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Full Length Research Paper

Anti-ulcerogenic effects of *Nagilla sativa* in ethanol-induced gastric injuries in rats

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Nagilla sativa **is a medicinal plant commonly used traditionally in the treatment of many aliments. The present study was undertaken to evaluate the protective effect of ethanolic extracts of *N. sativa* ethanolic seed extract (NSESE) against absolute ethanol-induced gastric mucosal damage in experimental rats. The rats were divided into five groups, respectively pre-treated orally with 10% Tween 20 solution (ulcer control groups), omeprazole 20 mg/kg (reference group), 250, 500 and 750 mg/kg of NSESE (experimental groups) 1 h before oral administration of absolute ethanol to generate gastric mucosal damage. After an additional hour, the rats were sacrificed and the ulcer areas of the gastric walls were determined. The ulcer control group exhibited severe mucosal injury, whereas groups pre-treated with NSESE exhibited significant protection of gastric mucosal damage. These findings were also confirmed by histology of gastric wall. Significant increases in gastric mucus production and decrease in acidity of gastric content were observed in treated groups with NSESE compare to ulcer control group. These results concluded that the treatment with NSESE prior to absolute alcohol has significantly protect gastric mucosa as ascertained grossly by significant reduction of ulcer area, increases in gastric mucus production and decrease the acidity of gastric content and histology by comparatively decreases in gastric mucosal damage, reduction or absence of edema and leucocytes infiltration of submucosal layer compared to ulcer control group. NSESE was able to decrease the acidity of gastric and increase mucus production of gastric mucosa, there by justifying its use as an anti-ulcerogenic agent.**

Key words: *Nigella sativum*, ulcer, cytoprotection, histology.

INTRODUCTION

Gastric ulcer is an illness that affects a considerable number of people worldwide. The pathogenesis of gastro-duodenal ulcers is influenced by various aggressive and defensive factors, such as mucus secretion, mucosal barrier, acid-pepsin secretion, blood flow, cellular regeneration and endogenous protective agents (Mizui et al., 1987). It has been shown that long term use of ulcer drugs may be associated with ineffectiveness of different drug regimens and even resistance to drugs are emerging (Al-Mofleh et al., 2007). Thus, there is an urgent need to identify more effective and safe anti-ulcer agents.

A widespread search has been launched to identify new anti-ulcer therapies from natural sources. Herbs, medicinal plants, spices, vegetables and crude drug substances are considered to be a potential source to combat various diseases including gastric ulcer. In the scientific literature, a large number of medicinal plants with gastric anti-ulcer potential have been reported (Abdulla et al., 2010; Ketuly et al., 2011; Mahmood et al., 2010; Wasman et al., 2010). *N. sativa*, commonly known as black seed or black cumin, belongs to the botanical

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Abbreviations: NSESE, *Nagilla sativa* ethanolic seed extract; **UMMC,** university Malaya medical centre; **UA,** anti-ulcer area.

family of Ranunculaceae. The seeds of this plant *N. sativa* have been used as a natural remedy for a number of diseases and conditions such as asthma, cough, bronchitis, headache, eczema, fever, dizziness and influenza (Bayrak et al., 2008).The plant has been investigated to justify its broad traditional therapeutic value (Ilhan et al., 2005). Many favorable biological properties of *N. sativa* have been reported such as anti-inflammatory (Hajhashemi et al., 2004), antioxidative (Meral et al., 2001), anti-tumor (Salomi et al., 1991), and anti-hepatoprotective properties (Kanter et al., 2005). The aim of this study was to investigate the cytoprotective properties of NSESE against ethanol-induced gastric damage in rats.

MATERIALS AND METHODS

Omeprazole

In this study, omeprazole was used as the reference anti-ulcer drug, and was obtained from the University Malaya Medical Centre (UMMC) Pharmacy. The drug was dissolved in 10% Tween 20 and administered orally to the rats in concentrations of 20 mg/kg body weight (5 ml/kg) according to the recommendation of Abdulla et al. (2010).

Tween 20

Tween 20 (10% v/v) in distilled water was used as vehicle for dosing in all animals.

