THE POSSIBLE PROTECTIVE ROLE OF CURCUMIN ON NICOTINE INDUCED DAMAGE OF GASTRIC MUCOSA OF ADULT MALE ALBINO RATS

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Received: January 1, 2017; Revised: January 10, 2017; Accepted: January 25, 2017

Abstract: Nicotine has been reported to cause deleterious changes on gastric mucosa such as gastritis, gastric ulcer and even has been well documented to be closely related to the development of gastric cancer. This study was carried out to evaluate the possible gastroprotective effect of curcumin along with its effect on cyclooxygenases against cytotoxicity of nicotine in gastric mucosa. Forty five adult male albino rats were randomly divided into three groups of fifteen animals in each group; group I (Control), group II: each rat was given oral nicotine (50 μg/ml) once daily, group III: each rat was given oral curcumin (200mg/kg) body weight once daily concomitant with nicotine with similar dose as group II. All animals were treated for 21 days and were sacrificed at the end. The stomach was processed for histological and immunohistochemical studies. Nicotine administration induced erosion and disruption of fundic gastric mucosa with exfoliated remnant of gastric glands associated with dilated, congested blood vessels and apical new vascularization. Marked decrease in PAS positive mucus with highly significant decrease in COX-1 expression and a highly significant increase in COX-2 expression were observed. Concomitant use of curcumin with nicotine showed considerable degree of preservation of gastric mucosa and PAS positive mucus. Anon significant decrease in COX-1 expression as well as a significant increase in COX-2 expression were observed. In conclusion, curcumin showed a protective role against the harmful effects of nicotine on the gastric mucosa.

Key words: Nicotine, Gastric mucosa, Curcumin, Cyclooxygenase

INTRODUCTION

Gastric ulcer is one of the harmful effects of nicotine on the gastrointestinal tract. Currently, nicotine use is a growing global problem as it is widely advocated for use in different ways such as cigarettes, cigars, pipe as well as electronic cigarettes and shisha. Nicotine is one of the most heavily addictive drugs and continues to be the leading cause of preventable death and is the main risk factor for major diseases such as chronic obstructive pulmonary disease [1,2]. Nicotine has been shown to increase the risk of infection with the bacterium Helicobacter pylori, reduction in the healing rate of gastric and duodenal ulcers. Also, long term oral nicotine induced gastric bleeding ulcers [1,3]. Nicotine potentiates gastric aggressive factors and attenuates defensive factors. It increases acid and pepsin secretions, gastric motility, duodenogastric reflux of bile salts, platelet-activating factor, endothelin generation, vasopressin secretion, vascular endothelial growth factor and fibroblast growth factor [4]. Other researchers added that...
nicotine increases the free radical-mediated mutations, and carcinogenic changes [5]. Also, it was discovered that nicotine induces proliferation of human adenocarcinoma cells via a cyclooxygenase (COX)/lipoxygenase–dependent pathway to metabolize fatty acids into eicosanoids [4,6].

Cyclooxygenase (COX), exists as two isoforms (COX-1 and COX-2). COX-1 enzyme is continuously stimulated by the body and expressed in many tissues, including the gastrointestinal tracts. Accordingly, COX-1 mRNA and protein are abundant in the gastric mucosa and maintain mucosal integrity through continuous generation of prostaglandins [7,8]. On the other hand, COX-2 characterized by a rapid inducibility in response to various proinflammatory stimuli or mitogenic agents, including cytokines, endotoxins, and tumor promoters. COX-2 mRNA and protein in inflamed tissues are mainly located in inflammatory cells but are also expressed in endothelial cells, fibroblast-like cells and epithelial cells [9]. In animal models, there is increasing evidence that COX-2 expression can be induced by mucosal injury. Moreover, COX-2 is responsible for pathological prostaglandins production at inflammatory sites [9,10].

Many studies had been conducted to evaluate the effect of curcumin as a gastro-protective material [11-13]. Curcumin (diferuloyl methane), is a phytochemical which is a major active ingredient of turmeric and giving turmeric its characteristic radiant orange–yellow color. The turmeric crystalline powder derived from the rhizome of the Curcuma longa plant. It is extensively used as a spice, food preservative and colouring material in India, China and South East Asia [14].

