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Research Article

Study of the Protective Effects of Flaxseed Oil on Ethanol Induced Gastric Mucosal Lesions in Non Ovariectomized and Ovariectomized Rats

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Abstract

Gastric and duodenal ulcers affect a considerable number of people in the world. Young women have a lower prevalence of ulcer than age-matched men. Female hormones have modulatory role on the vascular permeability and increase the mucus secretion. Administration of progesterone to female rats decreases duodenal ulcerations. Flaxseed oil has anti-inflammatory properties. The aim of this study was to show the protective effect of flaxseed oil against ethanol induced gastric ulcer. Rats were classified into non ovariectomized and ovariectomized negative control groups, positive control groups where ulcer was induced by 10 mL kg^{-1} ethanol 50% p.o., flaxseed oil (1.8, 3.6, 7.2 mL kg^{-1} p.o.) groups in addition to a non ovariectomized ranitidine (50 mg kg^{-1} p.o) group and an ovariectomized progesterone (18 mg kg^{-1} p.o.) group. Flaxseed oil had antioxidant and protective effect on gastric mucosa against ethanol ulcerogenic effect in both non ovarectomized and ovarectomized rat groups. Flaxseed oil can be used for prophylaxis against gastric ulcer in susceptible groups.

Key words: Gastric, ulcer, flaxseed oil, progesterone, ranitidine

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Gastric ulcer is considered as the new plague of the 21st century (O'Malley, 2003). The mechanisms which trigger gastric lesions have been studied in various experimental models (Sanchez *et al*., 2006). Ethanol has been shown to produce gastric damage by impairing gastric defensive factors, such as mucus and mucosa circulation (Szabo *et al*., 1992), as well as stress, smoking, nutritional deficiencies and frequent ingestion of nonsteroidal anti-inflammatory drugs (NSAIDs) (Belaiche *et al*., 2002). Moreover it has been stated in previous studies that oxidative stress plays an important role in the pathogenesis of gastric ulcer (Arslan *et al*., 2005).

Most studies have shown that young women have a lower prevalence of ulcer than age-matched men (Wu *et al*., 2008). Administration of progesterone to female rats decreases duodenal ulcerations. The mechanisms of this protective effect include the modulatory role of female hormones on the vascular permeability and an increase in the mucus secretion (Drago *et al*., 1999).

Ranitidine is one of the most potent histaminic H_2-receptor antagonists for inhibiting excessive histamine-induced acid secretion and is currently used worldwide to treat gastric ulcers (Ahmadi *et al*., 2011).

Flax plant, was originally cultivated in Egypt (Wisseman and Williams, 2013). Flaxseed oil comes from flaxseeds. The most common folk or traditional use of flaxseed is as a laxative. There are three groups of compounds in the flaxseeds, characterized by specific biological activity and functional properties: Alpha-Linolenic Acid (ALA) which is omega-3 family, linoleic acid (omega-6) and oleic acid (omega-9) soluble dietary fiber in the form of mucus and lignans, which have phytoestrogen properties. Use of flax seed oil in domestic food preparation has been able to reduce production of inflammatory cytokines (James *et al*., 2000). The hypolipidemic and anti-atherogenic effects of flaxseed are due to its content of ALA, dietary fibers and phytoestrogen lignans.

Flaxseed is highly rich in lignans which constitute up to 0.7-1.5% of the dry weight of the seed. When ingested in relatively large amounts, phytoestrogens have been shown to have significant estrogen modulating effects in animals and humans. Previous investigations have shown that flaxseed lignans have antioxidant properties (Chen *et al*., 2002). Flaxseed lignans can be used as natural antioxidants and may have a role in the prevention of oxidative stress. Flax seeds under the conditions of storage and processing technologies are harmless food product. Consumption of 50 g day^{-1} of flaxseed showed no adverse effects in humans (Martinchik *et al*., 2014).

The present study aimed at investigating the effect of flaxseed oil in three graded doses on ethanol induced gastric ulcer in normal and sex hormone depleted (ovariectomized) female rats, the latter are used to represent animal model mimicking postmenopausal females.

MATERIALS AND METHODS

Materials

Animals: Female albino Wistar rats (150-175 g b.wt.) obtained from Animal House Colony, National Research Centre, Dokki, Giza, Egypt were used in the experiment. All animals were housed in stainless steel cages in a temperature controlled (23±1°C) and artificially illuminated (12 h dark/light cycle) room free from any source of chemical contamination and were provided with a standard laboratory diet and water *ad libitum*. Animal procedures were performed in accordance with the Ethics Committee of the National Research Centre and followed the recommendations of the National Institutes of Health Guide for Care and Use of Laboratory Animals (NIH., 1985).

Drugs and chemicals: Ranitdine was purchased from Pharco, Egypt and was prepared as suspensions in 1% tween 80. Pure fortified flaxseed oil was purchased from Medizen Pharmaceutical Industries for NAPHA. Progesterone was purchased from Pharco, Egypt. Ethanol was purchased from MERCK Co. Inc-Rahaway, NJ, USA. Diethyl ether was purchased from Sigma Chemical Co., St. Louis, MO, USA.

Diagnostic kits: Kits for determination of reduced glutathione (R-GSH) in tissue homogenate and kits for determination of lipid peroxides (MDA) content in tissue homogenate were purchased from Biodiagnostic company, Egypt.

Methods

Ovarectomy procedure: The animals were anesthetized with thiopental. Bilateral ovarectomy was performed using a double-dorso-lateral approach according to the method described by Alkofahi and Atta (1999). All animals were left for four weeks before starting giving treatment with flaxseed oil, ranitidine or progesterone. Ovarectomy was done to ensure elimination of protective effect of female sex hormones on gastric mucosa.

