International Scholars Journals

International Journal of Medicine and Medical Sciences ISSN 2167-0404 Vol. 10 (4), pp. 001-008, April, 2020.
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Full Length Research Paper

An investigation of ulcerogenic activity of ethanol extract from nuts of *A. CATECHU* in ethanol induced gastric mucosal injury in rats

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Accepted 19 December, 2019

***ARECA CATECHU* L. (Arecaceae) nuts have been used in traditional medicine for the treatment of different diseases. The present study was performed to investigate the enhancement of ulcerogenic activity of ethanol extract from nuts of *A. CATECHU* in ethanol induced gastric mucosal injury in rats. Four groups of adult Sprague Dawley rats were orally pre-treated respectively with carboxymethyl cellulose (CMC) solution (ulcer control group), omeprazole 20 mg/kg (reference group), 250 and 500 mg/kg *A. CATECHU* nut extract in CMC solution (experimental groups) one hour before oral administration of absolute ethanol to generate gastric mucosal injury. After an additional hour, the rats were sacrificed and the ulcer areas of the gastric walls were determined. Grossly, the experimental groups exhibited significantly severe gastric mucosal injury compared to ulcer control group. Histological studies of the gastric wall revealed that experimental groups exhibited comparatively severe damage of gastric mucosa; along with edema and leucocytes infiltration of submucosal layer compared to ulcer control group. In conclusions, the present finding suggests that *A. CATECHU* nut extract enhanced ulcer production as ascertained grossly by significant increasing ulcer area, and histologically by comparatively increases in ulcer areas compared to ulcer control group.**

Key words: *Areca catechu* nut, omeprazole, enhancement gastric ulcer, histology.

INTRODUCTION

Betel nut (*Areca catechu* L., Arecaceae) is one of popular traditional herbal medicines and a common masticatory drug used in Far East Asia, India, and the South Pacific. Its use was recommended in many diseases, such as leucoderma, leprosy, anaemia and obesity. It has been used as a vermifuge (Sharan, 1996). The flowers are sometimes added to salads. The nuts, husks, young shoots, buds, leaves and roots are used in various medicinal preparations (Staples and Bevacqua, 2006). The seeds (nuts) contain alkaloids, tannins, polyphenols, sugar and lipid. The seed has anthelmintic, antifungal, antibacterial, anti-inflammatory and antioxidant activities (Wetwitayaklung et al., 2006). „Betel chewing" describes the practice of masticating a quid of ingredients, including the seed of the *A. catechu* palm (betel nut), the leaf of the creeping vine *Pier betel* and lime, usually in the form of burnt shell or coral. Betel nut is humanity"s fourth most widely used drug after nicotine, ethanol and caffeine, and is chewed by millions of people (Marshall, 1987). Significant illnesses can be associated with its use, including exacerbation of asthma, cholinergic crisis, cardiac arrhythmias, acute psychosis, milk-alkali syndrome, oropharyngeal tumors, and a variety of oral diseases (Nelson and Heischober, 1999). Chewing quid comprising areca nut and tobacco has adverse effects on periodontal tissues, oral hygiene and incidence of oral lesions (Parmar et al., 2008). Betel chewing has also been associated with reduced rates of dental caries and changes in the oral microbiological flora (Reichart et al., 2002) while contributing significantly to oral health-related morbidity and mortality (Trivedy et al., 2002), and