Plant materials and preparation of extracts

N sativa L seeds were purchased from local market, washed with distilled water and air dried in dark at room temperature. The seeds were grounded using a grinder and were extracted by maceration in methanol (100 g/1500 ml) in a conical flask for 5 days at 37°C. Afterwards, the solvents were filtered using filter paper and the solvents were dried under reduced pressure in an EYELA rotary evaporator (Sigma-Aldrich, USA). The NSESE was dissolved in 10% Tween 20 and administered to rats at the concentration of 250 $mgkg^{-1}$, 500 $mgkg^{-1}$, and 750 $mgkg^{-1}$ each recommendation of Abdulla 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/27/07/2010/MAA (R). The rats were divided randomly into 5 groups of 6 rats each. Each rat that weighed between 200 to 225 g was placed individually in a separate cage (one rat per cage) with wide-mesh wire bottoms to prevent coprophagia 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.

Gastric ulcer-induction by absolute ethanol

The rats fasted for 48 h before the experiment (Abdulla et al., 2010), but were allowed free access to drinking water up till 2 h before the experiment. Gastric ulcer was induced by orogastric intubation of absolute ethanol (5 ml/kg) according to the method described by Mahmood et al. (2010). Ulcer control groups were orally administered vehicle (10% Tween 20, 5 ml/kg). The reference group received oral doses of 20 mg/kg omeprazole in 10% Tween 20 as positive control. Experimental groups were orally administered NSESE in 10% Tween 20 (5 ml/kg) at doses of 250, 500 and 750 mg/kg. 1h after this pre-treatment all the groups of rats were administered with absolute ethanol (5 ml/kg) in order to induce gastric ulcers (Abdulla et al., 2010). The rats were euthanized 60 min later under an overdose of xylazin and ketamine anesthesia and their stomachs were immediately excised (Ketuly et al., 2011).

Measurement of mucus production

Gastric mucus production was measured in the rats that were subjected to absolute ethanol-induced gastric lesions. The gastric mucosa of each rat was obtained by gentle scraping the mucosa with a glass slide and the collected mucus were weighed by using a precision electronic balance (Abdulla et al., 2010).

Measurement of acid content of gastric juice (pH)

Samples of gastric contents were analyzed for hydrogen ion concentration by pH metric titration with 0.1 N NaOH solutions using digital pH meter (Abdulla et al., 2010).

Gross gastric lesions evaluation

Ulcers of the gastric mucosa appear as elongated bands of hemorrhagic lesions parallel to the long axis of the stomach. Gastric mucosa of each rat was thus examined for damage. The length and width of the ulcer (mm) were measured by a planimeter (10 × 10 mm^2 = ulcer area) under dissecting microscope (1.8×). The ulcerated area was measured by counting the number of small squares, 2 mm × 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 × 4 × 1.8 = UA (mm^2) according to the recommendation of Mahmood et al. (2010). The inhibition percentage (I.0%) was calculated by the following formula according to the recommendation of Wasman et al. (2010):

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

Histological evaluation of gastric lesions

Specimens of the gastric walls of 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 (Abdulla et al., 2010; Ketuly et al., 2011).

Statistical analysis

All values were reported as mean ± S.E.M. The statistical significance of differences between groups was assessed using one-way ANOVA. A value of $p<0.05$ was considered significant.

Table 1. Effect of NSESE on ulcer area and inhibition percentage in rats.

Animal group	Pre-treatment (5 ml/kg dose)	Mucus production (gram)	pH of gastric content	Ulcer area $(mm)^2$ (Mean ± S.E.M)	Inhibition (%)
1	10% Tween 20 (Ulcer control)	0.37 ± 0.12^a	3.77 ± 0.01^a	$985.17 \pm 9.1.92^a$	-
2	Omeprazole (20 mg/kg)	0.55 ± 0.11^b	6.82 ± 0.02^b	115.51 ± 3.15^b	88.28
3	NSESE (250 mg/kg)	0.52 ± 0.12^b	51.3 ± 0.01^c	175.84 ± 4.18^c	82.24
4	NSESE (500 mg/kg)	0.76 ± 0.15^c	6.55 ± 0.04^d	28.00 ± 1.22^d	97.16
5	NSESE (750 mg/kg)	0.79 ± 0.23^c	6.75 ± 0.04^d	0.00 ± 0.00^e	100

All values are expressed as mean ± standard error mean. Means with different superscripts are significantly different. The mean difference is significant at the $p>0.05$ level.

RESULTS

pH of gastric content and mucus production

The acidity of gastric content in experimental animals pretreated with NSESE was decreased significantly compared to that of the ulcer control group ($p<0.05$). The mucus production of gastric mucosa also increases significantly ($p<0.05$) in animals pretreated with NSESE compared to the ulcer control group (Table 1).