Experimental design: Ninety six animals were used and were divided into five groups. The first three groups were subdivided into non-ovariectomized (non OVX) and

ovariectomized (OVX). The forth group consisted of non OVX rats only and the fifth group consisted of OVX rats only. Each group contained eight animals and were classified as follows:

- **1st group serves as negative control group divided into two subgroups:** (a) Non OVX rats and (b) OVX rats were given 1 mL tap water orally daily throughout the experiment
- **2nd group serves as positive control group divided into two subgroups:** (a) Non OVX rats and (b) OVX rats where induction of animal model of gastric ulcer by single oral administration of ethanol, 10 mL kg^{-1} of 50% ethanol (Alvarez *et al*., 1999), without prior treatment with the antiulcer tested agents was done
- **3rd group flaxseed protected group divided into two subgroups:** (a) Non OVX rats and (b) OVX rats each subgroup was further sub divided into three groups pretreated with daily oral administration of flaxseed oil (Tanna *et al*., 2012), in doses (1.8, 3.6,7.2 mL kg^{-1}), respectively for 4 successive weeks followed by induction of ulcer by ethanol, 10 mL kg^{-1} of 50% ethanol
- **4th group ranitidine protected group:** Non OVX rats: Pretreated with oral administration of ranitidine used as a reference drug in dose of 50 mg kg^{-1} (Alvarez *et al*., 1999), daily for 4 successive weeks followed by induction of ulcer by ethanol, 10 mL kg^{-1} of 50% ethanol
- **5th group progesterone group:** OVX rats pretreated with progesterone replacement therapy in dose (18 mg kg^{-1}) given orally daily for 4 successive weeks followed by induction of ulcer by ethanol, 10 mL kg^{-1} of 50% ethanol. Human dose of progesterone was converted to rat dose using according to Paget and Barnes (1964)

All animals were sacrificed 1 h after ethanol administration by cervical dislocation and their stomachs were excised for macroscopic, microscopic and biochemical examination.

Macroscopic examination: The stomach was opened along the greater curvature and gently rinsed with 0.9% NaCl. Then the gastric mucosa were examined by the use of millimetric acetate paper (El-Dakhakhny *et al*., 2000). Gross mucosal lesions were recognized as hemorrhage or erosions with damage to the mucosal surface. The number and severity of mucosal lesions were noted and lesions were scaled as follows: Petechial lesions = 1, lesions less than 1 mm = 2, lesion between 1 and 2 mm = 3, lesions between 2 and 4 mm = 4, lesions more than 4 mm = 5. A total lesion score for each animal is calculated as the total number of lesions and respective severity scores (Mozsik *et al*., 1982).

Biochemical assays: Gastric tissues, cut into small pieces were homogenized (2 min at 5000 rpm) in 4 V of ice-cold tris-HCl buffer (50 mmol L^{-1}, pH 7.4). Levels of reduced glutathione (R-GSH) according to Beutler *et al*. (1963) and lipid peroxides (MDA) were determined in tissue homogenates according to Ruiz-Larrea *et al*. (1994).

Histopathological examination: Specimens of all animals were dissected immediately after death and fixed in 10% neutral-buffered formal saline for 72 h at least. All the specimens were washed in tap water for half an hour and then dehydrated in ascending grades of alcohol (70-80-90% and finally absolute alcohol) cleared in xylene, impregnated in soft paraffin wax at 55°C and embedded in hard parrafin. Serial sections of 6 µm thick were cut and stained with haematoxylin and eosin (Drury and Wallington, 1980) for histopathological investigation. In PAS stain, McManus and Cason (1950) were also performed, to demonstrate the mucopolysaccharids content of this tissue in different samples.

All sections were investigated by the light microscope. Images were captured and processed using Adobe Photoshop version 8.0.

Statistical analysis: The data are expressed as Mean±SEM for each group. Results were analyzed using one way analysis of variance (ANOVA) followed by Tukey Kramer test for multiple comparisons. The $p< 0.05$ was considered as being significant in all types of statistical tests. Graph Pad Software (version 6) was used to carry out the statistical tests.

RESULTS

Macroscopic examination of stomach revealed normal gastric mucosa in non OVX as well as OVX negative control rats which were given 1 mL tap water orally daily throughout the experiment.

On the other hand, lesions with evident borderlines in various forms and sizes were dispersed irregularly on all stomach surfaces of untreated non OVX rats that received ethanol as the mean number of ulcers were 5.83 and severity was 18.17 while in untreated OVX rats that received ethanol the mean number of ulcers were 9.16 and severity was 24.17.

The protective efficacy of ranitidine on severity of ulcer in non OVX group was 85.36%, while those of the three doses of flaxseed were 69.73, 83.48 and 80.73%, respectively. On the other hand the protective efficacies of progesterone and the three doses of flaxseed in OVX rats were 26.23, 69.67, 71.74 and 73.1%, respectively. Regarding the protective efficacy on the number of ulcers in non OVX rats the efficacies of ranitidine and the three doses of flaxseed were 74.27, 68.61, 65.69 and 71.52%, respectively, while in OVX rats the efficacies of progesterone and three doses of flaxseed were 54.58, 50.87, 58.18 and 65.5%, respectively.

The severity of ulcer paralleled with an increase in ulcer numbers was illustrated in Fig. 1a and b. Oxidative stress biomarkers in stomach homogenates, estimation revealed that ethanol administration significantly decreased stomach contents of R-GSH in non OVX and OVX by 30.85 and 24.84%, respectively, concomitantly with an increase in MDA content by 58.91 and 59.03%, respectively, when compared to the negative control groups.