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additionally, is an independent risk factor for hepatocellular carcinoma (Tsai et al., 2004). Furthermore, the areca nut (*A. catechu* nut) contains compounds with tumor-promoting activity (Jeng et al., 2001). *A. catechu* seed contain high polyphenols and flavonoids, and the seed is an excellent food material with potential nutrition and antioxidation (Zhang et al., 2009). Areca nut had been found to contain phenolics and alkaloids such as arecoline, arecaidine and guvacine (Zhang et al., 2008). Aqueous extract from seeds of *A. catechu* exhibited potent antioxidant and anti-inflammatory activities (Pithayanukul et al., 2009). Ingestion of large amount of areca nuts therefore can cause various cholinergic effects, such as salivation, lacrimation, urinary incontinence, sweating, diarrhea and cardiac arrhythmia. However, several reports have indicated that areca constituents have beneficial effects on skin, suggesting the possible use in cosmeceuticals (Lee et al., 2001; Ashawat et al., 2007). Betel nut alkaloids include potent muscarinic cholinomimetics that may be therapeutic in schizophrenia and psychosis (Sullivan et al., 2000) while the phenolic substance from *A. catechu* effectively inhibits hyaluronidase activity and has an anti-ageing effect by protecting connective tissue proteins (Lee et al., 2001). Azeez et al. (2007) showed that the alkaloid and polyphenols of areca could be used to enhance the healing of burn wounds, leg ulcers and skin graft surgery. Betel nuts also contain arecoline, a volatile cholinergic alkaloid and central nervous system stimulant, and arecaidine, a hydrolyzed product of arecoline. Both alkaloids possess the properties of acetylcholine at central ganglionic and peripheral nicotinic and muscarinic receptors (Chu, 1993). Arecoline, a major areca alkaloid, is cytotoxic agent to cultured fibroblasts (Chang et al., 1998) and shown to induce vasorelaxation as well as suppress endothelial cell growth (Kuo et al., 2005). Dasgupta et al. (2006) have reported that arecoline arrested splenic lymphocyte cell cycle at low concentration with induced apoptosis at high concentration thereby causing immunosuppression in arecoline recipients. Besides, it resulted in hepatotoxicity in arecoline recipient mice by disrupting the hepatocyte ultrastructure. The present study was undertaken to investigate the enhancement of ulcerogenic effects of *A. catechu* nuts ethanol extracts on ethanol-induced gastric mucosal injuries in rats.

MATERIALS AND METHODS

Omeprazole

Omeprazole was obtained from the pharmacy of University Malaya Medical Centre (UMMC) .The drug was dissolved in carboxymethy cellulose (CMC, 0.25% w/v) and administered orally to the rats in concentrations of 20 mg/kg body (Abdulla et al., 2010).

A. CATECHU nut and preparation of extracts

Dried *A. catechu* nuts were obtained from Ethno Resources Sdn Bhd, Selangor Malaysia, and identified by comparison with the Voucher specimen deposited at the Herbarium of Rimba Ilmu, Institute of Science Biology, University of Malaya, Kuala Lumpur. The nuts were tap washed followed by washing with distilled water and shade-dried for 7 to 10 days and was then finely powdered using electrical blender. 100 g of fine powder was soaked in 500 ml of 95% ethanol in conical flask for 3 days. After 3 days, the mixture was filtered using a fine muslin cloth followed by filter paper (Whatman No. 1) and distilled under reduced pressure in an Eyela rotary evaporator (Sigma-Aldrich, USA). The extract was dried at 45°C in incubator for two days and the dry extract was then dissolved in carboxymethyl cellulose (CMC, 0.25% w/v) and administered orally to rats in concentrations of 250 and 500 mg/kg body weight (Mahmood et al., 2010).

Experimental animals

Sprague Dawley healthy adult male rats were obtained from the Experimental Animal House, Faculty of Medicine, University of Malaya, and Ethic No. PM/27/07/2009/MAA (R). The rats were divided randomly into 4 groups of 8 rats each and the groups were recorded as Groups 1 to 4. Each rat that weighted between 200 - 225 g was placed individually in separate cage (one rat per cage) with wide-mesh wire bottoms during the experiment. The animals were maintained on standard pellet diet and tap water. The study was approved by the Ethics Committee for Animal Experimentation, Faculty of Medicine, University of Malaya, Malaysia. Throughout the experiments, all animals received human care according to the criteria outlined in the “Guide for the Care and Use of laboratory Animals” prepared by the National Academy of Sciences and published by the National Institute of Health.

