

**Effect of hunger and thirst stress on the fundic mucosa of the stomach of adult female albino rats
(Histological, histochemical and immunohistochemical study)**

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Abstract: Introduction: Long-term hunger is a problem that all living species in the nature has to cope with. Hunger has a great effect on the metabolism and structure of many body systems. Several studies reported variable effect of starvation on organs; stomach, liver, thyroid and intestine. **Aim of the work:** to throw more light on the effect of both hunger and thirst on the gastric fundus of female albino rats by using histological, histochemical and immuno-histochemical methods. **Materials and Methods** Thirty adult female albino rats were subjected to experiment for 6 days. The animals were divided into 5 groups, each of 6 rats as follow: **Group I:** (control group) were allowed free access to water and food *ad libitum* **Group II:** subjected to starvation for 1 day. **Group III:** starvation for 2 days. **Group IV:** starvation for 4 days. **Group IV:** starvation for 6 days. Animals were sacrificed by cervical dislocation. Specimens of the fundus of the stomach were excised, fixed in formal saline, processed to paraffin blocks and subjected to histological, histo-chemical and immune-histochemical studies. **Results:** Histologically and histochemically, congestion of some blood capillaries with extravasation of blood, shedding and desquamation of superficial epithelium were noticed after 1st day of starvation. A glandular cystic dilatation with flattening of their epithelial lining, degeneration and loss of some superficial epithelium was observed in the 2nd day of starvation. Some parietal showed vacuolated, pyknotic nuclei and distorted gland with gastric ulcer was noticed in fundic glands at 4th day of starvation. Multiple gastric ulcers with loss of gastric mucosa, destruction of muscularis mucosa and submucosa was reported on 6th day after starvation. Alcian blue stain showed strong reaction in mucus neck cells and faint reaction in the base of the gland which increased with increase hunger duration. Masson trichrome stain was expressed in collagen fibers in the lamina propria between the basal part of fundic gland which increased with more time of starvation. Immune-histochemical, all starvated group stained by caspas 3 showed positive immune reaction in the middle and basal part of the gland with increase intensity of reaction with increase hunger duration. **Conclusion :** Structural changes in the fundus of the stomach are in direct proportion with hunger duration.

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1. Introduction

Long-term hunger is a problem that all living species in the nature has to cope with. Hunger has a great effect on the metabolism and structure of many body systems. During starvation, as no food and water intake, body usually fails to meet the basic energy needs for maintenance and survival, potent adaptive such as growth, reproduction and lactation (Matteri *et al.*, 2000). Response to starvation conserves the health and function of key organs so first priority given to the central nervous system and erythrocytes to use glucose as energy source and redistributes resources toward essential biological functions (Wang *et al.*, 2006). During hunger, variable degree of protein destruction occurs as indicated by rapid drop of basal metabolism and increased amount of azoth urine (Koc and Muslu, 2007).

As a result of hunger, there is decrease in the level of serum insulin which is accompanied by increase of glucagons and thus gluconeogenesis, i.e.

the process of producing glucose from some amino acids and glycerol in the liver (Al-qudah, 2011). As a result of decrease the insulin level, blood cells cope with by absorbing less glucose. This forces fatty acids to enter into these cells to be used as energy source (Ganog, 2002). While the above mentioned changes occur during the first 2-3 days of starvation, the processes of gluconeogenesis reaches it's peak during the 3rd day (Sonmez and Ozan, 2005).

Starvation induced lowering of blood pressure, glucose level and body weight depending upon the hunger level and it's duration (Ucar *et al.*, 2004). Moreover, Food deprivation slow down the process of cell mitosis and prolong the cell cycle with some cells staying at G1 stage. Additionally, epithelium cells rate of renewing decrease (Colakolu *et al.*, 1999).

Under normal condition, the stomach and small intestine are protected from gastric juices; (hydrochloric acid and pepsin) secreted by secretory

cells to digest food. The later cells produce a layer of mucin, which help to neutralize gastric acid that may come in contact with gastric or intestinal lining epithelium. Moreover, mucosal cells lining the stomach and intestines are in continuous turnover and renewal every 3 days with subsequent removal and replacement of any damaged cells. Problems and pathological changes only happen when normal mucosal cell function is disturbed by starvation stress, non steroidal anti-inflammatory drugs, alcohol and nutrient deficiencies (Thirunavukkarasu *et al.*, 2010; Al-qudah, 2011).