Administration of ranitidine and flaxseed (1.8, 3.6 and 7.2 mL kg^{-1}) to non overiectomized rats prior to administration of 50% ethanol, protected against ethanol-induced reduction of R-GSH by: 18.61, 19.85, 22.89 and 21.34%, respectively. While in OVX rats that received progesterone and the three doses of flaxseed prior to induction of ulcer by ethanol, the efficacies of both medications in protection against reduction of R-GSH were 16.67, 14.63, 21.24 and 21.05%, respectively.

Regarding MDA levels in non OVX groups given ranitidine as well as the three doses of flaxseed prior to induction of ulcer by ethanol, the efficacies of both medications in protection against increase in MDA levels were 45.71, 37.52, 44.27 and 33.46%, respectively. On the other hand administration of progesterone and the three doses of flaxseed in OVX groups, in the same regimen as in non OVX groups protected against increase in MDA levels by 50.21, 39.48, 37.88 and 34.34%, respectively.

The results of oxidative stress biomarkers R-GSH and MDA levels are illustrated in Table 1.

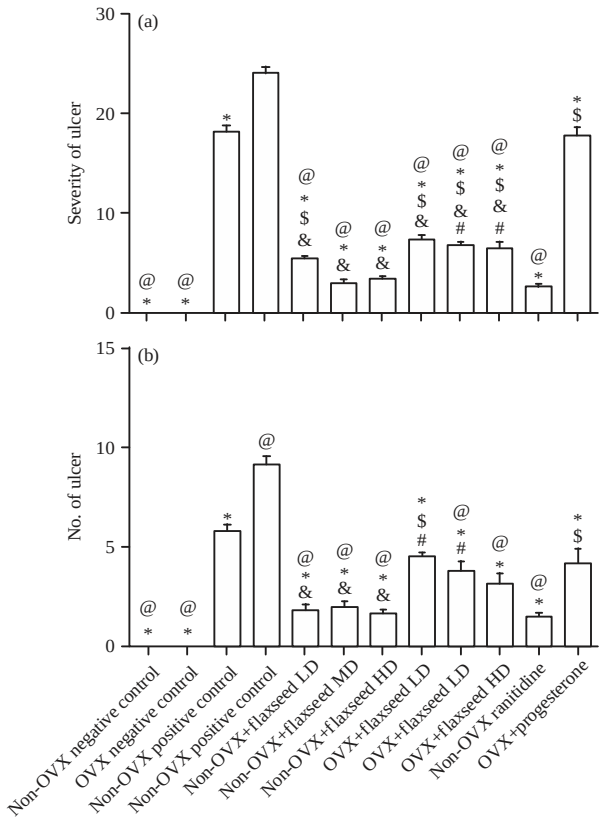

Fig. 1(a-b): Effect of flaxseed oil on severity and number of ulcers induced by ethanol (50%) in non-OVX and OVX rats, statistical analysis was done using one way analysis of variance (ANOVA) followed by Tukey Kramer test for multiple comparisons. N = 8, $p<0.05$, results are expressed as means of numbers and severity of ulcers+SEM, @: Significantly different from non-OVX ethanol group, *: Significantly different from OVX ethanol group, $: Significantly different from non-OVX Ranitidine group &: Significantly different from OVX progesterone group, #: Significantly different of OVX from non-OVX group receiving the same dose of flaxseed oil, flaxseed LD (1.8 mL kg^{-1}), flaxseed MD (3.6 mL kg^{-1}), flaxseed HD (7.2 mL kg^{-1}), LD: Low dose, MD: Medium dose and HD: High dose

Histopathological results: Examination of gastric mucosa in this study revealed that that in negative control rats the gastric tubular glands open in gastric pits lined with columnar Mucus Secreting Cells (MSC) (Fig. 2a). While ethanol had a damaging effect on this tissue as gastric mucosa of rats that received ethanol showed marked cellular infiltration either diffuse or aggregated and atrophy of cells in the upper half of gastric glands above (Fig. 2b). However, ovarectomy had a less damaging effect than that of ethanol (Fig. 2c) as gastric mucosa from rats subjected to ovarectomy ethanol showed multiple focal hemorrhagic areas, atrophy of superficial MSC and thickening of Muscularis Mucosa (MM). In Fig. 2d administration of ethanol after ovarectomy caused the

Table 1: Effect of flaxseed oil (1.8, 3.6 and 7.2 mL kg^{-1}) on R-GSH (μmol g^{-1} tissue) and MDA (nmol g^{-1} tissue) levels in ulcers induced by ethanol (50%, 10 mL kg^{-1}) in non-OVX and OVX rats

Groups parameters	R-GSH (μmol g^{-1} tissue)	MDA (nmol g^{-1} tissue)
Non-OVX negative control	67.15±1.30	13.75±0.34
OVX negative control	61.30±1.07$^{\pi@*}$	14.12±0.61
Non-OVX positive control (Ethanol 50%,10 mL kg^{-1})	46.43±0.27$^{\pi@*}$	33.47±0.46$^{\pi\beta}$
OVX positive control (Ethanol 50%,10 mL kg^{-1})	46.07±0.43$^{\pi@*}$	34.47±0.46$^{\pi\beta}$
Non-OVX flaxseed (1.8 mL kg^{-1})	57.93±1.41$^{\pi@*}$	20.91±0.74$^{\pi\beta@*\&}$
Non-OVX flaxseed (3.6 mL kg^{-1})	60.22±0.56$^{\pi@*}$	18.65±0.63$^{\pi\beta@*}$
Non-OVX flaxseed (7.2 mL kg^{-1})	59.03±1.33$^{\pi@*}$	22.27±1.01$^{\pi\beta@*\$\&}$
OVX+flax seed (1.8 mL kg^{-1})	53.97±1.86$^{\pi\beta@*}$	20.86±0.74$^{\pi\beta@*\&}$
OVX+flax seed (3.6 mL kg^{-1})	58.50±0.86$^{\pi@*}$	21.41±0.55$^{\pi\beta@*\$\&}$
OVX+flax seed (7.2 mL kg^{-1})	58.36±0.65$^{\pi@*}$	22.63±0.43$^{\pi\beta@*\$\&}$
Non-OVX ranitidine (50 mg kg^{-1})	57.05±1.11$^{\pi@*}$	18.17±0.59$^{\pi\beta@*}$
OVX+progesterone (18 mg kg^{-1})	55.29±1.46$^{\pi\beta@*}$	17.16±0.35$^{\pi\beta@*}$