Treatment

The rats were deprived of food for 48 h before the experiment (Abdulla et al., 2010), but were allowed free access drinking water (bottled tap water) up till 2 h before the experiment. Gastric ulcer in Sprague Dawley was induced by orogastric intubation of absolute ethanol according to the method described by (Mahmood et al. 2010). Ulcer control groups were orally administered with vehicle (CMC, 0.25% w/v). The reference group was received doses of 20 mg/kg omeprazole in CMC) as positive controls. Experimental groups were orally administered with 250 and 500 mg/kg of ethanol extract of *A. catechu* nuts in CMC solution, respectively. One hour after this pre-treatment; all groups of rats were gavaged with absolute ethanol in order to induce gastric ulcers. The rats were euthanized by cervical dislocation 60 min later (Paiva et al., 1998) under over dose of diethyl ether anesthesia and their stomachs were immediately excised.

Gross gastric lesions evaluation

Ulcers were found in the gastric mucosa, appearing as elongated bands of hemorrhagic lesions parallel to the long axis of the stomach. Each gastric mucosa was thus examined for damage. The length (mm) and width (mm) of the ulcer on the gastric mucosa were measured by a planimeter (10 x 10 mm^2 = ulcer area) under dissecting microscope (1.8x). The area of each ulcer lesion was measured by counting the number of small squares, 2 x 2 mm, covering the length and width of each ulcer band. The sum of the areas of all lesions for each stomach was applied in the calculation of the ulcer area (UA) wherein the sum of small squares x 4 x 1.8 = UA mm^2) as described by (Mahmood et al., 2010). The inhibition percentage (I %) was calculated by the following formula as described by (Abdulla et al. 2010).

Table 1. Enhancement of gastric ulcer by *A. catechu* nut extract on ethanol-induced gastric mucosal injury in rats.

Animal group	Pre-treatment (5 ml/kg)	Ulcer area (mm^2) Mean ± M.S.E)	Inhibition (%)
1	CMC (ulcer control)	856.67 ± 9.89^{a}	-
2	20 mg/kg omeprazole	184.17 ± 7.57^{b}	78.50
3	250 mg/kg *A. catechu*	1094.17 ± 59.45^{c}	-27.70
4	500 mg/kg *A. catechu*	1253.33 ± 40.65^{d}	- 46.30

All values are expresses as mean and S.E.M. Means with different superscripts are significantly different ($p<0.05$).