Curcumin possesses a broad spectrum of biological actions including anti-inflammatory with therapeutic properties that have very little or no toxicity in humans, anti-carcinogenic, anti-mutagenic, anti-coagulant, anti-diabetic, anti-bacterial, anti-venom, anti-protozoal, anti-fibrotic, hypocholesteremic and anti-aging properties [15,16]. It was found that curcumin offered protection against vascular dementia by exerting antioxidant activity as it was a potent scavenger of a variety of reactive oxygen species including superoxide anion radicals, hydroxyl radicals and nitrogen dioxide radicals [16]. In addition to that, it was able to ameliorate serum biochemical parameters, enzymatic and non-enzymatic antioxidant defense system, mucus secretion and prevent DNA fragmentation in gastric tissue [11].

Nowadays there is increase of using nicotine in modern life, so the aim of this work is to study the potential protective role of oral administration of curcumin on the cytotoxic effects of nicotine on gastric mucosa of adult male albino rats.

**MATERIALS AND METHODS**

**Experimental animals:** In this work, forty five adult male albino rats weighing between 150 and 200 g each were obtained from animal center in King Faisal University. All rats were placed in clean properly ventilated cages and were acclimatized for 7 days on a 12:12-h light-dark cycle before the experiment and were kept with ordinary laboratory animal diet and tap water. All ethical protocol for animal treatment were followed. The experimental protocol was accepted by the local Animal Care Committee of King Faisal University.

**Chemical:** Curcumin powder and nicotine hydrogen tartrate salt (SML 1236) were procured from Sigma Chemical Co., (St. Louis, Mo, USA). Curcumin powder was dissolved in corn oil and nicotine was dissolved in distilled water.

**Experimental design:** Rats were divided randomly into three groups included 15 rats for each as follows:

**Group I (control group) (n=15):** Animals were equally subdivided into three subgroups. Viz., Subgroup (1a) (n=5): animals received no treatment and were used to study the normal histological structure of the stomach (fundic gastric mucosa). Subgroup (1b) (n=5): each animal received corn oil orally at a single daily dose (2 ml) for 21 consecutive days. Subgroup (1c) (Curcumin-treated) (n=5): each animal received curcumin at an oral dose of 200 mg/kg body weight dissolved in corn oil as vehicle once daily for 21 consecutive days[17].

**Group II (nicotine-treated group) (n=15):** Animals received nicotine hydrogen tartrate salt dissolved in drinking distilled water at an oral dose of 50 μg/ml for 21 consecutive days [18].
**Group III (nicotine/curcumin-treated group)** *(n=15):* Animals received curcumin dissolved in corn oil at an oral dose of 200 mg/kg body weight once daily in concomitant with nicotine hydrogen tartrate salt dissolved in drinking distilled water at an oral dose of 50 μg/ml for 21 consecutive days.

**Histological study:** Twenty four hours following the end of the experiment. All animals were anesthetized with ip. injection of thiopental sodium at a dose of 40 mg/kg [19] and sacrificed. The abdomen was opened; the stomach was removed, opened along the greater curvature and washed with saline. After that the fundus divided into parts (each was about 1 cm x 1 cm in size), were placed in 10% neutral buffered formalin fixative for 24 hours then subjected to the normal procedures for paraffin sectioning. Sections of, 5 μm thickness were stained with hematoxylin and eosin stain for general structure and PAS stain for mucin [20]. The stained sections were examined and photographed by light microscopy.

**Immunohistochemical study:** Tissue sections were prepared for immunohistochemical staining for COX-1 and COX-2. Immunohistochemical staining was carried following the manufacturer instructions. Paraffin sections on charged slides were deparaffinized and rehydrated. They were incubated in hydrogen peroxide to block the endogenous peroxidase for 10-15 minutes, washed in buffer citrate and incubated for 30 minutes with rabbit serum. Then, some sections (from each group) were incubated with primary antibodies for COX-1 (RB-10687-R7) and other sections (from each group) were incubated with primary antibodies for COX-2 (RB-9072-R7) obtained from Lab Vision, USA; at a dilution of 1:200, at room temperature for 20 minutes followed by washing with PBS and co-incubated with biotinylated secondary anti-rabbit antibodies universal kits (K0673, Lab Vision, USA) at room temperature for 10 minutes. Thereafter, sections were stained with 3,3’-diaminobenzidine (DAB) solution to brown color as a chromogen, at room temperature for 10 min. The sections were washed, counterstained with Harris’s hematoxylin according to standard protocols, dehydrated and cleared in xylene and cover slipped. For each animal, five different sections were examined. Some sections were running without using the primary antibody and served as negative control.