Statistical analysis was done using one way analysis of variance (ANOVA) followed by Tukey Kramer test for multiple comparisons. N = 8, $p<0.05$, Results are expressed as means of numbers and severity of ulcers+SEM, π: Significantly different from non-OVX negative control group, β: Significantly different from OVX negative control group, @: Significantly different from non-OVX positive control group, *: Significantly different from OVX positive control group, $: Significantly different from non-OVX Ranitidine group &: Significantly different from OVX progesterone group, #: Significantly different OVX from non-OVX group receiving the same dose of flaxseed oil, MDA: Lipid peroxide and R-GSH: Reduced gluthathione

result to be worse as there was severe atrophy of gastric glands causing ulceration over a wide area. The damaging effect of ethanol on gastric mucosa in OVX rats pretreated with progesterone prior to ethanol administration was ameliorated (Fig. 2e and 3e) as gastric mucosa were intact but with mild atrophy of superficial MSC and mild cellular infiltration between gastric pits and blood capillary dilatation is observed in MM layer. Ranitidine had an incomplete ameliorating effect on the damaging effect of ethanol on gastric mucosa (Fig. 2f) rats that received ranitidine before ethanol showed distortion of MSC and gastric pits with diffuse cellular infiltration at the base of the glands.

Using flaxseed oil (1.8, 3.6 and 7.2 mL kg^{-1}) before ethanol revealed that this oil had an ameliorating effect, decreasing the damaging effect of ethanol on gastric mucosa and this effect was dose dependent in non OVX as well as OVX rats (Fig. 3). In Fig. 3a-c, gastric mucosa of non OVX rats that received low dose of flaxseed showed detachment of superficial MSC and hemorrhage in between gastric glands, while non OVX rats that received medium dose of flaxseed showed hemorrhage in between the upper halves of gastric glands with atrophy of cells and mild cellular infiltration at the base of glands with thickening of muscularis mucosa. Regarding non OVX rats that received high dose of flaxseed gastric mucosa showed mild regeneration of superficial MSC and mild cellular infiltration at the base of the glands. As for OVX rats pretreated with flaxseed oil in the three doses before ethanol administration: In Fig. 3d-f, gastric mucosa of rats that received low flaxseed dose showed cellular infiltration either diffuse or aggregated at the bottom of the glands and blood vessels dilatation. Gastric mucosa of rats that received medium dose of flaxseed showed beginning of regeneration of gastric pits with mild cellular infiltration beneath. While gastric mucosa of rats that received high dose of flaxseed before ethanol shows more or less normal gastric mucosa.

Using PAS stain to demonstrate the mucopolysaccharide content emphasize these results as noticed in Fig. 4a, which represents a negative control rat and shows the normal content of mucopolysaccharides in its tissue. There is decrease in the amount of this mucopolysaccharide noticed in non OVX rats that received ethanol and that were subjected to ovariectomy without receiving ethanol (Fig. 4b and c) and much decrease in the group subjected to ovariectomy then received ethanol (Fig. 4d). Using progesterone or ranitidine ameliorated this effect with different degrees (Fig. 4e and f).

By using the flaxseed oil three doses in non OVX rats before administration of ethanol got better results than that obtained with progesterone (Fig. 5a and b) and in high dose the result was slightly better than that of randitine (Fig. 5c).In OVX rats that were given flaxseed oil (1.8, 3.6 and 7.2 mL kg^{-1}) before ethanol low dose showed a very thin film of positive result on the surface of tissue with a very weak positive result in the gastric glands (Fig. 5d) while medium dose showed noticeable increase in positivity for the stain both on the surface and in the glands (Fig. 5e). Moreover, high dose showed thick mucous membrane on the surface and strong positive result for the stain in the neck of the gastric glands denoting marked increase in mucopolysaccharide content of the tissue.