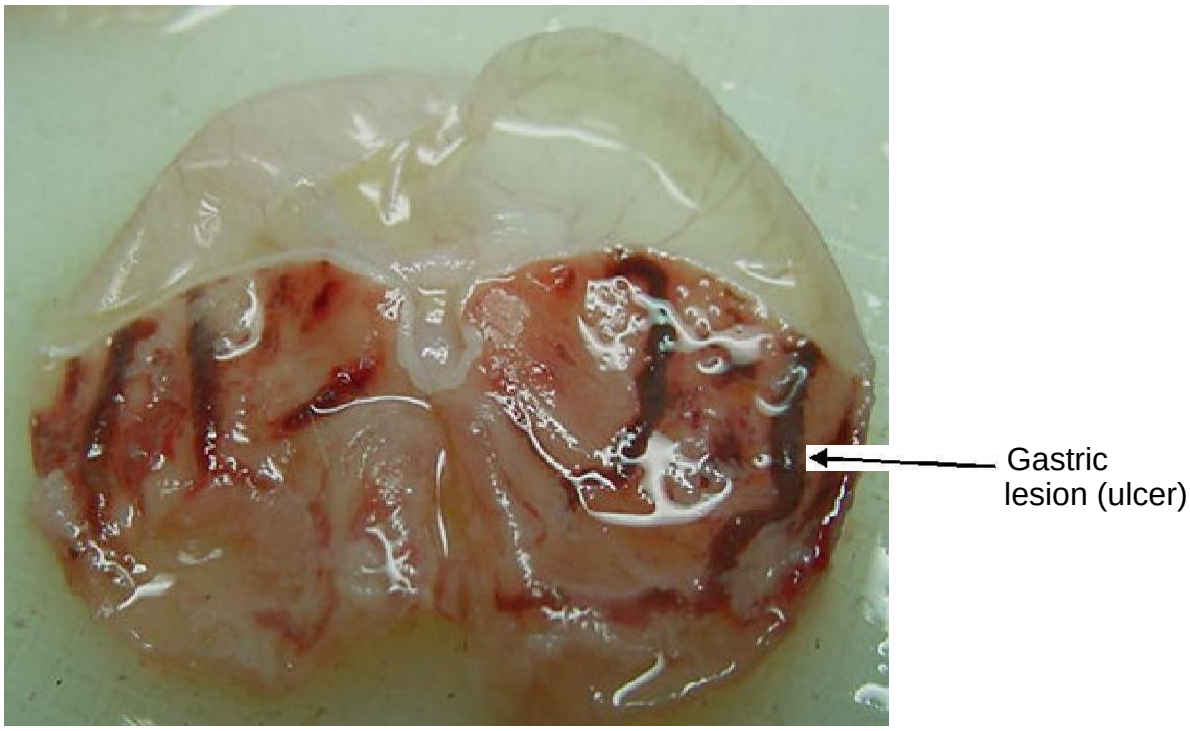

Figure 1. Macroscopical appearance of gastric mucosa in a rat pre -treated with CMC followed absolute ethanol gavages. Modrate to sever injuries are seen in the gastric mucosa.

$$(I\ \%) = [(UA_{control} - UA_{treated}) \mid UA_{control}] \times 100\%$$

Histological evaluation of gastric lesions

Specimens of the gastric walls from each rat were fixed in 10% buffered formalin and processed in a paraffin tissue processing machine. Sections of the stomach were made at a thickness of 5 µm and stained with hematoxylin and eosin for histological evaluation.

Statistical analysis

All values are reported as mean ± standard error mean (S.E.M.) and the statistical significance of differences among groups were assessed using one-way ANOVA. A value of $p<0.05$ was considered significant.

RESULTS

Gross evaluation of gastric lesions

The enhancement of ulcer activity of *A. catechu* nuts extract in ethanol-induced gastric lesion model is reported in Table 1. Results showed that rats pre-treated with *A. catechu* nuts extracts before being given absolute ethanol had significantly increased areas of gastric ulcer formation compared to rats pre-treated with CMC (ulcer control group) (Figures 1 and 2). Moreover, the nuts extract significantly increased the formation of the ulcers and significantly damaged the gastric mucosa in rats. It was also observed that damaged of gastric mucosa was more prominent in rats pre-treated with 500 mg/kg *A. catechu* nuts extract (Table 1). Beside, gastric mucosal

