Histological Effects of Gestational and Lactational Acrylamide Exposure on the Fundic Mucosa of Adult Albino Rat Offspring and the Possible Ameliorative Role of Quercetin

Original Article

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ABSTRACT

Introduction: Acrylamide is a toxic chemical used in many industrial uses. It is generated in carbohydrate-rich foods cooked using high temperature. Quercetin is amongst the most effective antioxidant of the flavonoids.

Objective: To assess the effects of acrylamide on the gastric mucosa in adult male albino rats after maternal exposure to it during pregnancy and lactation and the possible ameliorative role of quercetin.

Materials and Methods: Twenty-four pregnant albino rats were randomly divided into four equal groups. Group 1: (Control group): kept without treatment. Group 2: (Acrylamide treated group): they received acrylamide in a dose of 30 mg/kg body weight/day Group 3: (Quercetin and acrylamide treated group): they received quercetin in a dose 50 mg/kg body weight/day 2 hours before the acrylamide at the same dose of the previous group. Group 4: (Quercetin treated group): they received quercetin at the same dose as in the previous group. The drugs were received by gastric intubation from day 6 of gestation till the 21st day after birth. Adult offspring in each group were sacrificed. Specimens from the gastric fundus were processed for light microscopic, transmission and scanning electron microscopic examination in addition to morphometric study.

Results: Acrylamide induced mucosal injury, sloughing of surface mucosal cells, glandular disarrangement, and decreased mucosal thickness, as well as a decrease in the PAS-positive reaction. Scanning electron microscope showed exfoliation of the surface cells. Electron microscopic studies showed deteriorating changes in parietal cells, chief cells, mucous cells, and enteroendocrine cells. In contrast, most of the changes induced by acrylamide were improved in the acrylamide and quercetin treated group.

Conclusion: Quercetin exerted an ameliorative effect against acrylamide-induced gastric toxicity.

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Key Words: Acrylamide, electron microscope, gastric fundus, PAS, quercetin.

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INTRODUCTION

Acrylamide is a white odorless solid compound, soluble in water and several organic solvents. It is industrially produced as a precursor to polyacrylamides, which are used as flocculation agents and water soluble thickeners[1]. Acrylamide levels up to 3500 µg/kg have been reported in potato chips[2]. French fries is considered the main contributor to the dietary intake of acrylamide[3]. It is generated by the reaction between amino group asparagine and reducing sugars (fructose, glucose) or reactive carbonyls, this reaction is called Maillard reaction[4]. Humans are incessantly exposed to acrylamide through the diet, beginning with in utero exposures. Owing to its solubility in water, it can cross the placenta and 10% to 50% of dietary acrylamide has been discovered to course to the fetus[5]. The exposure continues through breast-feeding as well as breast milk was found to contain acrylamide up to 1 µg/L[6] in addition to the consumption of the baby food[7]. Consequently, particularly during intrauterine life and infancy, there is a greater likelihood of exposure to acrylamide in comparison to adults owing to the smaller body size of the fetuses and the babies[8].

Acrylamide causes oxidative stress with reactive oxygen species (ROS) generation that initiates cellular damage through the polyunsaturated fatty acids injury and lipid peroxidation of cell and organelles' membrane[9]. Oxidative stress and dysfunction of mitochondria are the major mechanisms in chemical induced cell injuries[10]. Likewise, the oxidative stress and antioxidants reduction play a crucial role in damage of gastric mucosa[11].

Quercetin (3,3_,4_5,7-pentahydroxyflavone) is one of the most abundant naturally occurring polyphenol in our foods[12]. Quercetin is distributed in fruits, vegetables and other dietary sources[13]. Onion has the highest amount of quercetin (about 300 mg/kg). Other vegetables, as broccoli and kale, are rich in quercetin[14]. Its daily intake in the human

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diet is estimated to be 50–500 mg[15]. Quercetin metabolites present in the circulation after quercetin consumption act as potent antioxidant and anti-inflammatory agents[16].

In this work, we aimed to study the structural changes in the fundic mucosa in the adult rat after gestational and lactational exposure to acrylamide and whether the quercetin can attenuate these changes.

MATERIAL AND METHODS

Chemicals

1. Acrylamide (purity: 99%): was purchased as a white powder from Sigma Chemical Co., USA. It was dissolved in sterile distilled water.
2. Quercetin: was obtained from Sigma Chemical Company, it was be dissolved in sterile distilled water

Animals

Twenty-four adult female albino rats (weighing 200-300 g) and 3 -4 months old were used in this study. Rats were gained from the Animal House, Faculty of Medicine, Assiut University. They were housed at room temperature with normal humidity, kept under normal light environment with standard pellet food and tap water ad libitum supply. All experiments were carried out in accordance with the approved protocols by the local experimental animal ethics committee.

Female rats' vaginal smear was examined daily to verify the estrous cycle. Presence of cornified cells in the vaginal smear was considered a sign for estrous females. The proestrous females were caged with male rats for mating. They were checked daily for a vaginal plug. The day of appearance of the vaginal plug (containing sperms) was considered day 0 of gestation. The pregnant rats were randomly divided into four equal groups (6 rats in each group):

Group 1: (Control group): They were kept without treatment.

Group 2: (Acrylamide treated group): They received acrylamide orally from day 6 of gestation till parturition and during lactation (up to the 21st day) in a dose of 30 mg/kg of body weight daily by gastric intubation[17].

Group 3: (Quercetin and acrylamide treated group): they received quercetin freshly dissolved in distilled water in a daily dose 50 mg/kg of body weight by gastric intubation 2 hours before the administration of acrylamide at the same dose of the previous group from day 6 of gestation till parturition and during lactation (up to the 21st day)[18].

Group 4: (Quercetin treated group): They received quercetin freshly dissolved in distilled water orally at the same dose and duration as in the previous group.

At day 21 postnatally, offspring were weaned. After weaning, the male offspring were monitored until the age of three months and sacrificed. Specimens were taken immediately from the gastric fundus and were processed for light microscopic and electron microscopic studies. Ten offspring were used from each group.

For Light microscopic examination

Specimens of the gastric fundus were immediately fixed in Bouin's solution, dehydrated, cleared then embedded in paraffin and sections at a 5 µm thickness were cut. The slides were stained by hematoxylin and eosin (H&E) stain for the cytoarchitecture examination[19] and Periodic Acid Schiff stain for detection of mucopolysaccharides[20].

Electron microscopic examination

a-For transmission electron microscopic examination

Specimens of gastric fundus were fixed in 2.5% 0.1 M phosphate- buffered glutaraldehyde, and then they were processed and embedded in epoxy resin mixture. One-micron thick semi- thin sections were cut, stained with toluidine blue stain and examined with the light microscope. Proper areas were selected and ultrathin sections (60–90 nm) were cut by ultramicrotome. Sections were mounted on copper grids and were stained with lead citrate and uranyl acetate and finally stained sections were examined with a Jeol-JEM-100 CXII electron microscope in Electron Microscope Unit, Assiut University[21].

b-For scanning electron microscopic examination

Specimens of gastric fundus were washed in phosphate buffer saline (PBS) and fixed in 1 ml of 2.5% glutaraldehyde in PBS or cacodylate. Specimens were washed in phosphate buffer and were post-fixed in 1% osmium tetroxide for 2 h, washed three or four times in the same buffer, dehydrated in ascending grades of ethanol, dried, stuck onto holders then coated with gold[22]. Examination and photographing were made with JEOL JSM-5400LV SEM operated at 15 kV in the Electron Microscopy Unit, Assiut University.

Morphometric study

The following parameters were assessed

1-Mean thickness (height) of the gastric mucosa (the perpendicular distance between the gastric mucosal surface and the muscularis mucosa). It was measured in 5 randomly non overlapping field in H & E stain sections in each group at a magnification ×200. This was measured using Olympus cell Sense standard software in Human Anatomy and Embryology Department, Faculty of Medicine, Assiut University.

2- Area percentage of PAS positive reaction The mean area percentage of PAS positive reaction was quantified in five images of randomly different non overlapping fields in PAS-stained sections of each group using image J Software (version 1.52, public domain) at × 400 magnification.

Statistical analysis

Data were presented as mean ± SD. Statistical analysis of data was tested for significance using One-way analysis of variance (ANOVA) and post hoc test "Tuckey" through "SPSS" software.

Finally, the results were believed to be statistically significant when the *P value* was <0.05 and non-significant when the *P value* was > 0.05[23].

RESULTS

1-light microscopic results

A-Hematoxylin and Eosin (H&E) stain

Hematoxylin & Eosin-stained sections of the control group showed that the fundic mucosa appeared to be formed of epithelium, lamina propria and muscularis mucosa. The lamina propria appeared packed with numerous, straight fundic glands which open by gastric pits into the surface (Figure 1a). Gastric glands were divided into isthmus, neck, and base. The epithelial lining was formed of columnar mucous secreting cells with pale apical cytoplasm and basal oval nuclei. The gastric pits were lined by surface mucous cells. Next to the pits, the isthmus part of the gland contained surface mucous cells mainly interspersed with parietal cells. The neck region had mucous neck cells that appeared cuboidal in shape with an apical vacuolated cytoplasm and a vesicular, rounded basal nucleus and numerous parietal cells. The basal part was composed of chief cells mainly with some parietal cells. The parietal cells were identified by their deeply eosinophilic cytoplasm and central nuclei. The chief cells had a basal basophilic cytoplasm and basal nuclei (Figure 1b). Fundic mucosa in the quercetin treated group showed a similar structure to the control group (Figures 1 c,d).

H&E-stained sections from the fundic mucosa of acrylamide treated groups showed that the fundic glands were disorganized (Figure 2a). Some surface epithelial cells were desquamated, shrunken with deeply stained cytoplasm and pyknotic nuclei. Most of mucous neck cells and parietal cells had a vacuolated cytoplasm and pyknotic nuclei. Mucosal glands basal region showed loss of normal architecture with numerous chief cells with pyknotic nuclei (Figure 2b).