Several previous studies reported the influence of food deprivation on tissue structures and several organs on experiments with special attention to the stomach (Al-qudah, 2011), intestine, liver (Al-qudah, 2012), thyroid (Abdillah., 2011) and pancreas (Kitagawa and Ono, 1986). During starvation, the gastric PH decrease as the amount of gastric acid increases as reported by koc and Muslu, (2007). While, Alvares (1987) observed inhibition of proliferation in gastric and intestinal epithelial cells in adult rat with food deprivation. Additionally, Other workers in their studies noticed decrease in the size of the crypts and villi and the crypt cell production rats during hunger stress (Oodlad *et al.*, 1988). On the other hand, Palanch and Alvares (1998) found that, starvation stimulates cell proliferation in the gastric mucosa of suckling rats, while no response on weaning rats.

In more recent works, Alquadah, (2011) reported only the effect of hunger stress on the stomach of male albino rats in different phases of hunger. However, this study aims to throw more light on the effect of both hunger and thirst on the gastric fundus of female albino rats by using histological, histochemical and immuno-histochemical methods.

2. Material and Methods

Animals:

Thirty adult female albino rats of an average weight of (250-300 gm) were obtained from breeding animal house in faculty of Medicine, Menofya University (Menofya, Egypt). They are kept in good hygienic conditions for one week before start of experiment and maintained at room temperature (22-25°C).

Experiment design:

All experiments in this study were carried out following the protocol recommended by Local Animal Care Ethical Committee. During the experiment, each group was kept in separate cage and in night-day periods with 12 hours. The animal were randomly divided to five groups:

Group I: The control group has free access to standard animal food and tap water ad libitum.

Group II: Animals were starvated (stopped food and water) for 1 day.

Group III: Animals were starvated (stopped food and water) for 2 days.

Group IV: Animals were starvated (stopped food and water) for 4 days.

Group V: Animals were starvated (stopped food and water) for 6 days.

At end of experiment, the animals were weighted with an electronic balance in gm. The mean weight of animals in each group were calculated and expressed as mean± SD. Statistical analysis was done using student t-test to compare animals weight between different groups, significant value $p \leq 0.05$. Animals were sacrificed by cervical dislocation. Specimens of the fundus of the stomach were excised then immersed in isotonic saline. The tissues were subjected to the following studies:

I-Histological study:

Specimens of the fundus of the stomach were fixed in 10% formal-saline and then processed to obtain paraffin blocks, from which 5µm thick sections were cut and stained with H&E (Hematoxylin and Eosin) for the general architecture of fundus of stomach (Stevens and Wilson, 1996).

II- Histochemical studies:

Paraffin sections were stained with Periodic Acid chief-alcian blue (PAS-AB) and Massone trichrome stains (M.T). PAS-alcian blue which is specific for estimation of neutral mucopolysaccharides. Masson trichrome stain is specific for collagen fibers (Stevens and Wilson, 1996).

III-Immunohistochemical study:

For this study, paraffin sections 4 µm thick were mounted on glass slides coated on pol-L-lysin, deparaffinized sections were dehydrated and then immersed in 10 M sodium citrate buffer (pH 6.0). Sections were then heated in a microwave oven at 60°C for 10 minutes. Endogenous peroxidase were inactivated by incubating sections with 3% hydrogen peroxide and nonspecific reactions were blocked by incubating sections in a solution containing 5% normal human serum and 1% normal goat serum. Then sections were incubated with primary antibody overnight at 4°C. Activated caspase-3 expression was assessed using a peroxidase-conjugated rabbit monoclonal antibody IgG (Cell signaling Technology, Ipswich, MA) (dilution 1:200). After 3 rinses with phosphate-buffered saline, the sections were incubated with a commercial kit (Pic-Ture TM, Zymed and South San Francisco, CA) for visualization of immunoreactions. Finally, they were rinsed with distilled water and counterstained with Mayer's hematoxylin. Normal lymphoid tissue was used as positive control. Negative control was performed by

omitting primary antibody step consequently no immune-staining occurred (Lee *et al.*, 2006).

