretreatment with *A. catechu* extract decreased the nitric oxide levels by its active compounds such as catechin, polyphenols that might have directly involved in scavenging NO radicals. Similar results were obtained with silibin [24] and quercetin [34]. The inhibition on NO during pretreatment with *A. catechu* extract showed its protective activity against NO radical, signifying the presence of flavonoidal compounds.

Activated neutrophils produce many enzymes and free radicals that damage the gastric mucosa, neutrophil is considered as an aggressive factor in ulcer formation [35]. MPx is an essential enzyme for normal neutrophil function, released into extracellular fluid as a response to various stimulatory substances. MPx activity is considered as an index for the evaluation of neutrophil infiltration. In the present study, the elevated activity of MPx in the gastric mucosa indicates oxidative injury induced by ethanol involves the

contribution of neturophil accumulation. Pretreatment with *A. catechu* extract significantly inhibited the MPx, which reflects the prevention of neturophil infiltration suggesting the protective effect against ethanol-induced ulcer. Inhibition of MPx activity by flavonoids results in the impairment of ROS production. Similar result was obtained in earlier studies showed that Cocoa liquor water-soluble polyphenol significantly prevented the increase of MPx activity on ethanol induced ulcer rats [36].

The production of oxygen free radicals via the xanthine-xanthine oxidase system and accumulation of activated neutrophils in gastric mucosal may be a source for free radicals [37]. Xanthine oxidase-derived superoxide radicals generate $H_2O_2$, which enters the cell and interacts with non-ferritin iron to produce cytotoxic hydroxyl radicals [38]. In the present observations xanthine oxidase activity was increased in ethanol group rats which is a source of oxygen free radicals is considered as a major factor responsible for gastric mucosal damage. Pretreatment with *A. catechu* showed decrease in the xanthine oxidase activity in gastric mucosa. The presence of flavonoids and procyanidin in the *A. catechu* might have elicited preventive action on xanthine oxidase in the gastric mucosa of ethanol model. Our finding is in agreement with earlier reports in which flavanol rich cocoa liquor has been shown to significantly reduce the activities of xanthine oxidase in ethanol-induced oxidative stress [36]. The flavonoids, quercetin and silibin, inhibit xanthine oxidase activity, thereby resulting in decreased oxidative injury [39].

In this present study, the gastric tissue GSH level of the ethanol-administered rats was lower than that of the control. Ethanol-induced generation of free radicals reduced the GSH synthesis, resulting in the decreased GSH content [40]. Endogenous sulfhydryl compounds are important to the maintenance of mucosal integrity in the gastrointestinal tract against oxidative stress [41], functioning as nucleophilic scavengers of reactive oxygen species. Ethanol lowers the concentration of non-protein sulphydryl specifically glutathione [3]. The depletion of GSH is leads to enhancement of LPO and thus increases the susceptibility of gastric mucosa to reactive oxygen species. Pretreatment with *A. catechu* was found to protect the GSH content depletion in gastric mucosa. This is in agreement with earlier reports that pretreatment with *Ginkgo biloba* (GbE) that contain flavonoids was inhibited ethanol-induced depletion in the non-protein -SH concentrations and MDA production [42]. *A. catechu* aqueous extract and polyphenol fraction treated to normal mice group showed an increase in glutathione levels supports our findings [43]. It also reported that relatively low concentrations of flavonoids stimulated transcription of a critical gene for GSH synthesis in cells [44]. Our finding with *A. catechu* extract also justifies the protective mechanism due to the presence of polyphenols.

Sialic acid is an important component of mucosal glycoprotein and it increases the viscosity of mucus and promotes the defensive mechanism [45]. Sialic acid content is known to be decreased in acute gastric ulcers [46]. In our studies, levels of sialic acid were found to be significantly reduced in ethanol treated ulcers. The decrease in the glycoprotein moieties in the gastric mucosa may be attributed to the decreased activity

of defense mechanisms as a result of damage to the gastric mucosa. *A. catechu* pretreatment (250mg/kg and 500 mg/kg) maintained the sialic acid level to near control rats. This finding is in good agreement with Jainu and Devi, [47].