The fundic mucosa of the acrylamide and quercetin treated group showed mild desquamation of surface epithelial cells in some areas (Figure 2c). The surface epithelial cells were apparently normal, but some cells were with deep acidophilic cytoplasm and basal dense nuclei. Majority of mucous neck cells appeared normal with some cells had vacuolation of their cytoplasm. Most of parietal cells appeared normal. Chief cells appeared normal, while few cells had vacuolated cytoplasm and pyknotic nuclei (Figure 2d).

B-PAS Stain

PAS-stained sections of the control group (Figure 3a) and the quercetin treated group (Figure 3b) showed a highly positive PAS reaction in the surface epithelial cells and gastric pit cells and extending to the isthmus and neck of the glands. The sections from acrylamide treated group showed a decrease in PAS positive reaction in comparison to the control group (Figure 3c). PAS-stained sections of Acrylamide and quercetin treated group showed a moderate PAS positive reaction of the surface mucous film extending to the gastric pits and isthmus of the gastric glands (Figure 3d).

C-Toluidine blue stain

Examination of semi-thin sections stained with toluidine blue of the control group revealed the mucous secreting cells which appeared between the isthmus and the base of gastric glands. They were cuboidal in shape, with a basal nucleus and a pale-staining cytoplasm. Apically in the cytoplasm there was a "packet" of small mucous granules which were stained dark blue. Parietal cells appeared pyramidal in shape with central nucleus, some of them appeared at the neck lining the glandular lumen and others appeared at the base of the gland peripheral to the chief cells, secretory canaliculi were seen as unstained intracellular spaces. Homogeneous cytoplasmic appearance resulted from the small area occupied by secretory canaliculi (Figure 4a). Chief cells appeared at the basal region with a regular, rounded nucleus and numerous apical zymogen granules (Figure 4b). The quercetin treated group showed a similar structure (Figures 4 c,d).

In the acrylamide treated group mucous secreting cell appear with pyknotic nuclei and lack in mucous granules. Parietal cells appeared with dilated intracellular canaliculi that appeared in the form of paler areas of the cytoplasm (Figure 4e). The chief cells appeared irregular with lack in zymogen granules (Figure 4f). In quercetin and acrylamide treated group cells lining the fundic glands retained the normal cell appearance to great extend with most of mucous secreting cells appeared columnar with a basal nucleus and pale-staining cytoplasm filled with small mucous granules which stained dark blue with toluidine blue. Most of parietal cells appeared pyramidal in shape with central nucleus, secretory canaliculi were seen as unstained intracellular spaces (Figure 4g). Most of the chief cells appeared with apical zymogen granules and regular, rounded nuclei (Figure 4h).

2- Electron microscopic results

A) Scanning electron microscopic results

In the control group, the cells of the luminal epithelial lining of the fundic mucosa appeared polygonal in shape and regular with well-defined borders and in between which gastric pits appeared. The mucus was seen either as small globular masses and white streaks over the cells or as migrating columns from the gastric pit (Figures 5a,b). In the quercetin treated group, the fundic mucosal surface examination showed the same appearance (Figures 5c,d).

The Acrylamide treated group showed the presence of cellular debris. Some cells appeared shrunken with mucus globules on the surface while some cells were swollen. Cells with fenestrations or erosions of their membranes along with cells with umbilicated apices were seen. A complete exfoliation of the cells in some areas was observed giving a honeycomb appearance of the gastric mucosa (Figures 5 e,f). In the quercetin and acrylamide treated group, an increased mucus secretion in fundic mucosa was observed that masked the gastric pits in many areas. Some cells appear shrunken as well as some swollen cells were detected in some areas (Figures 5 g,h).

B) Transmission electron microscopic results

In the control group, the mucous cells showed the presence of apical microvilli. The cell had a rounded basal nucleus with a regular double layered nuclear envelope with prominent nucleolus. The apical areas of these cells were loaded with numerous mucous granules. Golgi complex was consisting of small cisternae and several small vesicles. The mitochondria were distributed in the cytoplasm and the rough endoplasmic reticulum cisternae were observed in the basal part of cytoplasmic matrix. (Figure 6a). The parietal cell appeared pyramidal in shape with their apices directed towards the lumen of the gastric gland. It had rounded central euchromatic nucleus with regular distribution of chromatin, prominent nucleolus and regular nuclear membrane. The cell showed intercellular canaliculi lined by microvilli. The cytoplasm showed the presence of numerous mitochondria and abundant tubulovesicular system (Figure 6b). The chief cells showed basal euchromatic nucleus and accumulation of zymogenic granules at their apical cytoplasm. In the basal cytoplasm there were abundant rough endoplasmic reticulum in the form of numerous lamellae arranged in straight parallel rows in addition to great numbers of free ribosome distributed throughout the cytoplasmic matrix, Golgi apparatus and mitochondria (Figure 6c). The argentaffin cells had a pyramidal configuration with their broad bases resting on the basement membrane. The nucleus appeared heterochromatic with deeply infolded nuclear envelop. Numerous electron dense, spherical granules of different sizes were basally located. Sparse mitochondria, free ribosomes and rough endoplasmic reticulum were detected in the cytoplasmic matrix. (Figure 6d).

In the acrylamide treated group, the mucous cells showed loss of apical microvilli, the nucleus showed dense clumps of heterochromatin. The cytoplasm was rarified with a marked decrease and a disruption of the apical mucous granules, destroyed mitochondria and dilated rough endoplasmic reticulum cisternae (Figure 7a). The Parietal cells showed marked dilatation of intracellular canaliculi containing debris of microvilli. The nucleus was condensed and small. Mitochondria were irregular in size and markedly destroyed. The tubulovesicular system appeared irregular and dilated (Figure 7b). In the chief cells the nucleus appeared with irregular nuclear membrane and clumped chromatin. The cytoplasm was rarified with damage of most of the cytoplasmic organelles. The rough endoplasmic reticulum cisternae were dilated and there were lack and disruption of secretory granules. The Golgi apparatus were dilated and broken and the mitochondria appeared irregular and darkly stained (Figure 7c). The argentaffin cells showed a number of vacuolated secretory granules, irregular nucleus with condensation of chromatin, condensed mitochondria and the cytoplasm contained many vacuoles and dilated rough endoplasmic reticulum cisternae (Figure 7d).

In quercetin and Acrylamide treated group, the mucous cells showed the presence of euchromatic nucleus. The cytoplasm contained intact mitochondria, ribosomes and rough endoplasmic reticulum. Apical microvilli were preserved (Figure 8a). The parietal cells had central nucleus with prominent nucleolus. Intracellular canaliculi appeared slightly dilated. Mitochondria were numerous but some of them were disrupted (Figure 8b). The chief cells showed rounded basal nucleus with abundant rough endoplasmic reticulum cisternae, few of them were dilated. The cell showed some areas of vacuolated cytoplasm. There was an apparent decrease in zymogen granules. Mitochondria, free ribosomes and Golgi apparatus were observed (Figure 8c). The argentaffin cells appeared with large euchromatic nucleus. The cytoplasm showed preserved, basally located electron dense granules, dilated rough endoplasmic reticulum, free ribosomes, and some vacuoles (Figure 8d).

In the quercetin treated group the mucous cells, parietal cell, chief cells and argentaffin cells were similar to those in the control group (Figure s 9 a-d).

3-Morphometric results

A) Mean thickness(height) of the gastric mucosa

The mean thickness of the fundic mucosa in the acrylamide treated group showed a statistically significant ($P < 0.05$) decrease in comparison to the control group. In the quercetin and acrylamide treated rats, the mean thickness of the fundic mucosa showed a statistically significant ($P < 0.05$) increase in comparison to the acrylamide treated group (Table 1 and Histogram 1).

B) Area percentage of PAS positive reaction

There was a significant decrease in the mean area % of PAS positive reaction in the acrylamide treated group in comparison to the control group ($P < 0.05$). In the quercetin and acrylamide treated group, the mean area % of PAS positive reaction showed a statistically significant ($P < 0.05$) increase in the mean area % of PAS positive in comparison to the acrylamide treated group (Table 2 and Histogram 2).