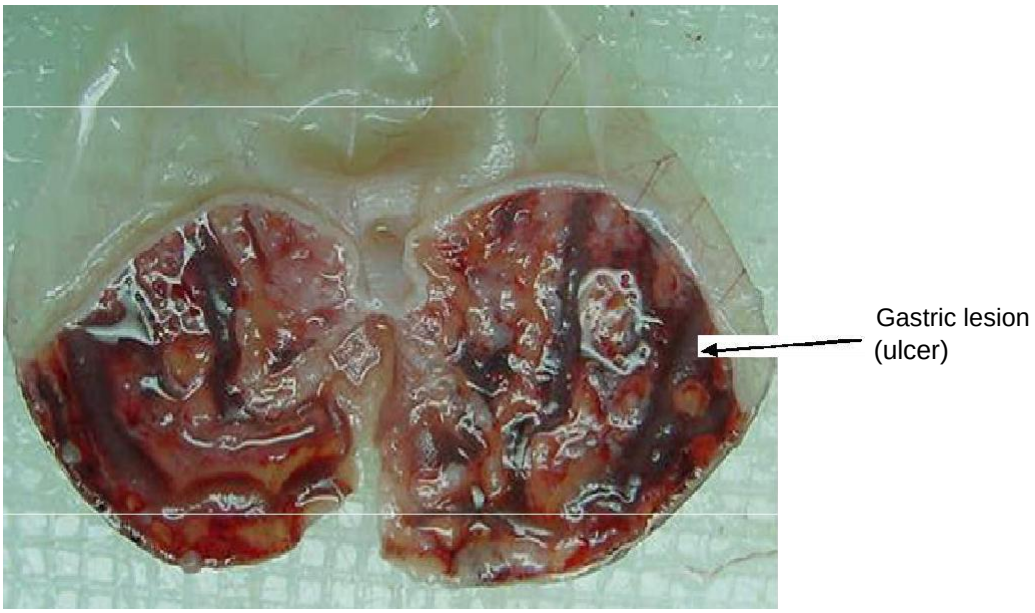

Figure 2. Macroscopical appearance of gastric mucosa in a rat pre-treated with *A. catechu* followed by absolute alcohol gavages. Sever injuries are seen in the gastric mucosa.

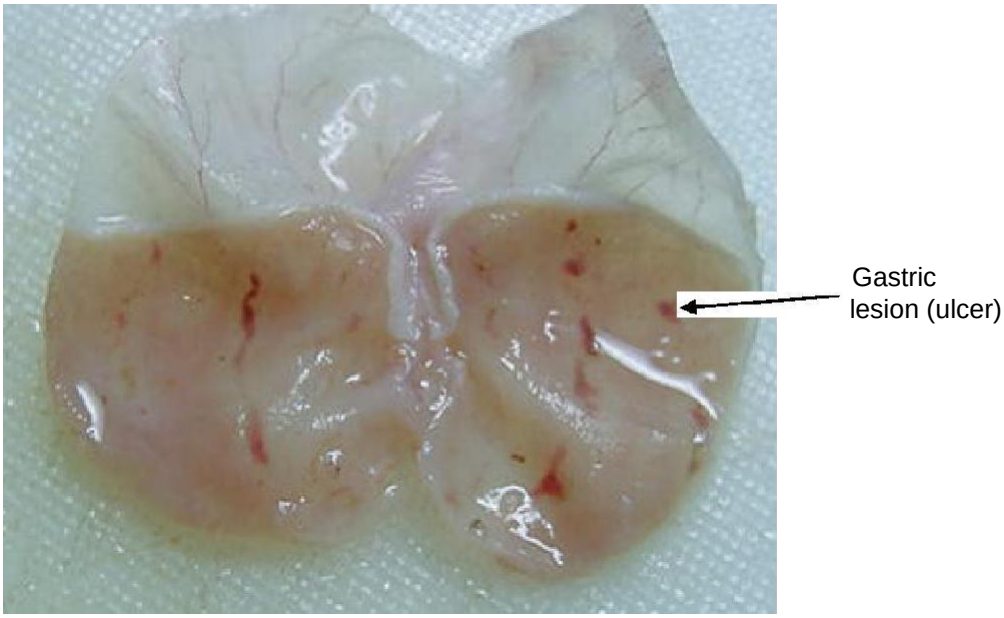

Figure 3. Macroscopical appearance of gastric mucosa in a rat pre-treated with 20 mg/kg omeprazole followed by absolute ethanol gavages. Mild injuries are seen in the gastric mucosa.

damage was significantly and dose dependently increased in the size and severity by pre-treatment of the animals with *A. catechu* nuts extract. Rats pre-treated with omeprazole before being given absolute ethanol had mild gastric ulcer and significantly protect gastric mucosa from necrotizing agent (Figures 3).