The DNA content of the tissue provides an estimate of the total number of cells in the tissues. In the present study, the decrease in the DNA content of gastric mucosa indicates the increased shedding of mucosal cells during ethanol-induced ulcer. Pretreatment with *A. catechu* increased the DNA content in gastric mucosa and implicating its effect in increasing mucosal resistance. Our finding is also supported by earlier evidence that *P. marsupium* treated group was found to be increased the DNA content in gastric mucosa [48].

In conclusion, the pretreatment with *A. catechu* significantly prevented the free radical induced damages and maintained the contents of GSH, DNA and sialic acid to near normalcy. The preventive activity of *A. catechu* extract against ethanol induced gastric mucosal ulcer might have been ascribed to the presence of phenolic compounds such as proanthocyanidins, polyphenolic compounds such as catechin and epictechin that have shown to exert direct antioxidant action on ethanol induced gastric ulcer. Thus the present finding suggested that the anti-ulcerogenic activity of *A. catechu* through its antioxidative mechanism.

## Acknowledgement

One of the authors M.S. is grateful to the Indian Council of Agricultural Research, New Delhi (ICAR) and Director, Central Plantation Crops Research Institute (CPCRI), Kasaragod, India for providing support to carry out this work.

## References

1. Cho CH, Pfeiffer CJ, Misra HP. Ulcerogenic mechanism of ethanol and the activation of sulphanilyl on the rat stomach *in vivo*. J Pharm Pharmacol 1991;43:495-498.
2. Hirokawa M, Miura S, Yoshida H, Kurose I, Shigematsu T, Hokari R, Higuchi H, Watanabe N, Yokoyama Y, Kimura H, Kato S, Ishii H. Oxidative stress and mitochondrial damage precedes gastric mucosal cell death induced by ethanol administration. Alcohol Clin Exp Res 1998;22:111S-114S.
3. Szabo S, Trier JS, Frankel PW. Sulfhydryl compounds may mediate gastric cytoprotection. Science 1981;214:200-202.
4. Büyükoglu ME, Taysı S, Polat F, Göçer F. Mechanism of the beneficial effects of dantrolene sodium on ethanol-induced acute gastric mucosal injury in rats. Pharmacol Res 2002;45:421-425.