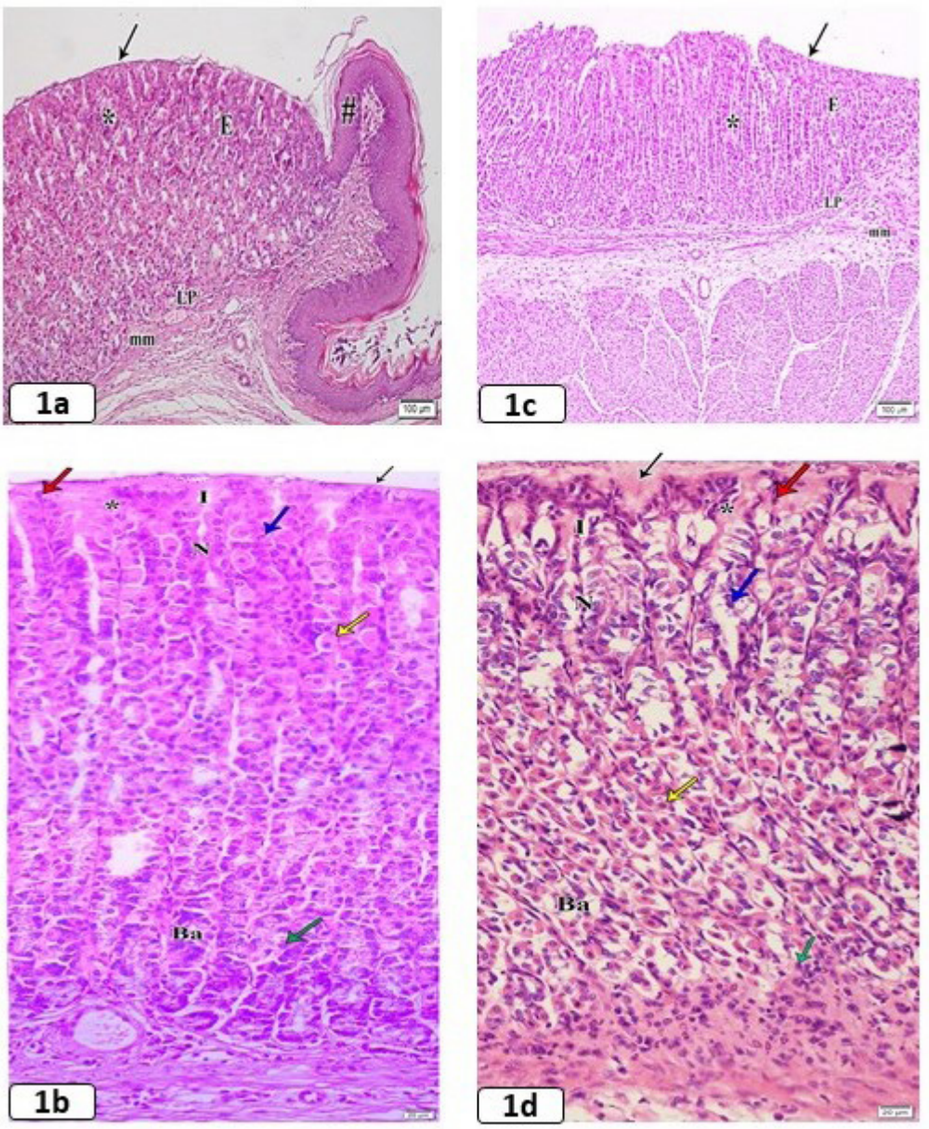


Fig. 1: Photomicrographs of the fundic mucosa in the control group (Figs. 1a-1b) and the quercetin treated group (Figs. 1c-1d) showing a similar normal fundic mucosa: (Fig. 1a and 1c) showing surface epithelium (E), lamina propria (LP) and muscularis mucosa (mm). Normal mucosa (↑) with a normal glandular architecture (*) is observed (H&E x100). Figs. 1b and 1d: The glands are formed of isthmus (I), neck (N) and base (Ba).The surface epithelial cells (red arrow) are columnar acidophilic cells with oval basal vesicular nuclei. Parietal cells (yellow arrow) are polyhedral with rounded vesicular nuclei and deep acidophilic cytoplasm. Mucous neck cells (blue arrow) are cuboidal with apical vacuolated cytoplasm and rounded vesicular basal nucleus. Chief cells (green arrow) are cuboidal with a basal basophilic and an apical acidophilic cytoplasm and rounded vesicular basal nuclei. (H&E x400).

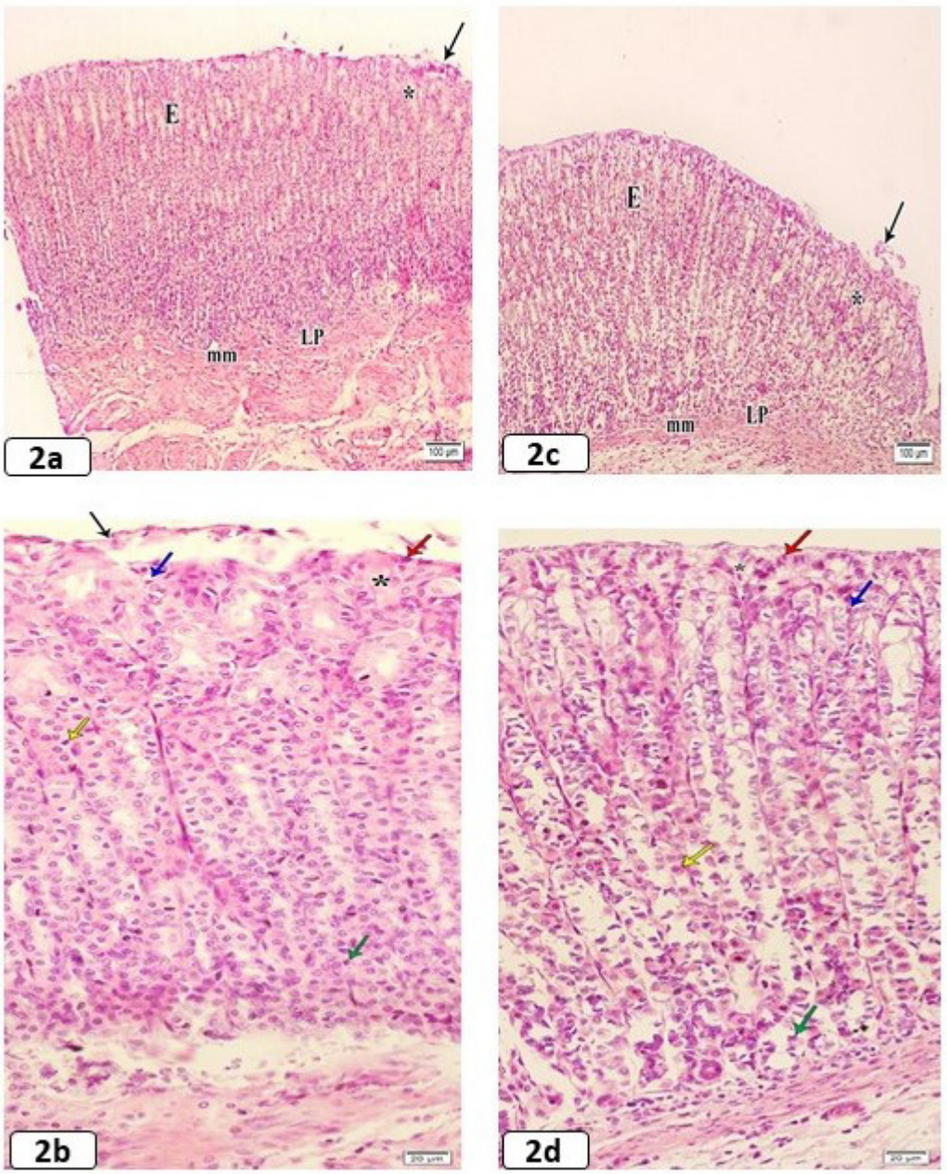


Fig. 2: Photomicrographs of the fundic mucosa in the acrylamide treated group (Figs. 2a-2b) and the quercetin and acrylamide treated group (2c-2d). 2a) Showing; surface epithelium (E), lamina propria (LP) and muscularis mucosa (mm). Apparently abnormal mucosa and desquamated surface epithelial cells in the lumen (↑) with loss of normal glandular architecture (*) is observed (H&E x100). Fig. 2b: Showing desquamated mucosa (↑) with loss of normal glandular architecture (*). The surface epithelial cells (red arrow) appear shrunken with deep acidophilic cytoplasm and pyknotic nuclei. Mucous neck cells (blue arrow) have vacuolated cytoplasm and pyknotic nuclei. Parietal cells (yellow arrow) have vacuolated cytoplasm and pyknotic nuclei. Chief cells (green arrow) appear with darkly stained nuclei (H&E x400). 2c) showing surface epithelium (E), lamina propria (LP) and muscularis mucosa (mm). Areas of abnormal mucosa and desquamated surface epithelial cells in the lumen (↑) with some loss of normal glandular architecture (*) are observed (H&E x100). Fig. 2d: Showing improvement in glandular architecture (*). Parietal cells (yellow arrow) appear normal. Majority of the other cells are normal but some of surface epithelial cells (red arrow), mucous neck cells (blue arrow) and chief cells (green arrow) have a vacuolated cytoplasm and dense nuclei. (H&E x400).

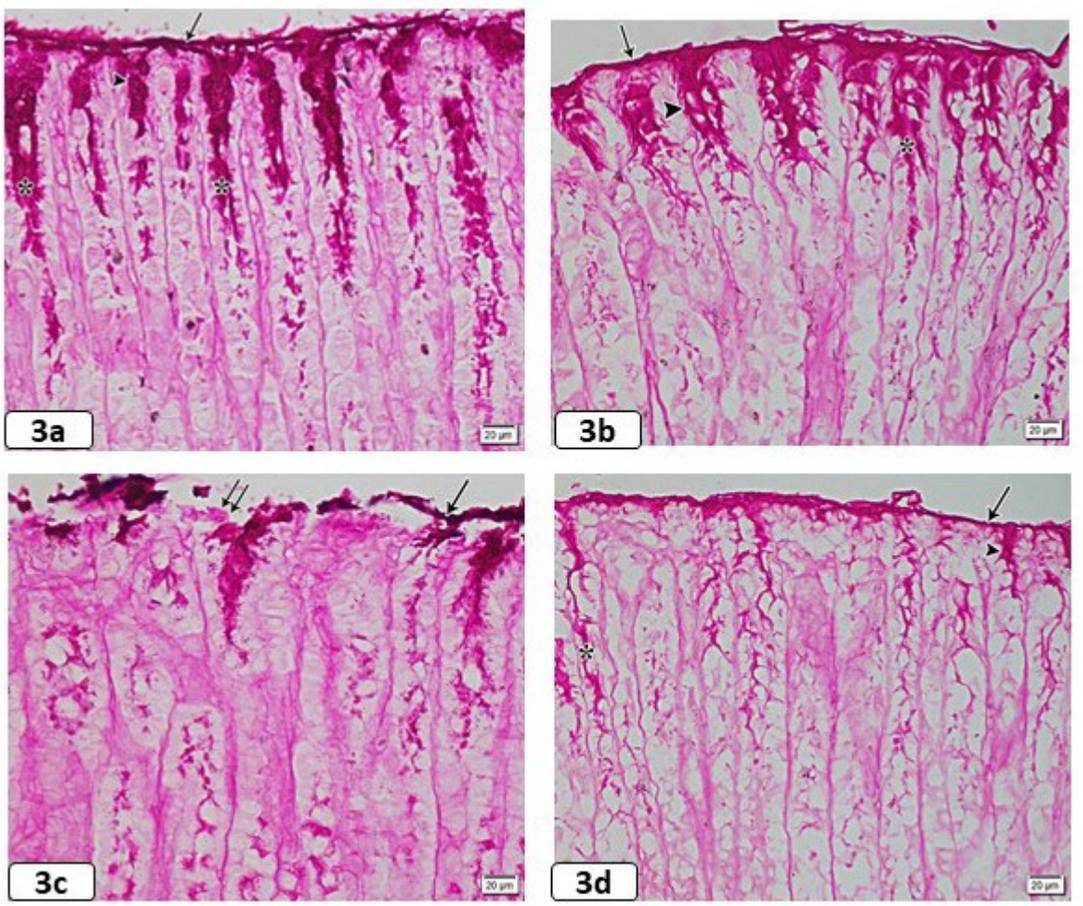


Fig. 3: Photomicrographs of the fundic mucosa in: the control group (3a) and the quercetin treated group (3b) showing a strong PAS positive thick continuous mucous film over the surface epithelium (↑). It extends to the gastric pits (◄) and isthmus of the glands (*). 3c) Acrylamide treated group showing a marked loss of PAS reaction in the surface epithelium (↑↑) with a positive thin PAS reaction in some areas (↑). 3d) Quercetin and acrylamide treated group showing a positive PAS reaction in the surface epithelium (↑) extending to the gastric pits (◄) and the isthmus of the gastric glands (*). (PAS x 400)