**Morphometric study:** Images were obtained using an Olympus light microscope (BX50, Tokyo, Japan) coupled to an Olympus digital camera (C-7070, Tokyo, Japan). Image analysis was performed using software “ImageJ” (National Institute of Health, Bethesda, Maryland, USA). Fundic mucosa specimens from each group were examined for the mean areas percentage of positive immunohistochemical reaction for COX-1 and COX-2 (in anti-COX-1 and anti COX-2 -stained sections). 10 different non-overlapping randomly selected fields at a magnification of 400 were examined in each slide. The areas percentage was calculated by subtracting the brown color from the different colors of positively stained specimens in serial sections [7].

**Statistical analysis:** The data were analyzed using one-way analysis of variance (ANOVA) test followed by Tukey’s procedure for comparison between the groups using the statistical package for the social sciences software (SPSS Inc., Chicago, IL).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control</th>
<th>Group II</th>
<th>Group III</th>
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<tbody>
<tr>
<td>Mean ± SD</td>
<td>11.892 ± 2.44</td>
<td>3.184 ± 0.67</td>
<td>9.401 ± 2.43</td>
</tr>
<tr>
<td>P value</td>
<td>0.000</td>
<td>&lt; 0.001**</td>
<td>0.15</td>
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**Table 1:** Showing the mean area percentage of COX-1/field in different groups compared to control group I. Data are expressed as mean ± standard deviation, *P < 0.05 is significant, **P < 0.001 is highly significant versus control respectively.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD</td>
<td>1.304±0.48</td>
<td>3.861±0.86</td>
<td>1.858±0.48</td>
</tr>
<tr>
<td>P value</td>
<td>0.000</td>
<td>&lt; 0.001**</td>
<td>&lt; 0.05*</td>
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**Table 2:** Showing the mean area percentage of COX-2/field in different groups compared to control group I. Data are expressed as mean±standard deviation, *P < 0.05 is significant, **P < 0.001 is highly significant versus control respectively.
Illinois, USA). All values were expressed as mean ± SD. Differences were regarded as significant if P value was less than 0.05 [21].

RESULTS

Histological results

Group I (control group): Hematoxylin and eosin and PAS stained sections from control subgroups Ia, Ib and Ic revealed similar normal histological structure of fundic gastric mucosa. The sections showed gastric mucosa composed of three layers; surface epithelium, lamina propria occupied by simple branched tubular fundic glands (isthmus, neck and base) and muscularis mucosa (Fig. 1). The luminal surface was covered by surface epithelium which is simple tall columnar cells (surface mucous cells) with mild basophilic cytoplasm, basal oval nuclei and apical vacuolated cytoplasm. Lamina propria is composed of thin loose connective tissue with smooth muscle fibers visible between the gastric glands. The gastric glands appeared narrow, straight, perpendicular to the surface epithelium and open into the surface by short narrow gastric pits (Fig. 2).

Each gastric gland was formed of isthmus region which was dominated with surface mucous cells and neck region which was dominated with both mucous neck cells and parietal cells. The parietal cells were polyhedral cells with acidophilic cytoplasm and central rounded nuclei (Fig. 2). The base of the gland was lined by small cuboidal chief cells with basal rounded nuclei and deeply basophilic cytoplasm together with occasional parietal cells and mucous secreting cells (Fig. 3). PAS stained sections showed intense PAS positive reaction on the surface of the mucosa, extended into the gastric pits and neck region of the glands (Fig. 4).

Group II (nicotine-treated group): H&E stained sections of this group showed, the luminal surface of gastric mucosa with many superficial areas of disruption and erosions at the surface epithelium. Dilated and congested blood vessels in lamina propria and submucosa were seen (Figs. 5,6). Mononuclear cellular infiltration and lysis of some parts of gastric glands were obviously noticed (Fig. 6). In the eroded areas the superficial cells showed discontinuity with pyknotic nuclei (Fig. 7). Focal areas of luminal surface showed surface mucous cell with foamy cytoplasm and others appeared with vacuolated cytoplasm.