Gross evaluation of gastric lesions

The anti-ulcer activity of NSESE in ethanol-induced gastric lesion model is shown in Table 1. Results showed that rats pre-treated with NSESE extracts before being given absolute alcohol had significantly reduced areas of gastric ulcer formation compared to rats pre-treated with 10% Tween 20 (ulcer control group) (Figure 1 and Table 1) ($p<0.05$). Moreover, the NSESE significantly suppressed the formation of the ulcers and it was interesting to note the flattening of gastric mucosal folds in rats pretreated with 500 and 750 mg/kg NSESE (Table 1 and Figure 1). Furthermore, ethanol-induced mucosal damage was significantly and dose dependently reduced in the size and severity by pretreatment of the animals with NSESE. The significant inhibition of gastric ulcer in pretreatment with NSESE was comparable with omeprazole which is a standard drug used for curing gastric ulcer.

Histological evaluation of gastric lesions

Histological observation of ethanol induced gastric lesions in ulcer control group pre-treated with 10% Tween 20, showed comparatively extensive damage to the gastric mucosa, oedema and leucocytes infiltration of the submucosal layer (Figure 2). Rats that received pre-treatment with NSESE had comparatively better protection of the gastric mucosa as seen by reduction in ulcer area, reduced or absent submucosal edema and leucocytes infiltration (Figure 2). The NSESE has been shown to exert the cytoprotective effects in a dose-dependent manner.

DISCUSSION

The present results demonstrated that pretreatment of rats with NSESE significantly protect the rat gastric mucosa against hemorrhagic lesion induced by absolute ethanol compared to control rats. NSESE showed prominent effects against ethanol-induced ulcer genesis in rats, which may be indication of the cytoprotective activity. Ethanol-induced gastric ulcers have been widely used for experimental evaluation of anti-ulcer activity. Disturbances in gastric secretion, damage to gastric mucosa, alterations in permeability, gastric mucus depletion and free-radical production are reported to be the pathogenic effects of ethanol (Salim, 1990). Ethanol-induced gastric lesion formation may be due to stasis in gastric blood flow, which contributes to the development of the hemorrhage and necrotic aspects of tissue injury (Guth et al., 1984). Omeprazole is a proton pump inhibitor which has been widely used as an acid inhibitor agent for the treatment of disorders related to gastric acid secretion for about 15 years (Li et al., 2004). Omeprazole has substituted benzimidazoles; it inhibits acid secretion by acting on the hydrogen-potassium exchanger ($H^{\pm}$, $K^{\pm}$-ATPase) for the apical plasma membrane of the gastric mucosa (Satoh et al., 1989).

Omeprazole is highly selective for the proton pump and undergoes catalyzed conversion into active form within the acid forming space. The active inhibitors react with SH (thiol) group of the proton pump, resulting in inhibition of acid formation (Nagaya et al., 1991). Results obtained in current study suggest that NSESE showed a protective action against ethanol-induced gastric mucosa damage as demonstrated by the reduction of the gastric ulcer area and increased gastric mucous production and decrease the acidity of gastric content. Ethanol produces necrotic lesions in the gastric mucosa by its direct toxic effect reducing the secretion of bicarbonate and production of mucous (Marhuenda et al., 1993). The