5. Chow JYC, Ma L, Cho CH. Involvement of free radicals and histamine in the potentiating action of cigarette smoke exposure on ethanol-induced gastric mucosal damage in rats. Free Radical Bio Med 1998;24:1285-1293.
6. Vanisree AJ, Mitra K, Shyamala devi CS. Antiulcerogenic effect of ul-409 against experimentally induced gastric ulcer in rats. Indian J Pharmacol 1996;28:265-268.
7. Hampton MB, Kettle AJ, Winterbourn CC. Inside the neutrophil phagosome: oxidants, myeloperoxidase, and bacterial killing. Blood 1998;92:3007-3017.
8. Szabo S, Szlenji S. Cytoprotection in gastro-intestinal pharmacology. Trends Pharma Sci 1987;8:149-154.
9. Nayar NM, Annamalai SJK. Alternative uses of Areca nut. In: Bavapaa KVA, Nair MK, Kumar TP, editors. The Arecanut Palm. Central Plantation Crops Researsh Institute: Kasragod; 1982. p. 263-280.
10. Lee K-K, Cho J-J, Park J, Choi JD. Anti-elastase and anti-hyaluronidase of phenolic substance from *Areca catechu* as a new anti-ageing agent. Int J Cosmetic Sci 2001;23:341-346.
11. Kim BJ, Kim JH, Kim HP, Heo MY. Biological screening of 100 plant extracts for cosmetic use (II): Anti-oxidative activity and free radical scavenging activity. Int J Cosmetic Sci 1997;19:299-307.
12. Ohsugi M, Fan W, Hase K, Xiong Q, Tezuka Y, Komatsu K, Namba T, Saitoh T, Tazawa K, Kadota S. Active-oxygen scavenging activity of traditional nourishing-tonic herbal medicines and active constituents of *Rhodiola sacra*. J Ethnopharmacol 1999;67:111-119.
13. Lee K-K, Cho J-J, Park J, Choi JD. The effects of *Areca catechu* L extract on anti-inflammation and anti-melanogenesis. Int J Cosmetic Sci 1999;21;275.
14. Hirozo Goto, Nobumitsu Tanaka, Kiyoaki Tanigawa, Yutaka Shimada, Takashi Itoh, Katsutoshi Terasawa. Endothelium-dependent vasodilator effect of extract prepared from the seeds of *Areca catechu* on isolated rat aorta. Phytother Res 1997;11:457-459.
15. Dar A, Khatoon S. Behavioral and biochemical studies of dichloromethane fraction from the *Areca cetchu* nut. Pharmacol Biochem Behav 2000;65;1-6.
16. Govindarajan VS, Mathew AG. Polyphenolic substances of arecanut I. Chromatographic analysis of fresh mature nut. 1963;2: 321-326.
17. Nonaka GI, Hsu FL, Nishioka I. Structures of dimeric, trimeric and tetrameric procyanidins from *Areca catechu*. J Chemical Soc Chemical Comm 1981;781-783.
18. de Miranda CM, van Wyk CW, van der Biji P, Basson NJ. The effect of areca nut on salivary and selected oral microorganisms. Int Dent J 1996;46:350-356.
19. Jeon SM, Kim HS, Lee TG, Ryu SH, Suh PG, Byun SJ, Park YB, Choi MS. Lower absorption of cholesteryl oleate in rats supplemented with *Areca catechu* L. extract. Ann Nutr Metab 2000;44:170-176.
20. Park YB, Jeon SM, Byun SJ, Kim HS, Choi MS. Absorption of intestinal free cholesterol is lowered by supplementation of *Areca catechu* L. extract in rats. Life Sci 2002;70:1849-1859.
21. Chempakam B. Hypoglycemic activity of arecoline in betel nut *Areca catechu* Linn. Indian J Exp Biol 1993;31:474-475.
22. Padmaja PN, Bairy KL, Kulkarni DR. Pro-healing effect of betel nut and its polyphenols. Fitoterapia 1994;LXV:298.