3. Results

Assessment of the rat weight:

Starvation for two days significantly reduced rat body weight as compared to control group ($p < 0.05$) while increased duration of starvation to four days highly significant reduced rat body weight as compared to control group ($p < 0.01$). On the other hand, food and water deprivation for 6 days showed markedly significant decreased rat body weight when compared to control group ($p < 0.001$) as shown in Table (1).

Table (1): Effect of starvation on the rat body weight:

Groups	Mean body weight of rat in (gm)
Control group (group I)	255 ± 1.5
Starvation for 1 day (group II)	230 ± 2.7 NS
Starvation for 2 day (group III)	206 ± 1.8 *
Starvation for 4 day (group IV)	181 ± 2.9 **
Starvation for 6 day (group V)	160 ± 1.49 ***

Values are expressed as mean ± SD (standard deviation).

Each group is compared with group I (control group)

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, NS: Non Significant

Histological results:

Group I (control group):

H&E stained sections of the fundic mucosa showed normal numerous parallel tightly packed straight fundic glands (Fig. 1). The lining epithelial cells were composed of mucous secreting columnar cells with basal oval nuclei and foamy cytoplasm. The isthmus part of the gland next to the pits was lined by surface mucous secreting columnar cells with basal oval nuclei and foamy cytoplasm. The isthmus part of the gland next to the pits was lined by surface mucous secreting cells and parietal cells. The neck region showed parietal cells and mucous neck cells which had flat basal nuclei and pale cytoplasm. The numerous parietal cells appeared rounded with deeply acidophilic cytoplasm and central rounded nuclei. The base of the gland predominantly had chief cells with basal nuclei and basophilic cytoplasm (Fig. 2).

PAS-AB stained sections showed positive mucous film over the surface epithelium and fill the gastric pits. Strong PAS-AB positive staining of mucus neck cells and faint reaction of scattered cells over the basal part of the gland was observed (Fig. 3). Masson trichrome (M.T) stained sections showed few collagen fibers in the lamina propria between the basal parts of the fundic gland (Fig. 4).

Fundic mucosa showed a negative caspas 3 immune reaction in most of the cells while only few glandular cells showed a positive caspas 3 immune reaction (Fig. 5).

Group II (starvation for 1 day):

Examination of H&E-stained sections of the fundic mucosa after starvation for one day showed congestion of some capillaries with extravasation of blood. Shedding (desquamation) of some superficial epithelium with dilatation of some glands were also noticed (Fig. 6).

PAS-AB- stained sections showed a faint PAS-AB-positive reaction of the surface mucous film. Very strong AB-positive mucous-secreting cells in the neck region and weak reaction was observed near the base of the gland (Fig. 7).

Masson's trichrome-stained sections showed increased collagen fibers between the fundic glands and thick lamina propria in comparison with that of the control (Fig. 8).

There was a strong caspase-3 immune positive reaction in the cytoplasm of some apical cells of the gland. Weak to moderate caspas 3 immune positive reaction in the middle and basal parts of the gland in comparison to control group (Fig. 9).

Group III (starvation for 2 days):

Examination of H&E stained sections of the fundic mucosa after starvation for two days showed dilatation and congestion of some blood vessels. Degeneration and loss of some superficial cells can be seen. Some superficial cells appear with pyknotic nuclei while others appear with fading nuclei (Fig. 10). A glandular cystic dilatation with flattening of their epithelial lining was observed. Some areas of the gland showed loss of their epithelium. The predominant chief cells in the base of the glands was also noticed (Fig. 11).

PAS-AB stained sections showed interrupted very thin and faint PAS-AB positive reaction of the surface film and very weak reaction of the rest of the gland (Fig. 12).

Masson's trichrome-stained sections showed increased thick strands of collagen fibers between the fundic glands in the lamina propria in comparison with that of the control group (Fig. 13).