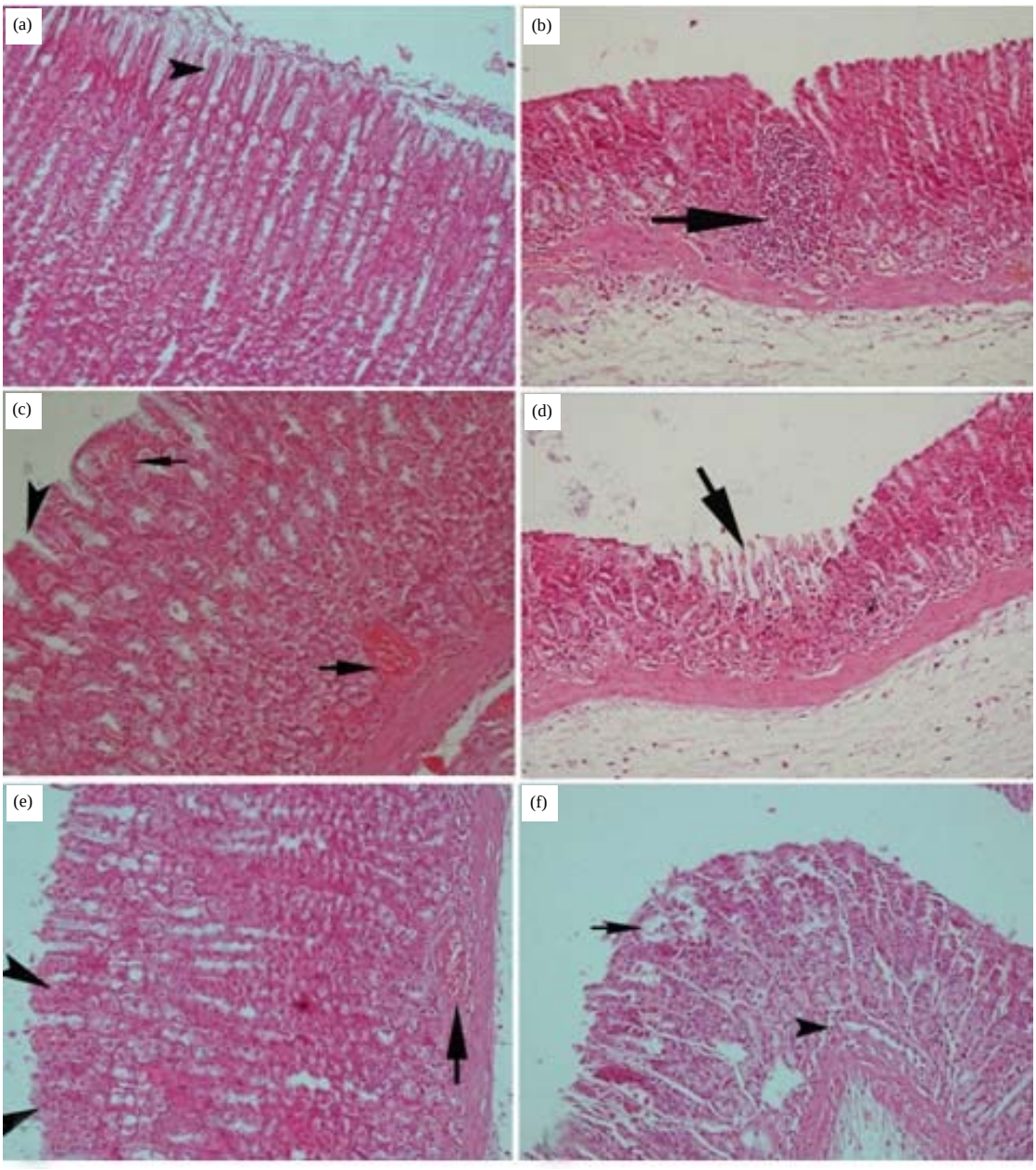

Fig. 2(a-f): Photomicrograph of sections of gastric mucosa from (a) Negative control rat shows the gastric tubular glands which open in gastric pits (arrowhead) lined with columnar Mucus Secreting Cells (MSC), (b) Rat received ethanol shows marked cellular infiltration either diffuse or aggregated (arrow) and atrophy of cells in the upper half of gastric glands above, (c) From a rat subjected to ovarectomy shows multiple focal hemorrhaged areas (arrow), atrophy of superficial MSC (arrowhead) and thickening of Muscularis Mucosa (MM), (d) From a rat subjected to ovarectomy and received ethanol shows severe atrophy of gastric glands causing ulceration over a wide area (arrow), (e) From a rat subjected to ovarectomy and received progesterone before ethanol shows intact gastric mucosa but with mild atrophy of superficial MSC and mild cellular infiltration between gastric pits (arrow head), Blood capillary dilatation is observed in MM layer (arrow) and (f) From a rat received ranitidine before ethanol shows distortion of MSC and gastric pits (arrow) with diffuse cellular infiltration at the base of the glands (arrowhead) and Hx and E x200