Histological evaluation of gastric lesions

Histological observation of ethanol induced gastric lesions in ulcer control group pre -treated with only CMC, showed comparatively extensive damage to the gastric mucosa, and oedema and leucocytes infiltration of the

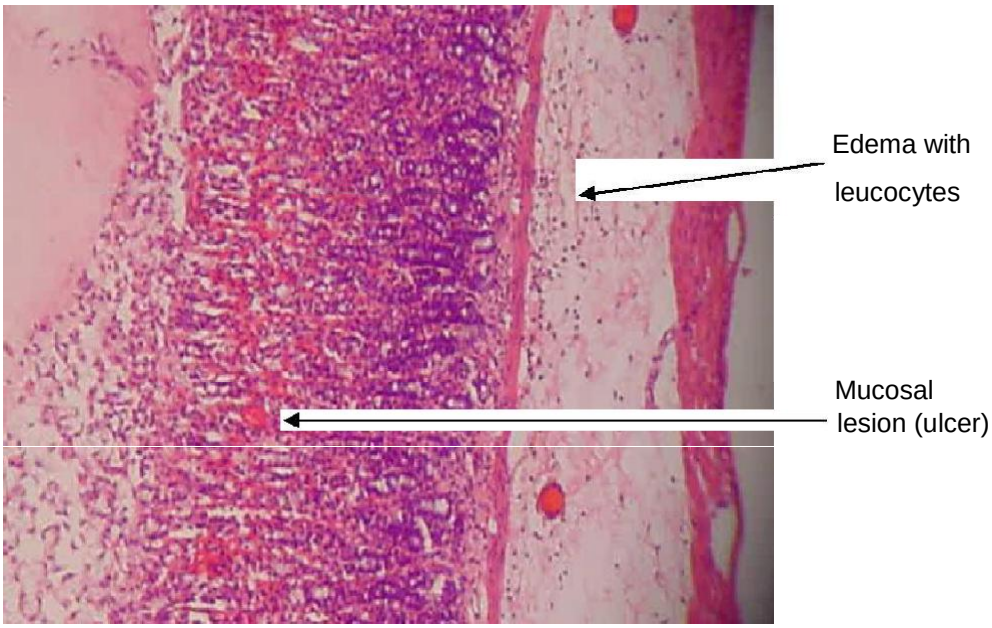

Figure 4. Histological section of the gastric mucosa in a rat pre-treated with CMC followed by absolute ethanol gavages. Severe disruption to the surface epithelium, and edema of the submucosal layer with leucocytes
infiltration (H&E stain, 10×).