Explanations of figures

Fig (1): A photomicrograph of a rat gastric mucosa from group I (control group) showing normal architecture of gastric mucosa, gastric pits (P) & gastric glands. The gastric glands are divided into isthmus, neck & base. Muscularis mucosa (M) is also seen. Notice short gastric pits (P) contain mucous. H&E X100

Fig (2): A photomicrograph of a rat gastric mucosa from group I (control group) showing apical part with surface mucous tall columnar cells (thin arrows) extending down into gastric pits (P). Mucous neck cells (arrow heads) contain obvious mucin, vacuolated cytoplasm and parietal cells (wavy arrows) with acidophilic cytoplasm and central rounded nuclei are also seen. Notice lamina propria with smooth muscle fibers (thick arrow). H&E X400

Fig (3): A photomicrograph of a rat gastric mucosa from group I (control group) showing bases of gastric glands contain chief cells (thin arrows) with basal basophilia and basal round nuclei. Also the parietal cells (wavy arrows) with granular acidophilic cytoplasm and central rounded nuclei and mucous secreting cells (arrow heads) with foamy cytoplasm are seen. Notice muscularis mucosa (M). H&E X400

Fig (4): A photomicrograph of a rat gastric mucosa from group I (control group) showing intensely stained film of PAS positive mucus reaction (thin arrows) covering the surface and extending down into the gastric pits (thick arrows). The surface columnar cells (wavy arrows) and mucous neck cells (arrow heads) are also containing PAS positive mucus reaction. PAS X400

Fig (5): A photomicrograph of a rat gastric mucosa from group II (nicotine-treated group) showing many superficial eroded areas (arrow heads) with shedded cellular debris into the lumen (thin arrow) and focal deep erosion (thick arrow). Dilated and congested blood vessels in the basal part of mucosa (V) and in the submucosa (Vm) are observed. H&E X100.

Fig (6): A photomicrograph of a rat gastric mucosa from group II (nicotine-treated group) showing lysis (S) of some parts of gastric glands, apical dilated and congested blood vessels (V). Mononuclear cellular infiltration (arrow) is also seen. H&E X200

Fig (7): A photomicrograph of a rat gastric mucosa from group II (nicotine-treated group) showing apical part with superficial and deep eroded areas (thin arrows). Surface mucous cells (arrow heads) showing vacuolated cytoplasm with pyknotic nuclei and no mucus granules. Abundant parietal cells (wavy arrows) with deeply acidophilic cytoplasm are seen. Notice some parietal cells appear with cytoplasmic vacuulation and pyknotic nuclei (thick arrows). H&E X400.
cytoplasm and pyknotic nuclei (Fig. 8). Abundant parietal cells were seen crowded and large. Some of them showed vacuolated cytoplasm with pyknotic nuclei and others appeared with deeply acidophilic cytoplasm and dark nuclei (Figs. 7, 8). The base of gastric glands showed focal areas with distorted shape and dilated spaces (Fig. 9a). Many chief cells and parietal cells appeared with vacuolated cytoplasm. Mononuclear cellular infiltration and dilated blood vessels also observed (Fig. 9b). PAS stained sections showed damage of surface of the gastric mucosa with decrease in PAS positive mucous on the surface of the gland, in both surface columnar epithelial cells and mucous neck cells (Fig. 10).

**Group III (nicotine and curcumin-treated group):** H&E stained sections of this group showed a picture nearly resembling those of the control group (group I). Some dilated and congested blood vessels in muscularis mucosa were observed (Fig. 11). Apical parts of the gastric glands showed preserved normal architecture. Parietal cells appeared less abundant as compared to the previous group (nicotine-treated) with acidophilic cytoplasm and central round nuclei (Fig. 12). The base of some gastric glands showed dilated lumen filled with acidophilic secretion. Parietal cells appeared with acidophilic cytoplasm and central round nuclei along with chief cells that showed deeply basophilic cytoplasm and central round nuclei (Fig. 13). PAS stained sections revealed intense positive reaction of surface mucosa and neck of glands as compared to control group (Fig. 14).