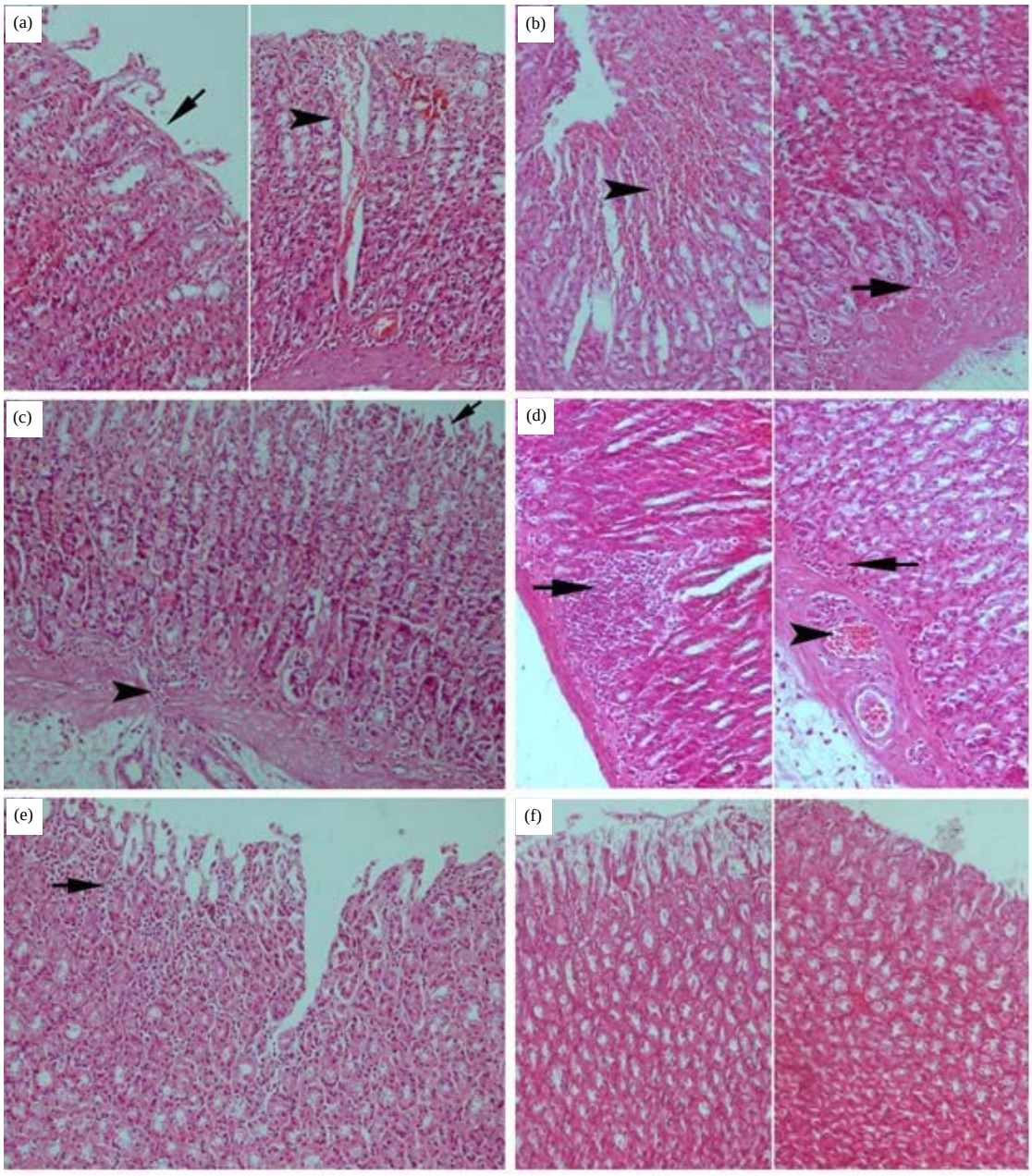

Fig. 3(a-f): Photomicrograph of sections of gastric mucosa, (a) From a non OVX rat received low dose of flaxseed before ethanol shows detachment of superficial MSC (arrow) and hemorrhage in between gastric glands (arrowhead), (b) From a non OVX rat that received medium dose of flaxseed before ethanol shows hemorrhage in between the upper halves of gastric glands with atrophy of cells (arrowhead) and mild cellular infiltration at the base of glands with thickening of muscularis mucosa (arrow), (c) From a non OVX rat received high dose of flaxseed before ethanol shows mild regeneration of superficial MSC (arrow) and mild cellular infiltration at the base of the glands (arrowhead), (d) From an OVX rat received low flaxseed before ethanol shows cellular infiltration either diffuse or aggregated at the bottom of the glands (arrow) and blood vessels dilatation (arrowhead), (e) From a rat received medium dose of flaxseed before ethanol shows beginning of regeneration of gastric pits with mild cellular infiltration beneath (arrow) and (f) from a rat received high dose of flaxseed before ethanol shows more or less normal gastric mucosa (Hx and E x200)