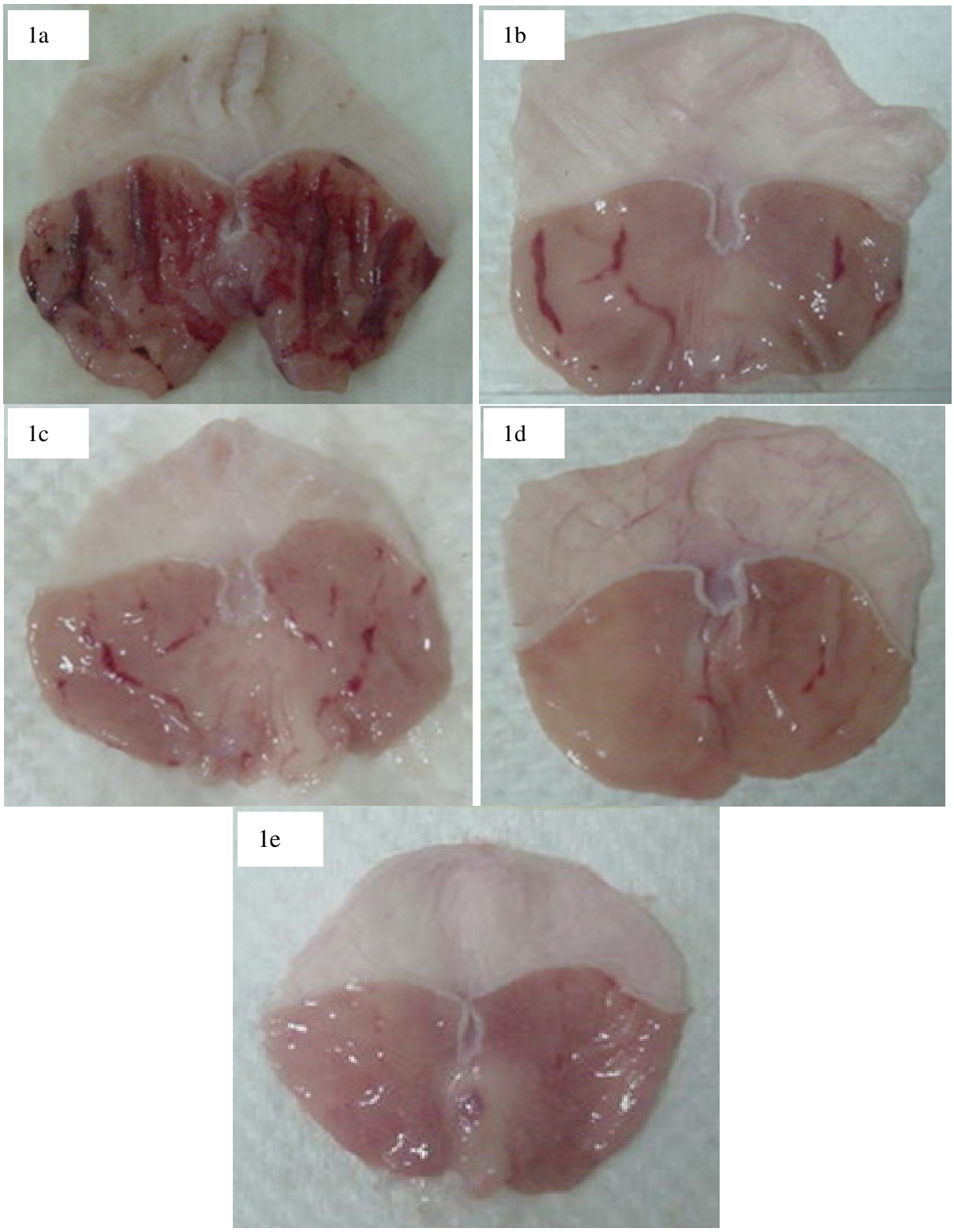

Figure 1. Gross appearance of the gastric mucosa in rats. 1a rats pre-treated with 5 ml/kg 10% Tween 20 (ulcer control). Severe injuries are seen in the gastric mucosa. Absolute ethanol produced extensive visible hemorrhagic necrosis of gastric mucosa. 1b rats pre-treated with of omeprazole (20 mg/kg). Injuries to the gastric mucosa are milder compared to the injuries seen in the ulcer control rats. 1c rat pre-treated with NSESE (250 mg/kg). Mild injuries are seen in the gastric mucosa. The extract reduces the formation of gastric lesions induced by absolute ethanol. 1d rat pre-treated with NSESE (500 mg/kg). Very mild injuries are seen in the gastric mucosa. 1e rats pre-treated with NSESE 750 mg/kg. No injuries to the gastric mucosa are seen; instead flattening of the gastric mucosa is seen.