Immune reaction for caspase-3 was strong positive in the parietal cells and Weak to moderate positive reaction in the base of the fundic glands in comparison with that of control (Fig. 14).

Group IV (starvation for 4 days):

Examination of H&E-stained sections of the fundic mucosa after starvation for four days showed gastric ulcer with atrophy of the lining epithelium and flattened nuclei. Some parietal cells were vacuolated with pyknotic nuclei (Fig. 15). Some fundic gland cells showed vacuolation with degeneration and loss of some other cells. Distortion of the gland was observed. Some parietal cells appeared with pale cytoplasm and pyknotic nuclei (Fig. 16).

There was moderate PAS-AB positive reaction in the apical part of the gland. Many patches of blue staining of acid mucin appeared in the basal part of the gland (Fig. 17).

Masson's trichrome-stained sections showed thick strands of collagen fibers which increased between the fundic glands in the lamina propria in comparison to that of control group (Fig. 18).

Immune reaction for caspase-3 showed strong positive immune reaction in the cytoplasm of most of glandular cells specially parietal cells and the basal part of the gland (Fig. 19).

Group V (starvation for 6 days):

Examination of H&E-stained sections of the fundic mucosa after starvation for six days showed multiple ulcers and loss of gastric mucosa. Exfoliation of the superficial epithelium was observed (Fig. 20). some cells in the basal part of the fundic gland were lost with destruction of the muscularis mucosa and submuco. Capillaries in the lamina propria appeared dilated and congested (Fig. 21).

PAS-AB stained sections showed very weak PAS-AB positive reaction in the apical part of the gland and Weak to moderate PAS positive reaction in the base of the gland (Fig. 22).

Masson's trichrome-stained sections showed very thick collagen fibers between the fundic glands in comparison to that of control group (Fig. 23).

Immune reaction for caspase-3 showed weak to moderate positive reaction in apical part of the fundic mucoasa and very strong positive reaction in the basal part of the gland (Fig. 24).



Fig. 1. A photomicrograph of a section in fundic mucosa of control adult female rat showing normal numerous parallel tightly packed fundic glands. Each gland has luminal pit (→), isthmus (I), neck (N) and base (B). (H&E, X 100)

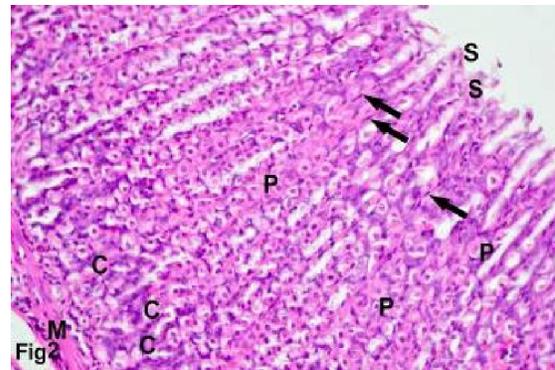


Fig. 2. A photomicrograph of a section in fundic mucosa of control adult female rat showing fundic gland with apical surface epithelium (S), mucus neck cells (→) with flat basal nuclei. Parietal cells (P) appear with acidophilic cytoplasm and central rounded nuclei. Chief cells (C) with basal nuclei and basophilic cytoplasm are predominant in the basal part of the gland giving it a darker appearance. Notice muscularis mucosa (M) can be seen. (H&E, X 400)

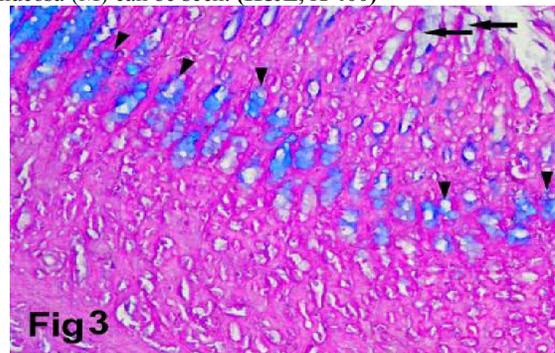


Fig. 3. A photomicrograph of a section in fundic mucosa of control adult female rat showing PAS-AB (Periodic Acid Chief – Alcian Blue) positive mucus film over the surface epithelium (→) filling the gastric pits. Note the strong AB positive staining of mucous neck cells (▶) with a faint reaction over scattered cells over the gland. (PAS-AB, X 400)