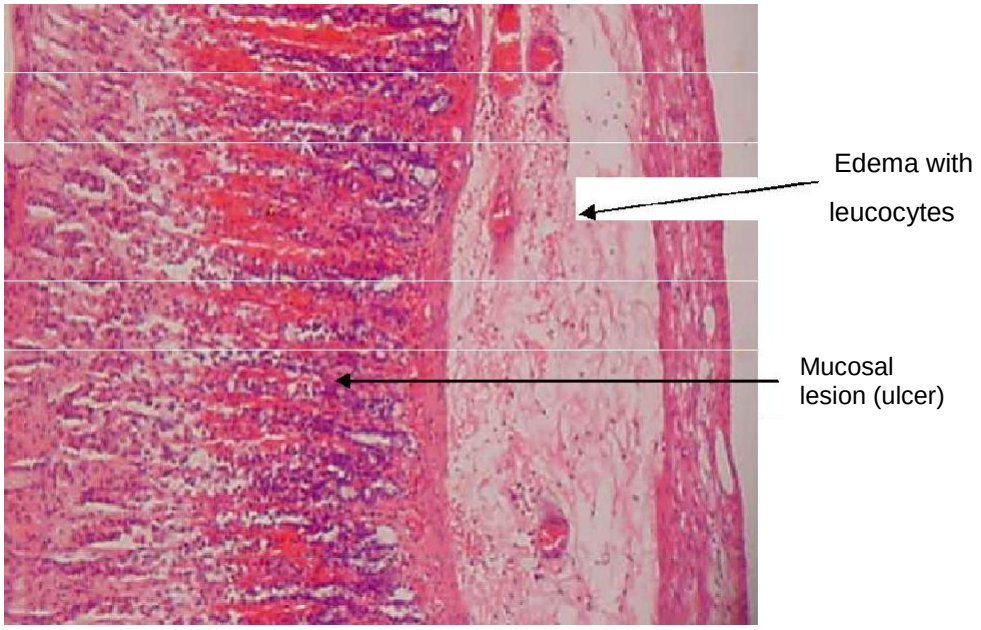

Figure 5. Histological section of the gastric mucosa in a rat pre-treated with 500 mg/kg *A. catechu* followed by absolute ethanol gavages. Severe disruption of gastric mucosa (extended deeply), and edema of the submucosal layer with leucocytes infiltration (H&E stain, 10x).

submucosal layer (Figure 4). Rats that received pre-treatment with *A. catechu* nuts extract had comparatively more extensive damage of the gastric mucosa as seen by increasing in ulcer area, submucosal oedema and leucocytes infiltration (Figure 5). Rats pre-treatment with omeprazole had mild damage to surface epithelium and

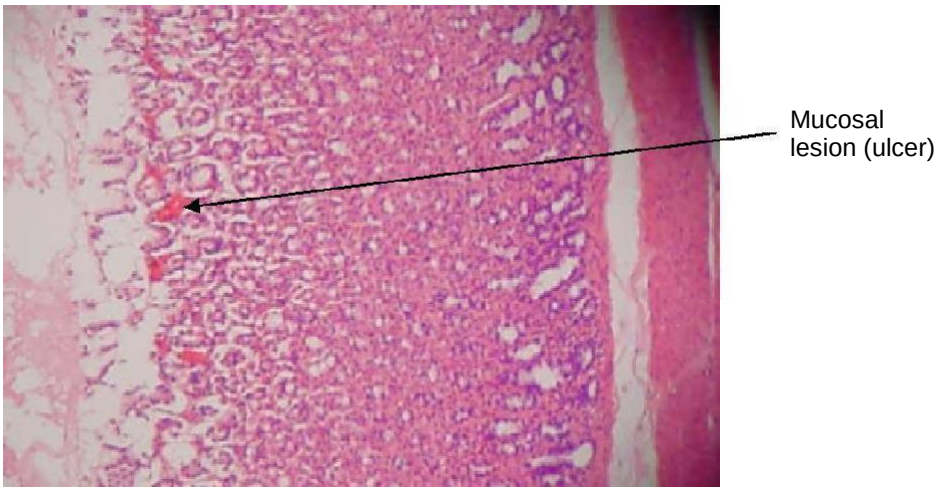

Figure 6. Histological section of the gastric mucosa in a rat pre-treated with 20 mg/kg omeprazole followed by absolute ethanol gavages. Mild disruption to surface epithelium, and no edema and leucocytes infiltration to submucosal layer (H&E stain, 10x).

no submucosal edema and leucocytes infiltration (Figure 6). The *A. catechu* nuts extract has been shown to exert the cytotoxic effects of gastric mucosa in a dose-dependent manner.

DISCUSSION

Ethanol-induced gastric ulcers have been widely used for the experimental evaluation of anti-ulcer activity. This method of inducing gastric lesions is a rapid and convenient way of screening plant extracts for anti-ulcer potency. It is known that gastric lesions produced by ethanol administration appeared as multiple-hemorrhagic red bands of different size along the glandular stomach. Studies suggest that the ethanol damage to the gastrointestinal mucosa starts with micro vascular injury, namely disruption of the vascular endothelium resulting in increased vascular permeability, edema formation and epithelial lifting (Szabo et al., 1995). Disturbances in gastric secretion, damage to gastric mucosa, alterations in permeability, gastric mucus depletion and free-radical production have been reported as the pathogenic effects of absolute ethanol (Salim, 1990).