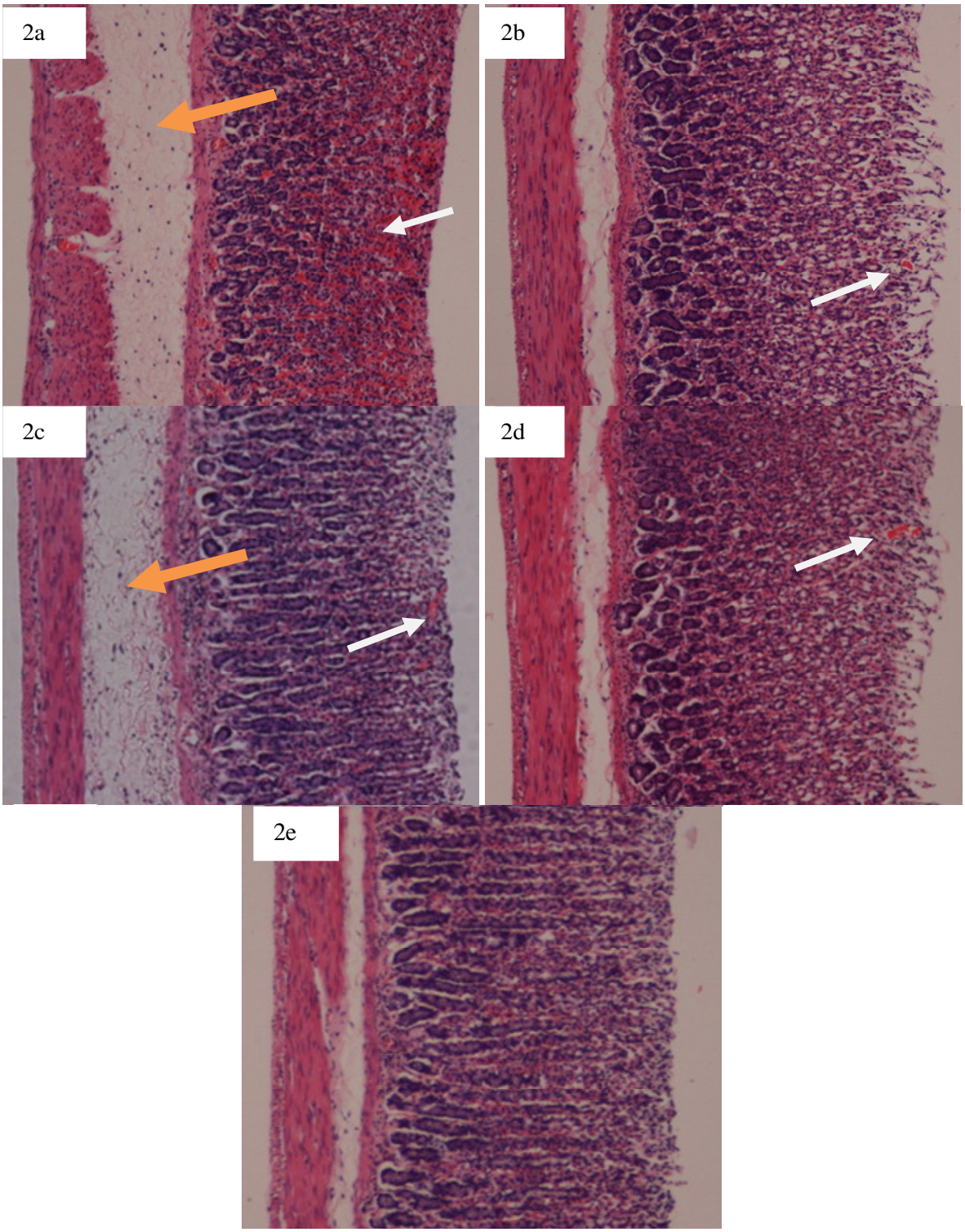

Figure 2. Histological study of the absolute ethanol-induced gastric mucosal damage in rats. 2a rats pre-treated with 5 ml/kg of 10% Tween 20 (ulcer control). There is severe disruption to the surface epitheliumand necrotic lesions penetrate deeply into mucosa (white arrow) and extensive edema of submucosa layer and leucocyte infiltration are present (orange arrow). 2b rats pre-treated with omeprazole (20 mg/kg). Mild disruption of the surface epithelium mucosa are present but deep mucosal damage is absent. 2c rat pre-treated with NSESE (250 mg/kg). Moderate disruption of surface epithelium are present but deep mucosal damage is absent. There is edema and leucocytes infiltration of the submucosal layer. 2d rat pre-treated with NSESE (500 mg/kg). Mild disruption of surface epithelium are present. 2e rats pre-treated with NSESE (750 mg/kg). There is no disruption to the surface epithelium with no edema and no leucocytes infiltration of the submucosal layer (H and E stain 10×).

products of the 5-Lipooxigenase pathway may also play a key role in the development of ulcer induced by irritant agents such as ethanol (Lange et al., 1985). NSESE prevented ethanol induced-gastric damage with mucous production increase. This may be explained with a correlation to a strengthening of the defense factors of gastric mucosa. It is evident that increased mucus production must have largely contributed to preventive effect of the NSESE. Similar findings exist in the literatures, where plant extracts have been shown to prevent gastric mucosal ulceration in rats (Wasman et al., 2010; Ketuly et al., 2011). The mucus of the gastric wall is thought to play an important role as a defensive factor against gastrointestinal damage (Wasman et al., 2010). Pretreatment with NSESE significantly decreases the acidity of the gastric content and increases the gastric mucus production. This suggests that gastro-protective effect of is mediated partly by preservation of gastric mucus production. 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).