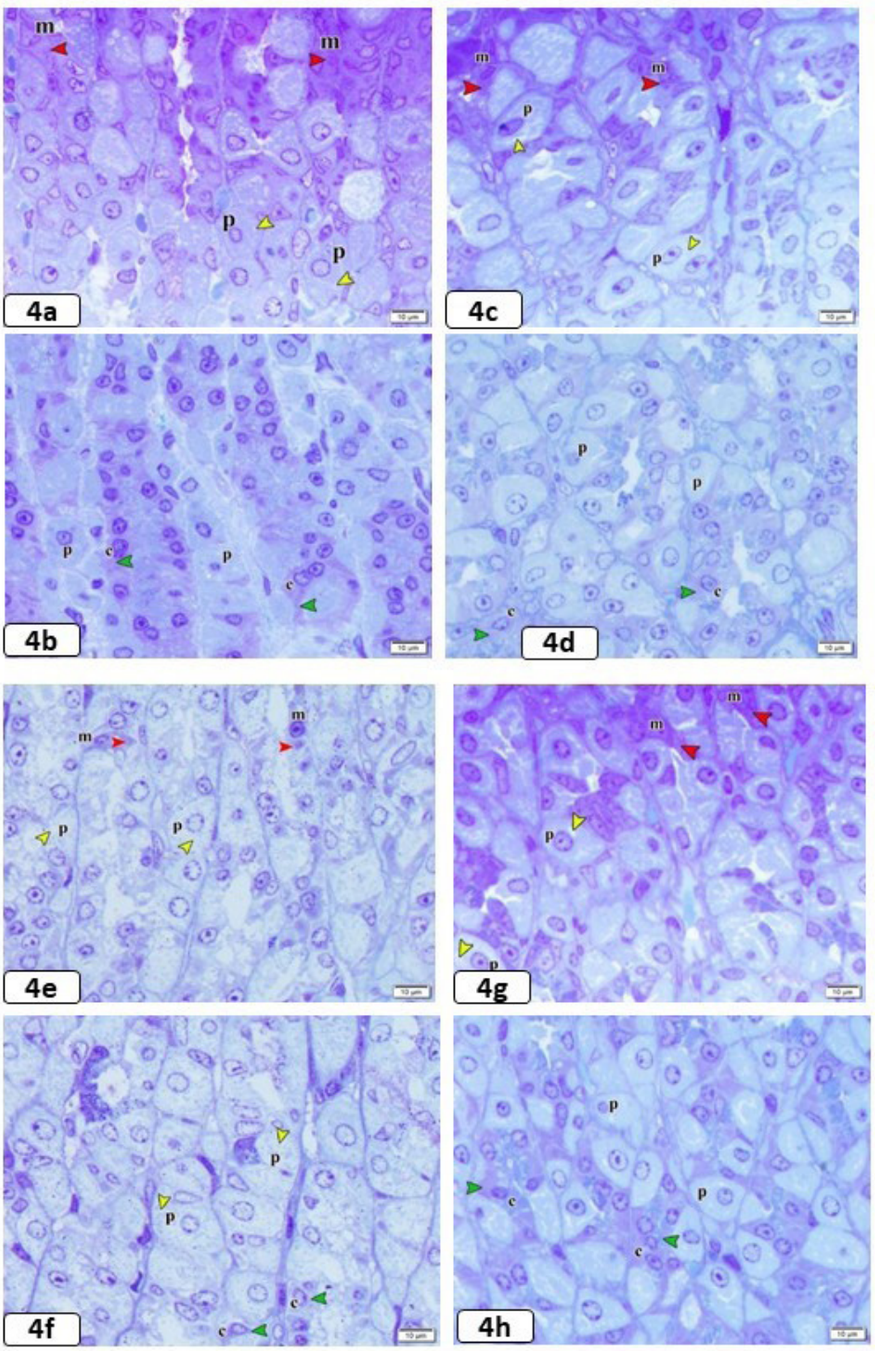


Fig. 4: Photomicrographs of semithin sections in the fundic mucosa in the control group (4a-4b) and the quercetin treated group (Figs. 4c, 4d). Figs. 4a and 4c: (isthmus and neck): Showing the mucous secreting cells (m) that are cuboidal with apical pale stained cytoplasm, darkly blue stained mucous granules (red arrowhead) and basal nucleus. Parietal cells (p) are pyramidal in shape with rounded vesicular nuclei, secretory canaliculi (yellow arrowhead) and homogenous cytoplasm. Figs. 4b and 4d (base of the gland): Showing chief cells (c) that are cuboidal in shape with basal nuclei and numerous apical zymogen granules (green arrowhead). Parietal cells could be seen (p). The acrylamide treated group (Figs. 4e and 4f), Fig. 4e (isthmus and neck): Showing deformed mucous secreting cells (m) having darkly stained nuclei and few mucous granules (red arrowhead), Parietal cells (p) are irregular with dilated secretory canaliculi (yellow arrowhead). Fig. 4f (base of the gland): Showing; chief cells (c) that lack zymogen granules (green arrowhead). Parietal cells (p) with dilated intracellular canaliculi (yellow arrowhead) could be observed. Figs. 4g and 4h Quercetin and acrylamide treated group. Fig. 4g (isthmus and neck): Showing mucous secreting cells (m) in which the apical cytoplasm containing darkly blue stained mucous granules (red arrowhead) and basal nuclei. Parietal cells (p) are pyramidal in shape with rounded vesicular nuclei, secretory canaliculi (yellow arrowhead) and homogenous cytoplasm. Fig. 4h (base of the gland) showing; chief cells (c) that appear with intact basal nucleus, numerous apical zymogen granules (green arrowhead) and apparently normal parietal cells (p).
(Toluidine blue x1000)