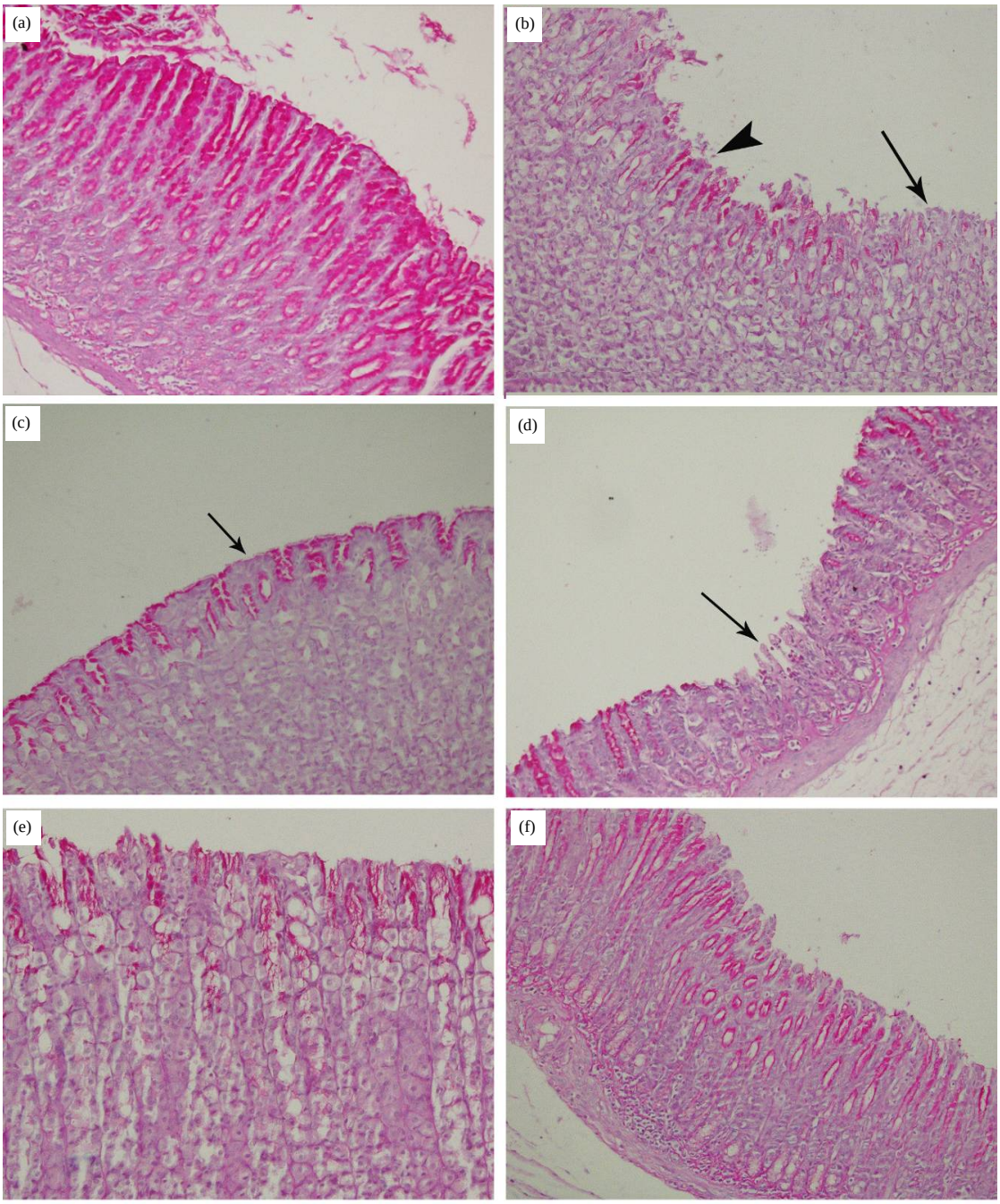

Fig. 4(a-f): Photomicrograph of sections of gastric mucosa from (a) A negative control rat shows the normal content of mucopolysaccharides in this tissue, (b) From a non OVX rat received ethanol shows marked decrease of the positive result of the stain in the gland (arrow head) and absence of positivity on the upper surface (arrow), (c) From a rat subjected to ovarectomy without receiving ethanol shows focal discontinuation of positivity [arrow] with noticeable decrease of positive result in the gland, (d) From a rat subjected to ovarectomy and received ethanol shows complete abolish of positive result to the stain at a wide area, (e) From a rat subjected to ovarectomy and received progesterone before ethanol shows absence of positive result at many places on the surface with marked decrease in positivity of the gland and (f) From a rat received ranitidine before ethanol shows amelioration if the mucopolysaccharide content of the glands (PAS x 100 and 200)