Administration of antioxidants inhibits ethanol-induced gastric injury in rat (Ligumsky et al., 1995). NSESE possesses a broad spectrum of biological activities, and the plant extract has been shown to contain antioxidant (Meral et al., 2001) and it is speculated that the gastroprotective effect exerted by NSESE could be attributed to its antioxidant property. Antioxidant property of the NSESE may possibly counteract oxidative damage caused by absolute ethanol toxicity. The observed anti-ulcerogenic activity may be due to its antioxidant effects and appears to strengthen the mucosal barrier, which is the first line of defense against endogenous and exogenous ulcerogenic agents. NSESE has been shown to contain flavonoids (Merfort et al., 1997). Previous studies have shown that flavonoids may be related to the antiulcer activity (Hiruma-Lima et al., 2006), and play a major role in the mechanism of gastroprotection (La Casa et al., 2000). It could be conceivable that the anti-ulcer activity of this plant could be linked to the flavonoids since flavonoids are reported to protect the mucosa by preventing the formation of lesions by various necrotic agents (Saurez et al., 1996). It is well known that many flavonoids display anti-secretory and cytoprotective properties in different experimental models of gastric ulcer (Zayachkivska et al., 2005). Flavonoids possess anti-oxidant properties in addition to strengthening the mucosal defense system through stimulation of gastric mucus secretion (Martin et al., 1994) and flavonoids can scavenge for the reactive oxygen species (super-oxide anions) and free radicals produced by ethanol.

These reactive intermediates are potentially implicated in ulcerogenicity (Lewis and Hanson, 1991). The result of the present study also revealed protection of gastric mucosa and inhibition of leucocytes infiltration of gastric wall in rats pretreated with NSESE. This plant has been shown to contain anti-inflammatory activity (Hajhashemi et al., 2004) and it is speculated that the gastroprotective effect exerted by this plant extract could be attributed to its anti-inflammatory activity. This anti-inflammatory activity could also be a key factor in the prevention of gastric ulcer as reported by Swarnakar et al. (2005). Similarly, Abdulla et al. (2010) and Wasman et al. (2010) demonstrated that the reduction of neutrophil infiltration into ulcerated gastric tissue promotes the prevention of gastric ulcers in rats. Mahmood et al. (2010) and Wasman et al. (2010) showed that oral administration of plant extract before ethanol administration significantly decreased neutrophil infiltration of gastric mucosa. Absolute alcohol would extensively damage the gastric mucosa leading to increased neutrophil 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 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, neutrophil accumulation in gastric mucosa has been shown to induce gastric ulceration (Abdulla et al., 2010; Wasman et al., 2010; Ketuly et al., 2011). In the present study, NSESE induced flattening of the mucosal folds. Relaxation of circular muscles may protect the gastric mucosa through flattening of the folds. This will increase the mucosal area exposed to necrotizing agents and reduce the volume of the gastric irritants on rugal crest (Mahmood et al., 2010; Wasman et al., 2010). Ethanol produces a marked contraction of the circular muscles of rat fundic strip. Such a contraction can lead to mucosal compression at the site of the greatest mechanical stress, at the crests of mucosal folds leading to necrosis and ulceration (Abdulla et al., 2010).

In conclusion, NSESE could significantly protect the gastric mucosa against ethanol-induced injury. Such protection was ascertained grossly by increase gastric mucus production and decrease the acidity of gastric content were significantly higher in treated groups compare to ulcer control group and also the reduction of ulcer areas in the gastric wall as well as histology by the reduction or inhibition of edema and leucocytes infiltration of submucosal layers. The data obtained confirm the traditional indications for this herb and present a new therapeutic option for the treatment of gastric ailments. The exact mechanism (s) underlying this anti-ulcerogenic effect remain unknown, but it seems that this extract contains pharmacologically active substances with potent antioxidant and anti-inflammatory activity which increase the mucus production and decrease the acidity of gastric content.

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