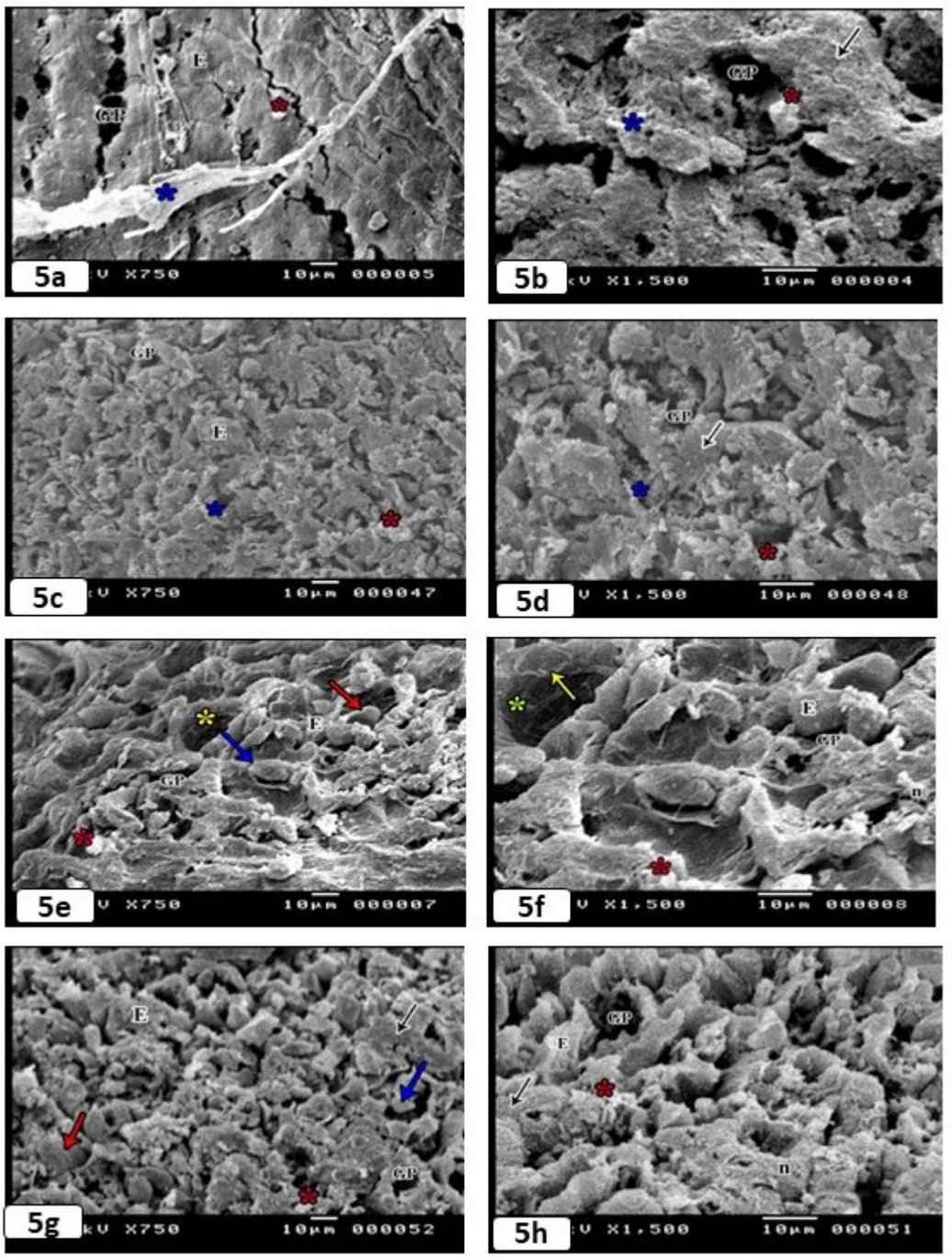


Fig. 5: Scanning electron micrographs of the fundic mucosa in: the control group (5a-5b) and the quercetin treated group (Figs. 5c-5d). Figs. 5a and 5c: Showing the luminal surface of gastric epithelial cells (E) that enclose gastric pits in between (GP), mucous appear on the surface in the form of white streak (blue star) or globules (red star) (x750). 5b) and 5d) showing luminal surface of gastric pits (GP) that are encroached with mucous with loss of cellular demarcation in some areas (↑) (x1500). Acrylamide treated group (5e-5f) showing 5e) surface gastric epithelial cells (E) with disturbed architecture enclosing gastric pits in between (GP). Some epithelial cells are shrunken (red arrow), while others are swollen (blue arrow). Surface mucous cells exfoliation over some areas giving honeycomb appearance (yellow star). Little mucous appear on the surface of the cells in the form of globules (red star) (x750). 5f) showing; some cells that are umblicated (yellow arrow) and necrotic debris (n) could be observed(x1500). Quercetin and acrylamide group (Figs. 5g-5h); Fig. 5g: Showing the luminal surface of the gastric epithelial cells (E) enclosing gastric pits in between (GP) which is encroached with mucous with loss of cellular demarcation in some area (↑). Mucous appear on the surface in the form of globule (red star). Few epithelial cells appear shrunken (red arrow) while some cells appear swollen (blue arrow) (x750). Fig. 5h: Showing in addition some necrotic debris (n) (x1500).

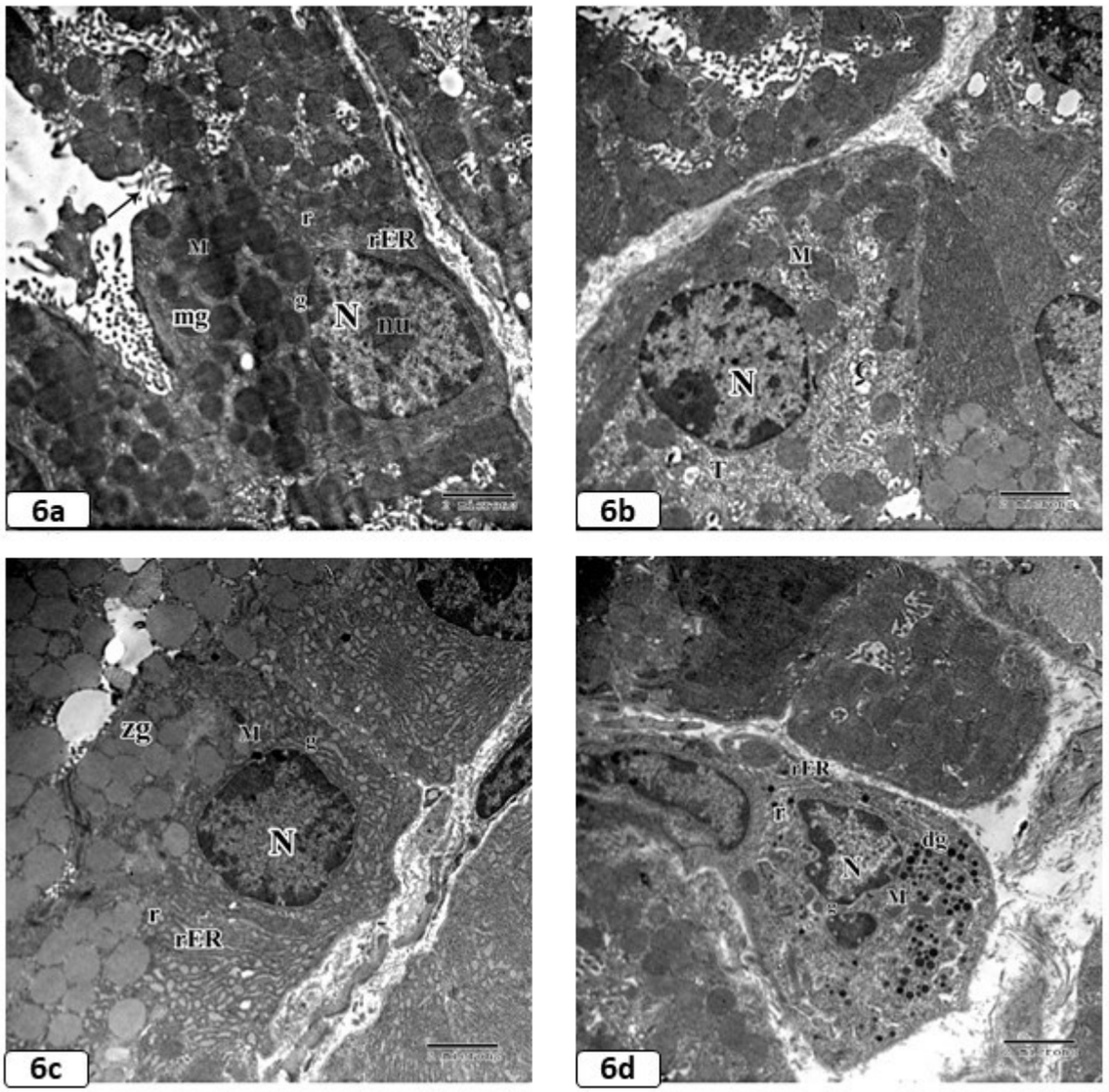


Fig. 6: Transmission electron micrographs of the fundic mucosa in the control group Fig. 6a: Showing a mucous secreting cell with a basal euchromatic nucleus (N), a prominent nucleolus (nu) and a regular nuclear membrane. The apical cytoplasm is filled with different sized mucous secretory granules (mg). Numerous ribosomes (r), rough endoplasmic reticulum (rER) and mitochondria (M) are noticed. Note the presence of microvilli (↑) and Golgi apparatus (g). Fig. 6b: Showing a parietal cell which is pyramidal shaped with central euchromatic nucleus (N) having regular distribution of chromatin and regular nuclear membrane. Numerous mitochondria (M) are observed. There are intracellular canaliculi (C) lined by microvilli and abundant tubulovesicles (T). Fig. 6c: Showing a chief cell having basal euchromatic nucleus (N) with regular nuclear membrane. The apical cytoplasm is filled with a different sized zymogen granule (zg). Abundant basal rough endoplasmic reticulum (rER), numerous free ribosomes (r), mitochondria (M) and Golgi apparatus (g) are noticed. Fig. 6d: Showing an argentaffin cell having large nucleus that is surrounded by infolded nuclear envelop (N). Electron dense granules (dg), rough endoplasmic reticulum (rER), mitochondria (M), Golgi apparatus (g) and free ribosome (r) are observed (x 5800).