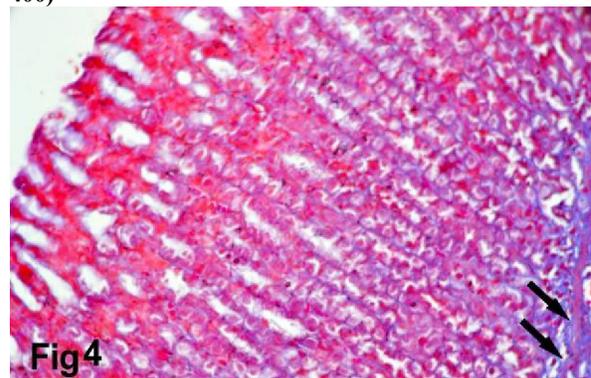


Fig. 4. A photomicrograph of a section in fundic mucosa of control adult female rat showing a few collagen fibers in the lamina propria (→) between the basal parts of the fundic glands. (M.T, X 400)

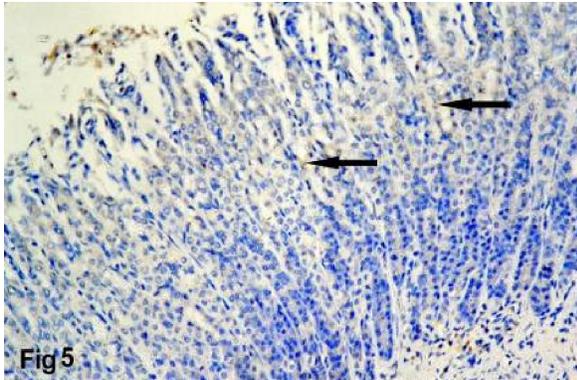


Fig.5. A photomicrograph of a section in fundic mucosa of control adult female rat showing negative caspas 3 immune reaction in most of the fundic gland cells. Note the positive caspas III immune reaction in a few glandular cells (→) (**Caspas 3 immunostain, X400**)

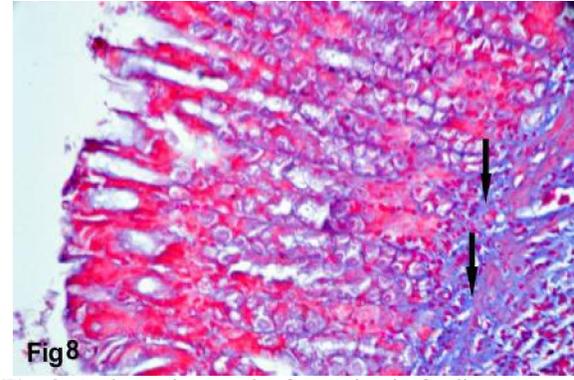


Fig. 8. A photomicrograph of a section in fundic mucosa of adult female rat following starvation for one day showing many collagen fibers between the fundic glands and thick lamina propria (→). (**M.T, X 400**).

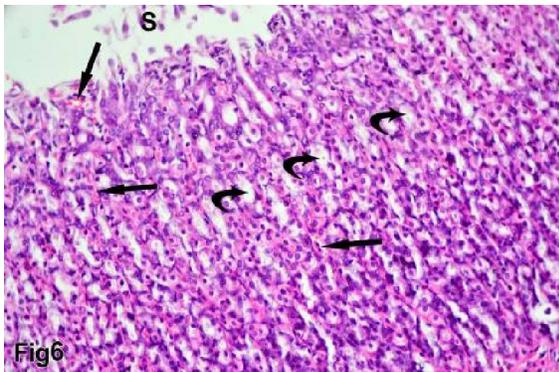


Fig. 6. A photomicrograph of a section in fundic mucosa of adult female rat following starvation for one day showing some congested capillaries with extravasation of blood (→). Notice the shedding (desquamation) of some superficial epithelium (S). Notice the dilatation of some glands (curved arrows). (**H&E, X 400**)