**Immunohistochemical results:**

**COX-1 expression:** Sections of the control group (subgroups Ia, Ib and Ic) stained immuno histochemically for COX-1 expression revealed similar pictures of a strong positive reaction in the lamina propria of the gastric mucosa. Apical areas of gastric mucosa (Fig. 15), basal areas of gastric mucosa and the endothelial cells lining the blood vessels showed intense positive COX-1 immunoreaction (Fig. 16). In group II (nicotine-treated group) showed marked decrease of COX-1 expression in the apical part of gastric mucosa (Fig. 17) and basal part of gastric mucosa (Fig. 18). However, there was a positive COX-1 immunoreaction in the endothelium of the blood vessels (Fig. 18). While group III (nicotine/curcumin-treated group) showed

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**Explanations of figures**

**Fig (8):** A photomicrograph of a rat gastric mucosa from group II (nicotine-treated group) showing some superficial surface mucous cells (thin arrows) containing mucin in their cytoplasm and others have vacuolated cytoplasm (arrow heads). Some parietal cells show vacuolated cytoplasm and pyknotic nuclei (thick arrows) and others contain granular acidophilic cytoplasm (wavy arrows). Congested blood capillaries (V) are seen in the lamina propria. H&E X 400.

**Fig (9):** A photomicrograph of a rat gastric mucosa from group II (nicotine-treated group) showing base of gastric glands (a) with focal disorganization of the basal cells (asterisk) and dilated spaces (S) inbetween them. H&E X 400 (b) chief cells showing vacuolated cytoplasm (thin arrows) and some parietal cells show vacuolated cytoplasm (thick arrows). Notice mononuclear cellular infiltration (arrow head) in the lamina propria with dilated blood vessels (V). H&E X400

**Fig (10):** A photomicrograph of a rat gastric mucosa from group II (nicotine-treated group) showing damage of mucosa and marked depletion of PAS positive mucus (thin arrows) on the surface of the glands as well as the surface columnar mucous secreting cells. Notice the neck region is containing weak PAS positive mucus (arrow heads) in mucous neck cells. PAS X400

**Fig (11):** A photomicrograph of a rat gastric mucosa from group III (nicotine / curcumin treated group) showing architecture nearly similar to that of control group. Dilated and congested blood vessels (V) in the muscularis mucosa (M) are seen. Notice mucin covering the luminal surface of gastric mucosa and extending down into the gastric pits (thin arrow). H&E X200

**Fig (12):** A photomicrograph of a rat gastric mucosa from group III (nicotine / curcumin treated group) showing the apical part of gastric gland architecture is nearly preserved. Mucous neck cells (thin arrows) and less abundant parietal cells (wavy arrows) with acidophilic cytoplasm and round nuclei are seen. H&E X400

**Fig (13):** A photomicrograph of a rat gastric mucosa from group III (nicotine / curcumin treated group) showing base of gastric glands nearly normal appearance. Dilated lumen of gastric gland (D) with acidophilic secretion inside it is seen. Focal areas of the bases of gastric glands show chief cells with vacuolated cytoplasm and pyknotic nuclei (arrow head) and others cells have basophilic cytoplasm and central nuclei (thin arrows). Parietal cells show acidophilic cytoplasm and round nuclei (wavy arrows). H&E X400

**Fig (14):** A photomicrograph of a rat gastric mucosa from group III (nicotine / curcumin treated group) showing PAS positive mucus on the luminal surface (arrows) and neck region of the gland (arrow heads) nearly similar to that of control group. PAS X400
marked positive COX-1 immunoreaction in the apical and basal gastric mucosa nearly similar to control (Figs. 19a,b, 20).

**COX-2 expression:** Immunohistochemically stained sections of control group (subgroups Ia, Ib and Ic) for COX-2 expression showed negative immune reaction for COX-2 in gastric mucosa almost in all fields (Fig. 21). Sections of group II (nicotine-treated group) showed a positive immunohistochemical reaction of COX-2 in the lamina propria around the gastric glands in the basal part of the gastric mucosa and focal areas of mononuclear cellular infiltration (Fig. 22). Moreover sections of group III (nicotine/curcumin-treated group) showed few cells with positive COX-2 immunoreaction especially in the

**Explanations of figures**

Fig (15): A photomicrograph of a rat gastric mucosa from group I (control group) showing marked positive–COX-1 immunoreaction in the lamina propria of the apical part (thin arrows) of gastric mucosa. COX-1 immune stain & DAB, Harris’s Hematoxylin counterstaining X400