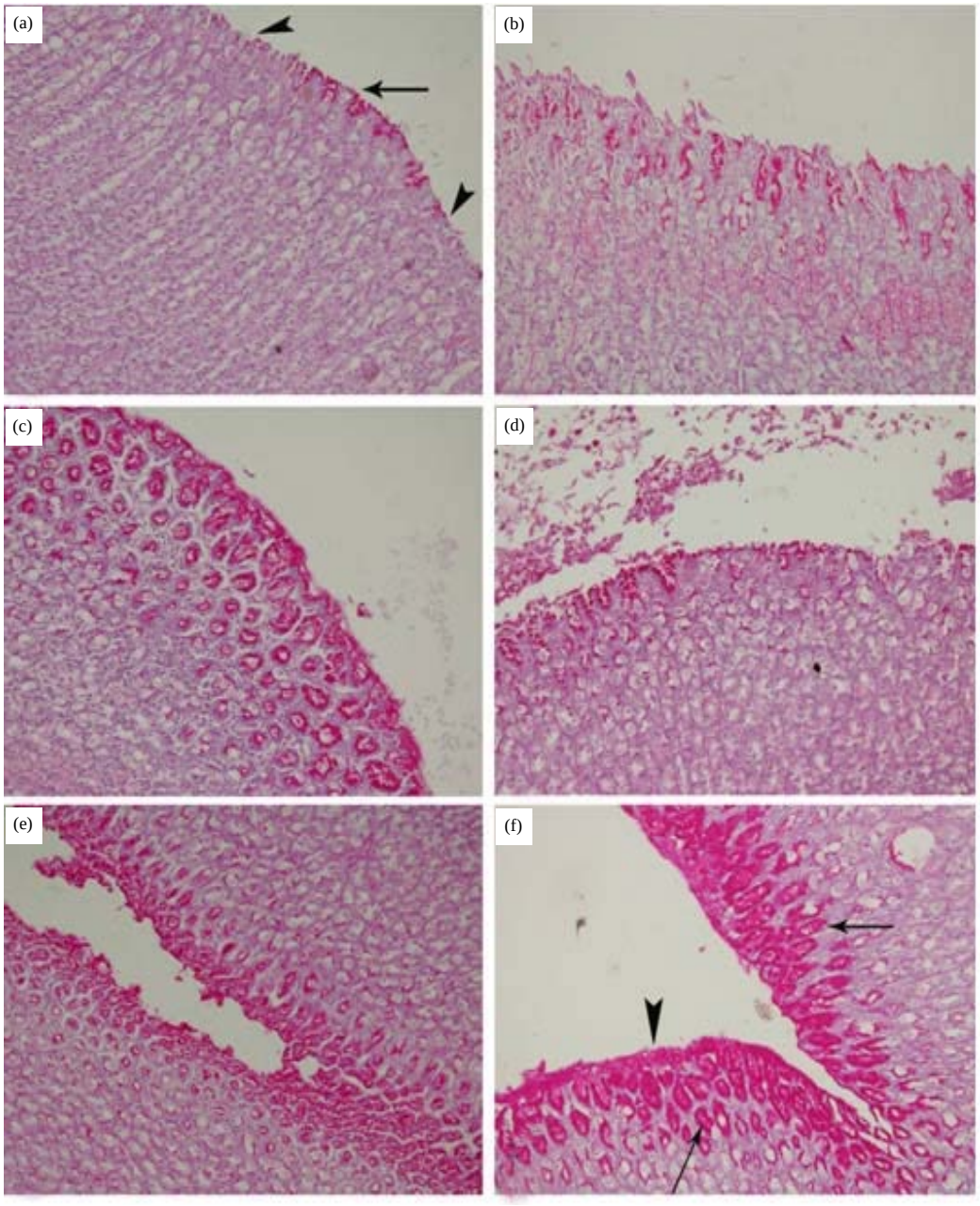

Fig. 5(a-f): Photomicrograph of sections of gastric mucosa, (a) From a non OVX rat received flaxseed low dose before ethanol shows absence of mucous membrane on the surface at many places (arrow head) and decrease of mucopolysaccharide content of the glands (arrow), (b) From a non OVX rat received medium dose of flaxseed before ethanol shows mild improvement of mucopolysaccharide content, (c) From a non OVX rat received high dose of flaxseed before ethanol shows strong positive result for the stain in the upper half of gastric glands and on the surface denoting increase in mucopolysaccharide content, (d) From an OVX rat received low dose of flaxseed before ethanol shows a very thin film of positive result on the surface of tissue with a very weak positive result in the gastric glands, (e) From an OVX rat received medium dose of flaxseed before ethanol shows noticeable increase in positivity for the stain both on the surface and in the glands and (f) from an OVX rat received high dose of flaxseed before ethanol shows thick mucous membrane on the surface and strong positive result for the stain in the neck of the gastric glands denoting marked increase in mucopolysaccharide content of the tissue (PAS x200)

DISCUSSION

In the present study, the ameliorating effect of flaxseed oil on gastric ulcer induced by ethanol, was studied in two models non OVX female rats and OVX female rats representing animal model mimicking postmenopausal females who are more subjected to gastrointestinal disorders as a result of stress and absence of the protective effect of female sex hormones.

In the present study, the results of macroscopic examination as well as histopathologic examination of gastric mucosa and oxidative stress biomarkers were concomitant with each other.

The ulcerogenic effect of ethanol associated with increase in MDA and decrease in R-GSH level in both non OVX and OVX rats in this study are in accordance with Szelenyi and Brune (1988), who stated that oxygen-derived free radicals generated by ethanol may cause gastric damage. Moreover the ulcerogenic effect of ethanol is most probably due to direct toxic action, reduction of the secretion of bicarbonate and depletion of gastric wall mucus. It causes hemorrhagic ulceration of the stomach by causing extensive damage to mucosal capillaries resulting in increased vascular permeability, oedema formation and epithelial lifting (Nordmann, 1994).

In the present study, ranitidine had a protective effect on ethanol induced gastric ulcer as it is a histaminic H_2-receptor antagonist so it caused a reduction in total acid output and reduction in ulcer severity and number. Moreover, the antioxidant effect of ranitidine in our study may be due to what was previously reported by Ahmadi *et al*. (2011) who stated that ranitidine (1 mM) was able to reduce the iron-induced rise in lipid peroxidation in rat brain homogenates, proving that ranitidine has antioxidant properties.

Also progesterone had prophylactic effect on gastric mucosa against ulcers in rats who underwent ovariectomy, this may be due to increased blood flow at the edge of gastric ulcers as stated by Machowska *et al*. (2004). The protective activity of progesterone depends also upon the increase in the production of mucus by gastric mucosa (Montoneri and Drago, 1997). So, hormonal replacement with progesterone might be beneficial in the course of healing of gastric ulcers.

The three doses of flaxseed oil used in this study, were effective in preventing development of ethanol-induced gastric ulcers, hence, protecting the gastric mucosa against such lesions. The effects of flaxseed had significantly prophylactic effects in non OVX as well as OVX rats against ethanol induced ulcer in dose dependent manner. Since lignans with phytoestrogen properties are particularly abundant in flax seed and when ingested in relatively large amounts have been shown to have significant estrogen modulating effects in animals and humans and since lignans also possess antioxidant activity, according to Martinchik *et al*. (2014), this may explain the protective effects of flaxseed oil on gastric mucosa against ulcer and their effect on oxidative stress biomarkers in non OVX and OVX rats.

Another explanation for the prophylactic effects of flaxseed oil in this study is that supplementation with flax seed oil can effectively increase eicosapentaenoic acid (EPA; the same omega-3 found in fish oil) concentrations in tissues (Mantzioris *et al*., 1994), EPA, in turn, has the ability to convert into series-3 prostaglandins which have anti-inflammatory properties. Many factors contribute to the complex course of inflammatory reactions, including the omega-6 fatty acid, Arachidonic Acid (AA). The AA can be converted via an enzymatic process into proinflammatory substances, including series-2 prostaglandins, leukotrienes and cytokines. In states of inflammation, it seems that omega 3 fatty acid is able to compete with AA for enzymatic metabolism, which results in less production of inflammatory substances (Heller *et al*., 1998). Also use of flax seed oil in domestic food preparation has been able to reduce production of inflammatory cytokines (James *et al*., 2000).

CONCLUSION

The main conclusion in this study is that Flaxseed oil in three dose levels (1.8, 3.6 and 7.2 mL kg^{-1}) had protective effect against gastric ulcer in non OVX rats receiving ethanol 50%, representing animal model mimicking gastric ulcer in premenopausal human females as well as in OVX rats receiving ethanol 50%, representing animal model mimicking gastric ulcer in postmenopausal human females. However, the protective effect was more evident on non OVX groups comparable to OVX groups receiving the same doses, in most parameters. This could be due to the aiding protective effect of endogenous female sex hormones.

Also progesterone had some prophylactic effects in OVX rats against gastric ulcer induced by ethanol. This also may emphasize the importance of hormonal replacement therapy with progesterone after menopause for protection against gastric ulcer.

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