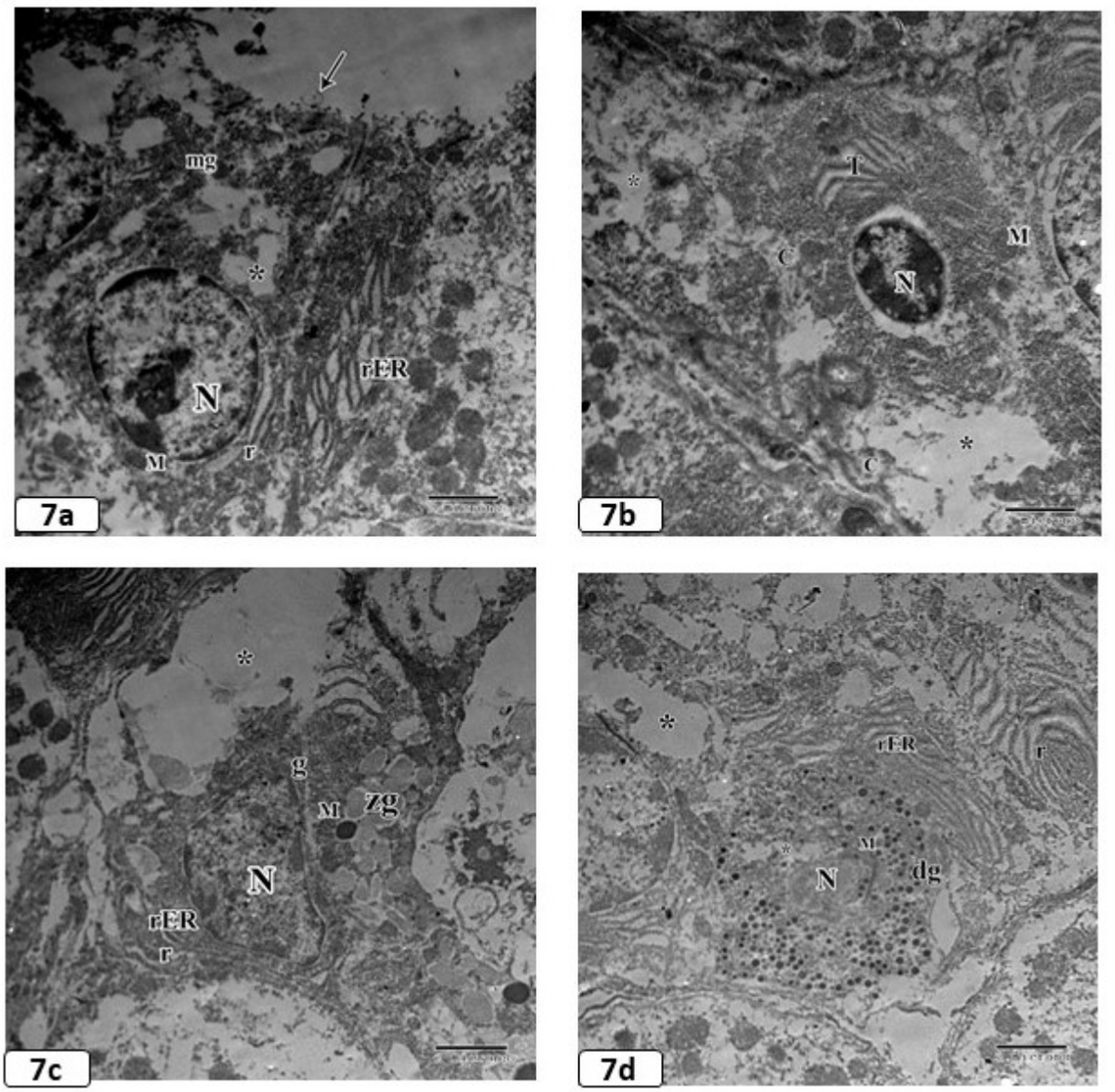


Fig. 7: Transmission electron micrographs of the fundic mucosa in the acrylamide treated group. Fig. 7a: Showing a mucous secreting cell with loss of microvilli and a disrupted cell membrane (↑) and rarefied cytoplasm (*). The nucleus showed dense clumps of heterochromatin (N). A marked decrease and disruption for apical secretory mucous granules (mg) are seen. Note the presence of destroyed mitochondria (M), dilated rough endoplasmic reticulum (rER) and few free ribosomes (r) (x 5800). Fig. 7b: Showing a parietal cell with rarified cytoplasm (*), shrunken nucleus (N) with dense clumps of chromatin. Mitochondria (M) are destroyed. Dilated intracellular canaliculi (C) and irregular dilated tubulovesicles (T) are noticed. Fig. 7c: Showing a chief cell having folded nucleus (N) with irregular nuclear membrane and rarified cytoplasm (*). Irregular mitochondria (M), free ribosomes (r), dilated rough endoplasmic reticulum cisternae (rER), irregular and broken Golgi apparatus (g) and lack and disruption of zymogen granules (zg) are noticed. Fig. 7d: Showing an argentaffin cell showing vacuolated cytoplasm (*), shrunken pale nucleus (N). Some of dense granules appear vacuolated (dg). There are condensed mitochondria (M), dilated rough endoplasmic reticulum cisternae (rER) and ribosome (r). (x 5800).

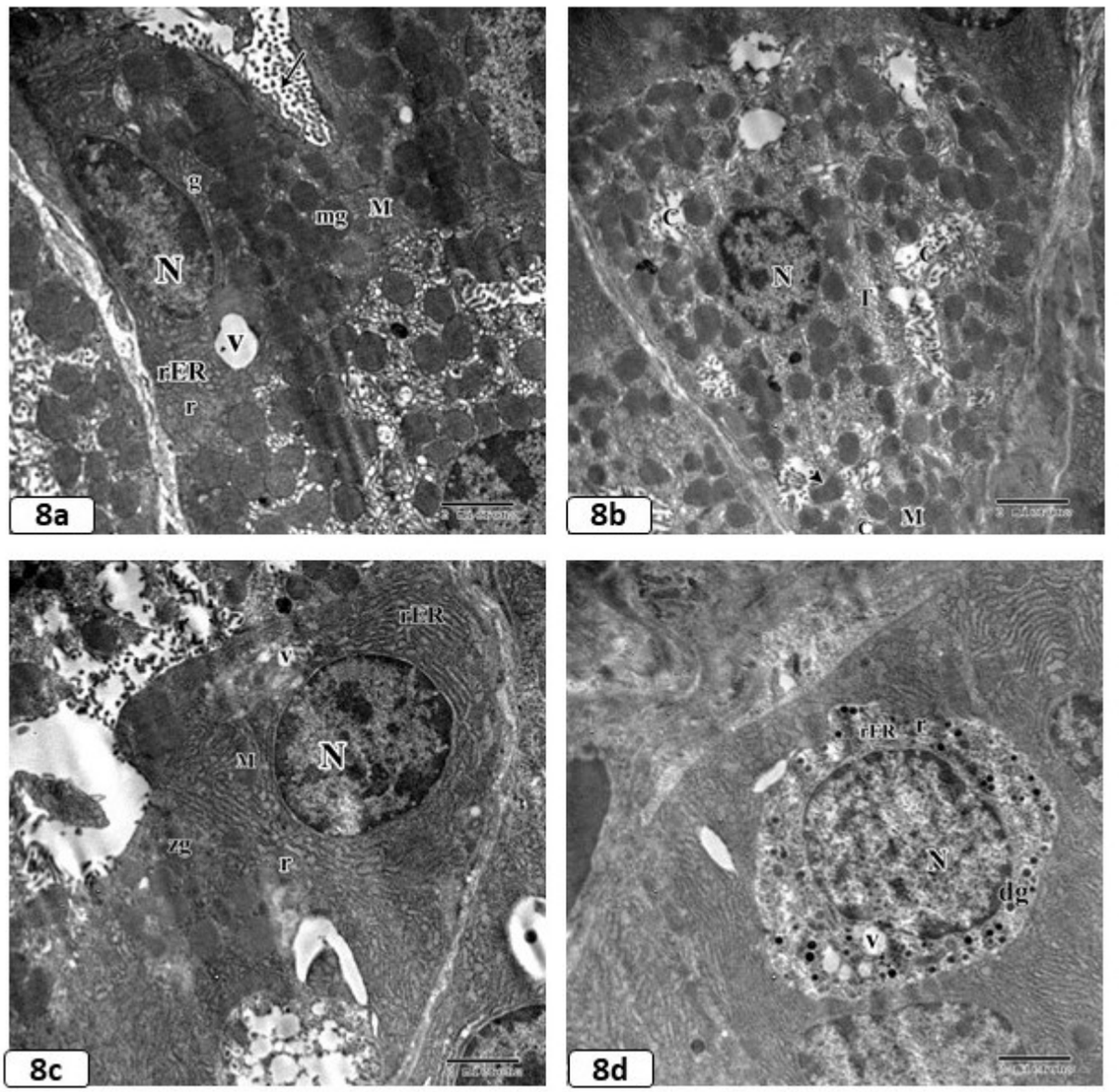


Fig. 8: Transmission electron micrographs of the fundic mucosa in the quercetin and acrylamide treated group. Fig. 8a: Showing a mucous secreting cell with euchromatic nucleus (N), apical secretory granules (mg), mitochondria (M) and Golgi apparatus (g). Rough endoplasmic reticulum (rER), ribosomes (r), microvilli (↑) and some vacuoles (v) are noticed. Fig. 8b: Showing a parietal cell showing central small nucleus (N) and numerous mitochondria (M) some of which were disrupted (arrowhead). Note dilated intracellular canaliculi (C) and tubulovesicular syśtem (T). Fig. 8c: Showing a Chief cell having large basal euchromatic nucleus (N), slightly vacuolated cytoplasm (V), free ribosomes (r), abundant rough endoplasmic reticulum ciśternae some of which are dilated (rER) and mitochondria (M). Few zymogen granules (zg) are noticed. Fig. 8d: Showing an argentaffin cell showing large euchromatic nucleus (N), preserved basal dense granules (dg), few dilated rough endoplasmic reticulum (rER), free ribosome (r) and some vacuoles (V). (x 5800).