Fig (16): A photomicrograph of a rat gastric mucosa from group I (control group) showing positive–COX-1 reaction in the lamina propria of the basal part of gastric mucosa (arrows). Notice positive reaction in the endothelium of blood vessel (arrow heads). COX-1 immune stain &DAB, Harris’s Hematoxylin counterstaining X400

Fig (17): A photomicrograph of a rat gastric mucosa from group II (nicotine-treated group) showing weak positive–COX-1 reaction in the lamina propria of gastric mucosa (thin arrows). COX-1 immune stain &DAB, Harris’s Hematoxylin counterstaining X400

Fig (18): A photomicrograph of a rat gastric mucosa from group II (nicotine-treated group) showing weak positive–COX-1 reaction in the basal part (arrows). Notice positive–COX-1 immunoreaction in the endothelium of blood vessel (arrow heads). COX-1 immune stain &DAB, Harris’s Hematoxylin counterstaining X400

Fig (19): A photomicrograph of a rat gastric mucosa from group III (nicotine/curcumin treated group) showing marked positive–COX-1 reaction in the lamina propria is similar to that of control group. (a) transvers section (thin arrows) and (b) longitudinal section (thick arrows). COX-1 immune stain &DAB, Harris’s Hematoxylin counterstaining X400

Fig (20): A photomicrograph of a rat gastric mucosa from group III (nicotine/curcumin treated group) showing marked positive–COX-1 immunoreaction in the basal part (arrows). Notice positive–COX-1 immunoreaction in the endothelium of blood vessel (arrow heads). COX-1 immune stain &DAB, Harris’s Hematoxylin counterstaining X400

Fig (21): A photomicrograph of a rat gastric mucosa from group I (control group) showing negative–COX-2 reaction in gastric gland cells and lamina propria. COX-2 immune stain &DAB, Harris’s Hematoxylin counterstaining X400

Fig (22): A photomicrograph of a rat gastric mucosa from group II (nicotine-treated group) showing positive–COX-2 reaction in the lamina propria (arrows) of the basal part of the gastric mucosa. COX-2 immune stain &DAB, Harris’s Hematoxylin counterstaining X400

Fig (23): A photomicrograph of a rat gastric mucosa from group III (nicotine/curcumin treated group) showing weak positive–COX-2 reaction in the lamina propria (arrows) of the basal part of the gastric mucosa. COX-2 immune stain &DAB, Harris’s Hematoxylin counterstaining X400
lamina propria of the basal part of gastric mucosa (Fig. 23).

**Morphometric and statistical analysis:** Control group, showed a non-significant changes in the mean of area percentage of COX-1/field, in all subgroups (Ia, Ib and Ic). However, the mean area percentage of COX-1/ field of group II (nicotine-treated group) showed a highly significant decrease (P <0.001) as compared with control group I. As regards group III there was increase in the mean of area percentage of COX-1 immunoreaction compared to group II but, there was a non-significant decrease (P >0.05) as compared with control group I (Table 1, Histogram 1).

As regards, the means of area percentage of COX-2/ field of control group, there was a non-significant change in subgroups (Ia, Ib and Ic). However, there was a highly significant increase (<0.001) in the means of area percentage of COX-2/field in animals of group II compared to control group I. As regards group III there was decrease in the mean of area percentage of COX-2 immunoreaction as compared to group II but there was a significant increase (P <0.05) as compared with control group I (Table 2, Histogram 2).

**DISCUSSION**

The widespread use of nicotine in the modern life induced many hazards on the gastrointestinal system. Although the bulk of cigarette smoke is inhaled, many of its constituents specially nicotine are absorbed into the saliva and are swallowed, where they have topical effects on the gastric mucosa. Moreover, they are also absorbed into the bloodstream and have systemic effects [22]. Nicotine causes gastric mucosal lesions through a number of mechanisms including; inhibition of synthesis of prostaglandins, which is important for protection of the gastric mucosa against a wide variety of insults, via cyclooxygenase (COX) enzymes. Also nicotine inhibits the production of growth factors, including epithelial growth factor (EGF) and vascular endothelial growth factor (VEGF), that influence cell proliferation and blood vessel generation, which are important in the healing process. Other effects include reduced nitric oxide production by endothelial cells and suppression of immune cells, such as neutrophils and macrophages, all were contributed to abnormal cell turnover [23].