Ethanol produces necrotic lesions in the gastric mucosa by its direct toxic effect, reducing the secretion of bicarbonates and production of mucus (Marhuenda et al., 1993). Exposure to ethanol increases the extension of cellular damage in a dose-dependent way (Mutoh et al., 1990). Oxidative stress plays an important role in the pathogenesis of various diseases including gastric ulcer, with antioxidants being reported to play a significant role in the protection of gastric mucosa against various necrotic agents (Trivedi and Rawal, 2001). Kobayashi et al. (2001) reported that teprenone exerts a protective effect against mucosal lesions through inhibition of neutrophils infiltration in the ulcerated gastric tissue and Shimizu et al. (2000) demonstrated that the reduction of neutrophils infiltration into ulcerated gastric tissue promotes the healing of gastric ulcers in rats. Cheng and Koo (2000) showed that oral administration of plant extract before ethanol administration significantly decreased neutrophils infiltration of gastric mucosa.

Fujita et al. (1998) observed that an increase in neutrophils infiltration into ulcerated gastric tissue delayed the healing of gastric ulcers in rats. Absolute alcohol would extensively damage the gastric mucosa leading to increased neutrophils infiltration into the gastric mucosa. Oxygen free radicals derived from infiltrated neutrophils in ulcerated gastric tissues have inhibitory effect on gastric ulcers healing in rats (Suzuki et al., 1998). Neutrophils mediate lipid peroxidation through the production of superoxide anions (Zimmerman et al. 1997). Neutrophils are a major source of inflammatory mediators and can release potent reactive oxygen species such as superoxide, hydrogen peroxide and myeloperoxidase derived oxidants. These reactive oxygen species are highly cytotoxic and can induce tissue damage (Cheng and Koo 2000). Furthermore, neutrophils accumulation in gastric mucosa has been shown to induce microcirculatory abnormalities (Bou-Abboud et al., 1988). Suppression of neutrophils infiltration during inflammation was found to enhance gastric ulcer healing (Tsukimi et al., 1996).

Betel quid chewing can produce gastric hemorrhagic ulcers in rats through oxidative stress (Hung, 2004). Enhancement of acid back-diffusion, mast cell histamine release and micro vascular permeability are important in modulating gastric hemorrhagic and ulcer in betel quid-fed rats (Hung and Chen, 2004). Areca nut contained

arecoline, the main ingredient of areca fruit. Arecoline is a cholinergic agent that can cause gastric contraction and induce gastric secretion. Arecoline causes seizure and results in gastric mucosal damage (Hung, 2000). Areca nuts and arecoline take part in the pathogenesis of betel quid chewing-related oral mucosal lesions, possibly through both genotoxic and non-genotoxic mechanisms (Jeng et al., 1999). Reactive oxygen species, such as hydroxyl radicals, that are formed in the human oral cavity during areca quid chewing might cause oxidative DNA damage to the surrounding tissues (Chen et al., 2002). Arecoline, a major areca alkaloid, decreases cell growth and collagen synthesis and inhibits cell attachment, cell spreading and cell migration. These inhibitory effects were associated with intracellular depletion of glutathione (Jeng et al., 1996). In conclusion, *A. catechu* nuts extract could significantly enhanced ulcerogenic effect on ethanol-induced gastric ulcers. Such ulcerogenic effects was shown to be dose dependent as ascertained by the increasing of ulcer areas in the gastric wall as well as the increasing of edema and leucocytes infiltration of submucosal layers. Further studies are required to determine the active ingredients responsible for the mechanism of ulcer of *A. catechu* nuts.

ACKNOWLEDGEMENTS

The authors express gratitude to the staff of the Faculty of Medicine Animal House for the care and supply of rats, and to the University of Malaya for the financial support UMRG (RG102/09HTM).

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