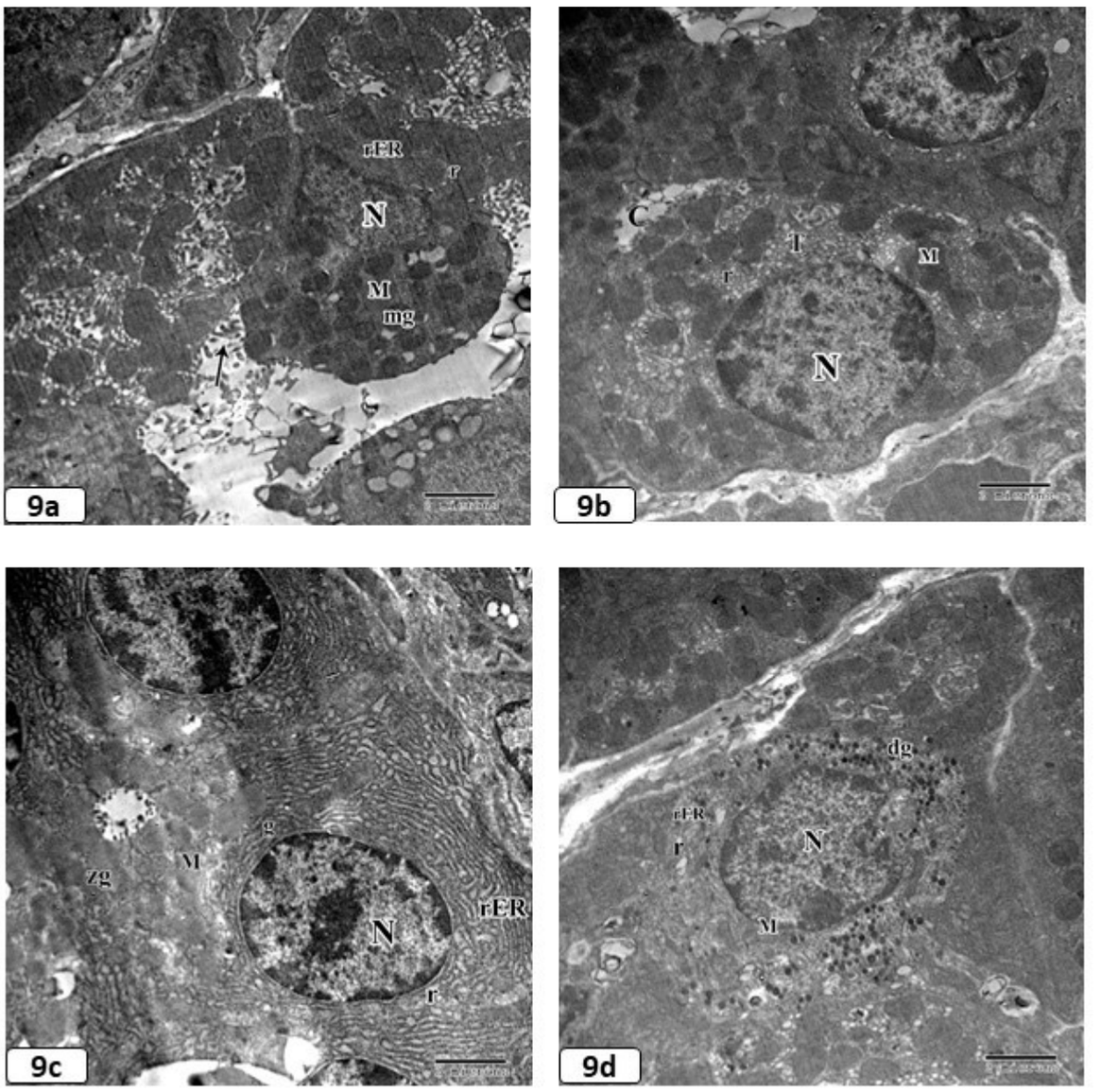


Fig. 9: Transmission electron micrographs of the fundic mucosa in the quercetin treated group. Fig. 9a: Showing a mucous secreting cell that has euchromatic nucleus (N). The apical cytoplasm is filled with mucous secretory granules (mg). Mitochondria (M), numerous free ribosomes (r) and rough endoplasmic reticulum (rER) are noticed. Note the presence of microvilli (↑). Fig. 9b: Showing a parietal cell with central euchromatic nucleus (N), numerous mitochondria (M) and free ribosomes (r) were observed. Intracellular canaliculi (C) and well-developed tubulovesiclular system (T) are noticed. Fig. 9c: Showing a chief cell having basal euchromatic nucleus (N) with regular nuclear membrane. The apical cytoplasm is filled with different sized zymogen granules (zg). Numerous ribosomes (r), numerous basal rough endoplasmic reticulum (rER), mitochondria (M) and Golgi apparatus (g) are noticed. Fig. 9d: Showing an argentaffin cell showing large oval nucleus surrounded by infolded envelop (N). Small dense granules (dg), mitochondria (M), rough endoplasmic reticulum (rER) and free ribosome (r) are noticed. (x 5800).

Table 1: Showing the mean thickness of the fundic mucosa in the different studied groups

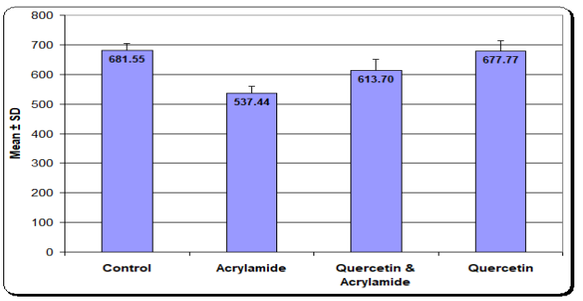
	Control	Acrylamide	Quercetin & Acrylamide	Quercetin
Mean ± SD	681.55±23.39	537.44±22.90	613.70±37.10	677.77±35.99
Range	642.3-700.5	505.0-566.0	575.1-660.5	631.0-722.3
*P-value*1		0.000*		
*P-value*2		0.000*	0.018*	1.000
*P-value*3			0.007*	0.000*
*P-value*4				0.026*

*P-value*1: Comparison among all groups.
*P-value*2: Comparison with the control group.
*P-value*3: Comparison with the acrylamide treated group
*P-value*4: Comparison with the quercetin and acrylamide treated group.

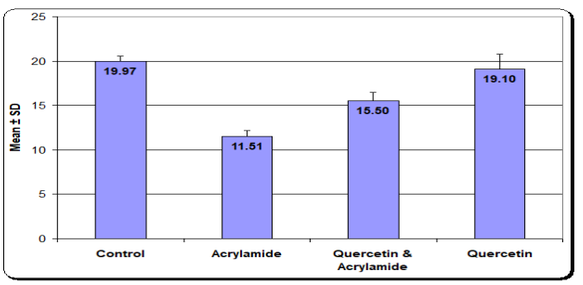
Table 2: Showing the mean area % of PAS positive reaction in the different studied groups

	Control	Acrylamide	Quercetin & Acrylamide	Quercetin
Mean ± SD	19.97 ± 0.56	11.51 ± 0.63	15.50 ± 0.94	19.10 ± 1.67
Range	19.4-20.7	10.8-12.3	13.9-16.2	16.4-20.7
*P-value*1		0.000*		
*P-value*2		0.000*	0.018*	1.000
*P-value*3			0.007*	0.000*
*P-value*4				0.026*

*P-value*1: Comparison among all groups.
*P-value*2: Comparison with the control group.
*P-value*3: Comparison with the acrylamide treated group
*P-value*4: Comparison with the quercetin and acrylamide treated group.



Histogram 1: Showing the mean thickness of fundic mucosa in the different studied group.



Histogram 2: Showing the mean area % of PAS positive reaction in the different studied groups.

DISCUSSION

Perinatal nutritional environment is known to play a critical role in determining offspring's future outcomes. Growing evidence suggests that acrylamide has adverse effects on pregnancy and embryonic development[24]. Thus, this study was conducted to evaluate the effects of the acrylamide on the adult gastric mucosa of male albino rats after maternal exposure to it during pregnancy and lactation and the possible protective role of the quercetin against these effects.

In this study, the oral route was used as it was found that time to attain maximum serum levels of acrylamide in rats depend on the route of administration. the ratio of the active acrylamide metabolite, glycidamide to acrylamide was greatest during oral exposure through the diet or gavage in comparison to intraperitoneal exposure[25].

The results of the present study indicated that exposure to acrylamide during pregnancy and lactation lead to obvious histologic alterations in the adult offspring gastric fundic mucosa. These include disorganized glandular architecture; desquamation and shedding of surface epithelial cells were observed. Most of epithelial cells, mucous cells, parietal cells and chief cells appeared with vacuolated cytoplasm and pyknotic nuclei. This was in agreement with El-Mehi and El-Sherif[26] who studied the effect of acrylamide on the gastric mucosa of the adult rat and found that the epithelial and the parietal cells appeared ballooned with vacuolated cytoplasm. Similar results were observed by Kermani-Alghoraishi *et al*.[27] who found that the acrylamide caused vacuolations as well as apoptosis in the cells of the seminiferous tubules of the testes in adult rats after intraperitoneal exposure to acrylamide for 6 weeks. In addition, semithin sections stained by toluidine blue of the acrylamide treated group consistently demonstrated lack of mucous granules in mucous secreting cells, dilated intracellular canaliculi in parietal cells and lost zymogen granules in chief cells.

The present morphometric study indicated that the mean thickness of the fundic mucosa in the offspring of the mothers treated with acrylamide showed a statistically significant decrease than in those of the control mothers. This could be explained by the observations of El-Sayyad *et al*.[28] who reported that maternal treatment with acrylamide or feeding with fried potato chips caused a significant inhibition in the proliferation and maturation process of the cerebellar cortex during postnatal growth. Furthermore, it was documented that acrylamide inhibited proliferation as well as induced apoptosis of placentas after gestational exposure in the mouse[24].

In the present work the offspring of the acrylamide treated mothers showed decreased PAS positive reaction in comparison to those of control group. The morphometric results confirmed this finding as the mean area percentage (%) of the PAS positive reaction decreased significantly. This was in accordance with El-Mehi and El-Sherif[26] who observed thin, slight PAS positive reaction of the surface

mucous film in the majority of sections in the acrylamide treated adult rat stomach. This could be attributed to the damaging effect of acrylamide on the mucus cells due to excessive oxidative stress[29]. In line with our study, Koledin *et al*.[30] studied the effect of sub chronic acrylamide treatment on the mucin secretion of the colon goblet cell and observed that acrylamide produce changes in the mucin synthesis, differentiation as well as distribution which have adverse effect on the colon mucosa.

In this work the scanning electron microscope of the fundic mucosa of the adult offspring of acrylamide treated mothers showed the presence of cellular debris on the cell membranes, cells with small erosions or fenestrations and cells with umblicated apices. There was an exfoliation of the surface cells completely in some areas of the gastric mucosa giving a honeycomb appearance. These observations were in agreement with El-Mehi and El-Sherif[26] who detected by the scanning electron microscopic examination, surface mucous cells shedding exposing the underlying lamina propria with some cells appeared shrunken and others appeared swollen. They noted the presence of cells with fenestrations in fundic mucosa of acrylamide treated adult rats. In addition, El Sayyad *et al*.[28] observed the loss of the ordinary normal structures and deformity of both the fungiform and filiform papillae on the tongue by scanning electron microscopic examination in neonate maternally treated with acrylamide.

By transmission electron microscopic examination of the fundic mucosa of the adult offspring of the acrylamide treated mothers, this study revealed that mucous epithelial cells exhibited disrupted cell membrane and lost microvilli. These findings could be attributed to the ability of acrylamide to increase lipid peroxidation of biological membranes in which reactive oxygen species (ROS) give rise to the per-oxidative deterioration of lipids, increasing its permeability to ions that lead to disruption of the membrane structure[31]. The nucleus showed dense clumps of heterochromatin with vacuolated cytoplasm and most of the apical mucous granules were lost or disrupted. Destroyed mitochondria and dilated rough endoplasmic reticulum were detected. This was consistent with Ali *et al*.[32] who reported that generation of oxygen free radicals produce damage of mucosal barrier and tissue acidosis which attributed to marked decrease in number and disruption of apical secretory mucous granules in the mucous secreting cells in rat gastric mucosa exposed to indomethacin. These results are also in line with Mohamed and Selim[33] who found disruption in hepatocytes organelles and vacuolation of their cytoplasm in the liver of albino rat offspring after perinatal acrylamide exposure.