23. Wank YC, Huang TL. Screening of anti-*Helicobacter pylori* herbs deriving from Taiwanese folk medicinal plants. FEMS Immunol Med Microbiol 2005;43:295-300.
24. Dehmlow C, Erhard J, de Groot H. Inhibition of Kupffer cell functions as an explanation for the hepatoprotective properties of silibinin. Hepatology 1996;23:749-754.
25. Sastry K, Moudgar RP, Mohan J, Tyagi JS, Rao GS. Spectrophotometric determination of serum nitrite and nitrate by copper-cadmium alloy. Anal Biochem 2002;306:79-82.
26. Bradley PP, Piebat DA, Cristensen RD, Rstein G. Measurement of coetaneous inflammation: estimation of neutrophil content with an enzyme marker. J Invest Dermatol 1982;78:206-209.
27. Stripe F, Della Corte E. The regulation of rat liver xanthine oxidase. J Biol Chem 1969;244:3855.
28. Sedlak J, Lindsay RH. Estimation of total, protein bound, and non-protein sulfhydryl groups in tissue with Ellman's reagent. Anal Biochem 1968;25:192-205.
29. Warren L. The thiobarbituric acid assay of sialic acids. J Biol Chem 1959;234:1971-1975.
30. Bregman AA. Laboratory Investigation and Cell Biology. New York: John Wiley and Sons; 1983. p. 51-60.
31. Ishii M, Shimizu S, Nawata S, Kiuchi Y, Yamamoto T. Involvement of reactive oxygen species and nitric oxide in gastric ischemia-reperfusion injury in rats: protective effect of tetra hydrobiopterin. Dig Dis Sci 2000;45:93-98.
32. Fridovich I. The biology of free radicals. Science 1978;201:875-880.
33. Czinner E, Haymasi K, Kery A, Szoke E, Lemberkovics E. The in vitro effect of helichysi flos on microsomal lipid peroxidation. J Ethnopharmocol 2001;77;31-35.
34. Shutenko Z, Henry Y, Pinard E, Seylaz J, Potier P, Berthet F, Girard P, Sercombe R. Influence of the antioxidant quercetin in vivo on the level of nitric oxide determined by electron paramagnetic resonance in rat brain during global ischemia and reperfusion. Biochem Pharmacol 1999:57:199-208.
35. Fukumura D, Kurose I, Miura S, Tsuchiya M, Ishii H. Oxidative stress in gastric mucosal injury: role of platelet-activating factor-activated granulocytes. J Gastroenterol 1995;30:565-571.
36. Osakabe N, Sanbongi C, Yamagishi M, Takizawa T, Osawa T. Effects of polyphenol substances derived from *Theobroma cacao* on gastric mucosal lesion induced by ethanol. Biosci Biotechnol Biochem 1998;62:1535-1538.
37. Tepperman BL, Soper BD. Effect of sialoadenoectomy on ethanol-induced gastric mucosal damage in rat: role of neutrophils. Canad J Physiol Pharmacol 1990;68:207-210.
38. Kvietys PR, Inauen W, Bacon BR, et al. Xanthine oxidase-induced injuryto endothelium: role of intracellular iron and hydroxyl radical. Am J Physiol 1989;257:H1640-H1646.
39. Shoskes DA. Effect of bioflavonoids quercetin and curcumin on ischemic renal injury: a new class of renoprotective agents. Transplantation 1998;66:147.
40. Bast A, Haenen GRMM, Doelman CJA. Oxidants and antioxidants: state of the rat. Am J Med 1991;91:2S-13S.

41. Olson CE. Glutathione modulates toxic oxygen metabolite injury of canine chief cell monolayers in primary culture. Am J Physiol Gastrointest Liver Physiol 1988;254:G49-G56.
42. Sheng-Hsuan Chen, Yu-Chih Liang, Jane CJ Chao, Li-Hsueh Tsai, Chun-Chao Chang, Chia-Chi Wang, Shiann Pan. Protective effects of *Ginkgo biloba* extract on the ethanol-induced gastric ulcer in rats. World J Gastroenterol 2005;11:3746-3750.
43. Shivapurkar NM, Bhide SV. Effect of betel nut constituents on sulfhydryl metabolism. Indian J Pharmacol 1978;10:257-264.
44. Myhrstad MC, Carlsen H, Nordstrom O, Blomhoff R, Moskaug JO. Flavonoids increase the intracellular glutathione level by trans activation of the γ-glutamylcysteine synthetase catalytical subunit promoter. Free Radic Biol Med 2002;32: 386-393.
45. Spiro RG. Glycoproteins, their biochemistry, biology and role in human disease. New Engl J Med 1969;281:1043-1056.
46. Dekanski JB, Macdonald A, Sacra P, Parke DV. Effects of fasting, stress and drugs on gastric glycoprotein synthesis in rats. Br J Pharmac 1975;55:387-392.
47. Jainu M, Devi CS. Gastroprotective action of *Cissus quadrangularis* extract against NSAID induced gastric ulcer: role of proinflammatory cytokines and oxidative damage. Chem Biol Interact 2006;161:262-270.
48. Joshi MC, Dorababu M, Prabha T, Kumar MM, Goel RK. Effects of *Pterocarpus marsupium* on NIDDM-induced rat gastric ulceration and mucosal offensive and defensive factors. Indian J Pharmacol 2004;36:296-302.