Intended for limitations of gastric ulcer, a search for natural herbal substance with a protective effect and potent antiulcer became in sight. Some studies confirmed that curcumin have chemo-preventive properties against malgenancies. Moreover it can ameliorate and improve gastric lesions associated with drug-induced gastric erosions [12].

In the present study, nicotine-treated animals (group II) showed erosions in the gastric mucosa and decrease of PAS reaction with lysis of gastric glands and remnants of exfoliated cells in the lumen. The most striking change is overcrowding of parietal cells in the gastric mucosa and dilated and congested blood vessels in the lamina propria. This was in agreement with the results of previous investigators, who have reported that nicotine increase the incidence of gastric ulceration and cancer through reduced levels of epidermal growth factor and inhibition of prostaglandin synthesis and production [18,24]. This had been resulted in increased acid secretion by stomach and prevents production of bicarbonate ions that interfere with the neutralization of acids and leaves the mucus layer susceptible to erosion and disruption [25].

Nicotine induced damage in the gastric mucosal barriers and adversely affect the defensive mechanism of the stomach as it decrease synthesis of mucous which is the first line of defense in the stomach. It also decreases mucosal blood flow as it enhances the release of vasopressin, a potent constrictor, and induces angiogenesis as well as it have the power to suppress cell proliferation. These factors collectively have been thought to be the factors responsible for the loss of the mucosal integrity in the upper gastrointestinal tract [13,26]. Furthermore, other factors such as increase of tissue free radical production and presence of free radicals in smoke, release of pro-inflammatory cytokines, interleukin-1β and tumour necrosis factor-α all together lead to gastric mucosal damage [6,13]

The present study revealed that giving nicotine induced highly significant decrease in COX-1 expression associated with highly significant increase in COX-2 expression in the gastric mucosa when compared with control group. In line with this finding, the researchers found
that, severe gastrointestinal injuries and lesions due to acetylsalicylic acid are mainly caused by the inhibition of COX-1 [27]. It was reported that COX-1 a further line of defense for the gastrointestinal mucosa necessary for maintenance of mucosal integrity and ulcer healing through continuous generation of prostaglandin [28]. On the other side, high COX-2 correlated with gastric mucosal injury which facilitates the development of tumors and degradation of extra-cellular matrix (ECM). Moreover, less gastrointestinal ulceration and bleeding have been observed with the use of selective COX-2 inhibitors [29,30].

In the present study, concomitant administration of curcumin with nicotine (group III) showed,amelioration of the damage effect induced by nicotine on the gastric mucosa including gastric glands which appeared with normal architecture and strong PAS positive mucus. This was in agreement with Haider et al. [12] who explained that curcumin has the potential to act against ulcers in the stomach. Curcumin longa has a selective and competitive antagonistic activity towards the H2 receptor located on gastric parietal cells and suppression of proinflammatory cytokines [31]. Several mechanisms were suggested for curcumin antiulcer activity as attenuation of different ulcerative effectors including gastric acid secretion, total peroxides, myeloperoxidase activity, interleukin-6 (IL-6) and inhibitor activity for pepsin [32]. Moreover, curcumin plays efficient role in preventing the formation of ROS and scavenging free radicals. It protects cells from peroxidative stress as well as increase blood flow. Moreover, it enhances the activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase and reduced glutathione peroxidase [33].

The present work demonstrated that giving nicotine with curcumin enhanced the increase of immuno-reactivity of COX-1 in gastric mucosa as compared with group II (nicotine-treated group). Whereas, immune-reactivity of COX-2 showed a decrease of the immunoreaction as compared with group II (nicotine-treated group) but still significantly increased when compared with control group. Curcumin had a potent anti-inflammatory effect as it inhibited the activity of enzymes such as cyclooxygenase-2 (COX-2), lipooxygenase (LOX) and the inducible isoform of nitric oxide synthase (iNOS) [14]. Furthermore, it was believed that COX-1 causes production of prostaglandins, which have cytoprotective effects on gastric mucosa [7].

In conclusion, people who are constantly exposed to the administration of nicotine are advised to increase their intake of curcumin in food and drinks because of its valuable protective effect on gastric mucosa.

ACKNOWLEDGEMENTS

Authors are grateful to College of Medicine, King Faisal University, Saudi Arabia for providing all facilities and equipment to complete this work.

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