This study showed that the cytoplasm of parietal cells, was markedly vacuolated. The nucleus was condensed and small. Mitochondria were irregular in size and markedly destroyed with irregular cristae. Mahmoud[34] explained mitochondrial alterations by the formation of free radicals leading to lipid peroxidation and oxidative stress. The free radicals bind to mitochondrial DNA, leading to impaired mitochondrial structure. Moreover, oxidative stress causes inner mitochondrial membrane depolarization with ensuing release of cytochrome c into the cytosol leading to caspase mediated apoptosis induction[35]. Zamani *et al*.[36] indicated that acrylamide induce the generation of reactive oxygen species and cellular apoptosis through the mitochondria-dependent pathways. The rough endoplasmic reticulum cisternae were dilated and broken. Marked dilatation of intracellular canaliculi, as noticed in the semithin sections, with luminal debris of microvilli was observed. This was reported with El Husseiny *et al*.[37] who explained that dilatation of intracellular canaliculi of parietal cell might be due to the stimulation of the cell by histamine leading to rearrangement of cytoskeleton and recruitment of (H+/K+-ATPase) rich tubulo- vesicles to the apical membrane expanding the intracellular canaliculi with loss of luminal microvilli.

Damage of the fundic mucosal cells could be attributed to the acrylamide ability to form hemoglobin adducts which lead to oxygen transport defect and cell hypoxia. That is in addition to sodium influx and osmotic fluid shifts into the cells that become swollen[5].

The chief cells showed severe damage in both the cytoplasmic organelles and the nucleus. The nucleus appeared with a folded membrane and clumped chromatin. Dilated rough endoplasmic reticulum was observed. Similar findings were observed by Mahmoud[34] who explored the effect of 2,3,7,8 tetrachlorodibenzo-p-dioxin on the gastric mucosa and attributed these changes to oxidative stress. Endoplasmic reticulum stress leads to up regulation of generation of reactive oxygen species[38]. Meyer *et al*[39] suggested reprogramming of chief cells into a metaplastic cell line as a consequence of the damage to the gastric epithelium. In the present work there were lack of their secretory granules. These changes were in parallel with Allam *et al*[40] who found that prenatal and perinatal exposure to acrylamide affected the ultrastructure of Purkinje cells of cerebellum and led to disruption at cell organelles.

The argentaffin cells showed empty and vacuolated secretory granules. The nucleus was irregular with condensation of chromatin. The mitochondria appeared condensed. In agreement with these findings, it was reported that ethylene glycol treated rats showed damage of mitochondria, endoplasmic reticulum and Golgi apparatus in the peptic, the parietal cells and the enteroendocrine cells and this could be clarified by oxidative stress[41].

Yáñez *et al*.[42] reported that flavonoids and flavones, particularly quercetin, are well-known to prevent important cytotoxicity process against cultured human cells through intracellular reactive oxygen species rising. Quercetin is known to have anticarcinogenic, anti-inflammatory, antioxidant and free radical scavenging properties[43]. Naturally occurring quercetin is present in many fruits (e.g., grapes, apples, cranberries, cherries) and vegetables (e.g., onion, asparagus, peppers) as well as in green or black tea[44].

In the present study the quercetin at a dose of 50 mg / Kg /day was used to assess its protective effects against damaging effect of acrylamide on the gastric glands. By examination of haematoxylin and eosin-stained sections, this study revealed improvement in the general architecture and glandular arrangement in the fundic mucosa in rats maternally exposed to quercetin and acrylamide. Most of the cells appeared normal. The present results of semithin sections examination confirmed these observations. This was consistent with Hu *et al*.[45] who observed that quercetin significantly reduces the apoptotic nuclei in H2O2-treated gastric cells. This was in agreement also with EL-Beltagi and Ahmed[46] who found that administrations of quercetin could result in a reduction of the acrylamide induced hepatic cellular damage. It was noted by Huang *et al*.[47] that quercetin suppressed inflammation and oxidative stress induced by lipopolysaccharide effectively. In this work treatment with quercetin was found to increase significantly the mean mucosal thickness in offspring of mothers treated with quercetin and acrylamide in comparison to those of mothers treated with acrylamide only. These results are compatible with those of Uthra, *et al*[31] who reported that acrylamide noticeably led to increased lipid peroxidation, a decrease in the levels of reduced glutathione and antioxidant enzymes superoxide dismutase and catalase in liver, kidney and brain. It also increased the activities of serum transaminases, urea, uric acid, creatinine, lipid profile, bilirubin in serum. With quercetin treatment tissue and serological indices were restored towards normal levels. Likewise, these results are in agreement with Coşkun *et al*.[48] who studied the antioxidant effects of quercetin on ethanol-induced gastric lesions in rats and approved that it protected gastric mucosa against ethanol induced-damage.

Furthermore, it has been found from the present work that a significant increase in area percentage of PAS positive reaction of surface mucous film on the surface epithelium in rats maternally exposed to quercetin and acrylamide in comparison with those maternally exposed to acrylamide only. This can be explained by the study of Yan *et al*.[49] who found that quercetin induces secretion of mucus as a mechanism of it in protecting gastric damage induced by indomethacin.

By scanning electron microscope, it was observed that quercetin caused obvious decrease in the disfigurement of surface cells which caused by acrylamide with increase in mucous in comparison to maternally exposed to acrylamide only. This was in accordance with El-Mehi and El-Sherif[26] who used rosemary as antioxidant against acrylamide toxic effects and they observed that rosemary treatment showed preserved cellular lining of the fundic glands. Likewise, the present ultrastructural results supported these observations and indicated improvement in all cells of the gastric mucosa as the cytoplasm appeared less vacuolated and organelles appeared less damaged. These results can be explained as quercetin is one of the most prominent and bioactive flavonoids with antioxidant and radical scavenging activity[50].

The results of this work are supported by the study that was conducted by Hu *et al*.[45] who found that quercetin can protect epithelial cell of gastric mucosa in *vitro* and in *vivo* from oxidative damage and attributed this to its ability to inhibit oxidative stress, and to regulate mitochondrial dysfunction, and to inhibit apoptosis. In agreement with these results, Lebda *et al*.[51] showed that acrylamide lowered testosterone hormone levels, alters sperm motility as it induced damage to testicular cells and testicular atrophy. Nevertheless, quercetin treatment led to testicular cells preservation and increase of testosterone levels again.

CONCLUSION

The quercetin could be useful for the inhibition and treatment of acrylamide induced gastric toxicity and could be also valuable in the supportive care of persons subjected to acrylamide.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

التأثيرات الهستولوجية الناتجة بسبب التعرض لمادة الأكريلاميد أثناء الحمل والرضاعة على الغشاء المخاطي لقاع المعدة فى الفئران البيضاء البالغة والدور المحسن المحتمل للكيرسيتين

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المقدمة: مادة الأكريلاميد مادة كيميائية سامة تستخدم في العديد من الأغراض الصناعية تتكون في الأطعمة الغنية بالكربوهيدرات بعد تعرضها لدرجة حرارة عالية. يعتبر الكيرسيتين من أكثر مضادات الأكسدة فاعلية في مركبات الفلافونويد.

الهدف من البحث: أجريت هذه الدراسة لتقييم آثار مادة الأكريلاميد على الغشاء المخاطي في المعدة في ذكور الفئران البيضاء البالغة بعد تعرض الأم لها أثناء الحمل والرضاعة ايضا والدور المحسن المحتمل للكيرسيتين.

مواد وطرق البحث: تم استخدام عدد أربعة وعشرين من الفئران البيضاء الحوامل بشكل عشوائي إلى أربع مجموعات متساوية. المجموعة ١: (المجموعة الضابطة): لم تتلقى اى علاج. المجموعة ٢: (المجموعة المعالجة بالأكريلاميد): تلقوا مادة الأكريلاميد بجرعة ٣٠ مجم / كجم / يوم. المجموعة الثالثة: (المجموعة المعالجة بالكيرسيتين والأكريلاميد): تلقوا كيرسيتين بجرعة ٥٠ مجم / كجم / يوم قبل ساعتين من مادة الأكريلاميد بنفس جرعة المجموعة السابقة. المجموعة ٤: (المجموعة المعالجة بالكيرسيتين): تلقوا كيرسيتين بنفس جرعة المجموعة السابقة. تم تلقي الأدوية عن طريق الانبوبة المعدية من اليوم السادس من الحمل حتى اليوم الحادي والعشرين بعد الولادة. تم تجهيز عينات من قاع المعدة فى الفئران البالغة في كل مجموعة للفحص بالمجهر الضوئي والمجهر الإلكتروني بالإضافة إلى الدراسة االمورفوميترية.

النتائج: تسببت مادة الأكريلاميد في إصابة الغشاء المخاطي ، وفقد الخلايا المخاطية السطحية ، وعدم انتظام فى الغدد، وانخفاض سماكة الغشاء المخاطي ، بالاضافة الى نقص فى صبغة بيرايودك شيف الحامضية للغشاء المخاطى . أظهر الفحص بالمجهر الإلكتروني الماسح تقشر الخلايا السطحية. وأظهرت الدراسات المجهرية الإلكترونية تغيرات متدهورة في الخلايا الجدارية والخلايا الرئيسية والخلايا المخاطية وخلايا الغدد الصماء المعوية. في المقابل ظهر تحسن لمعظم التغييرات التي أحدثتها مادة الأكريلاميد في المجموعة المعالجة بالأكريلاميد والكيرسيتين.

الاستنتاج: الكيرسيتين له تأثيرً محسنً ضد التأثير الضار على المعدة الناجم عن مادة الأكريلاميد.