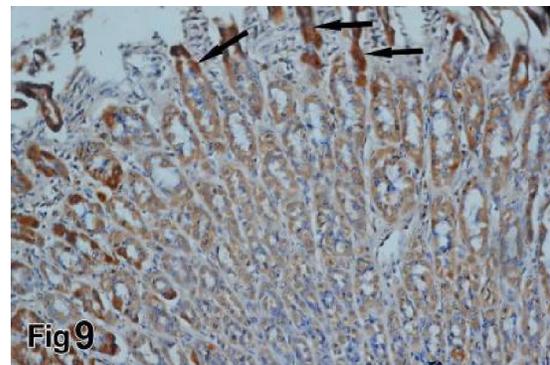


Fig. 9. A photomicrograph of a section in fundic mucosa of adult female rat following starvation for one day showing strong positive caspas 3 immune reaction in the apical cells of the gland (→). Weak to moderate caspas 3 immune reaction in the middle and basal parts of the gland in comparison to control group. (**Caspas 3 immunostain, X400**)

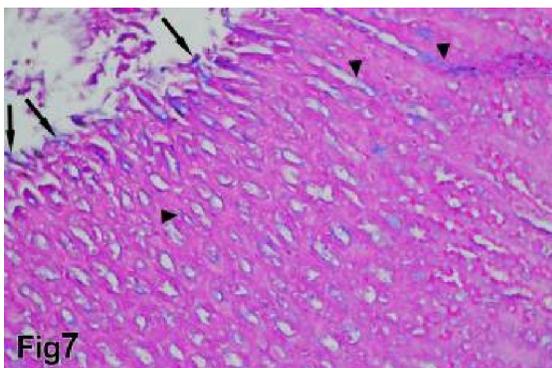


Fig. 7. A photomicrograph of a section in fundic mucosa of adult female rat following starvation for one day showing a PAS-AB positive reaction over the surface epithelium (→). Notice the strong reaction in mucous neck cells (▶) and weak reaction in the base of the glands in comparison with that of control. (**PAS-AB, X 400**)

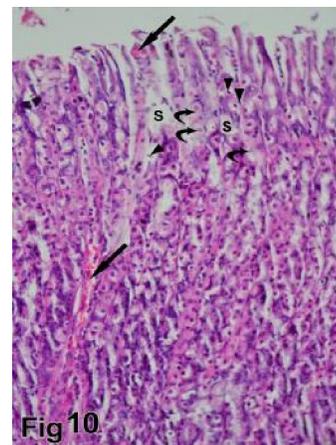


Fig. 10. A photomicrograph of a section in fundic mucosa of adult female rat following starvation for two days showing dilated congested blood vessels (→). Degeneration and loss of some superficial cells (S) can be seen. Some superficial cells appear with pyknotic nuclei (▶) while Others appear with fading nuclei (curved arrow). (**H&E, X 400**)

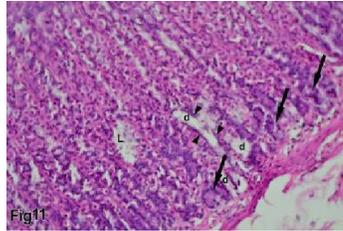


Fig. 11. A photomicrograph of a section in fundic mucosa of adult female rat following starvation for two days showing glandular cystic dilatation (d) with flattening of their epithelial lining (▶). Some areas of the gland showed loss of their epithelium (L). The predominant chief cells in the base of the glands (→) were also noticed (H&E, X 400)

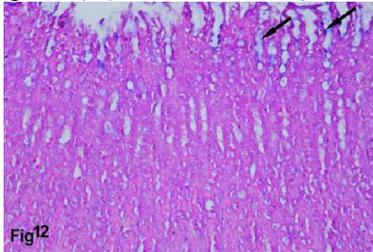


Fig. 12. A photomicrograph of a section in fundic mucosa of adult female rat following starvation for two days showing the interrupted very thin and faint PAS-AB positive reaction of the surface film (→). Note: the very weak reaction of the rest of the gland. (PAS-AB, X 400)

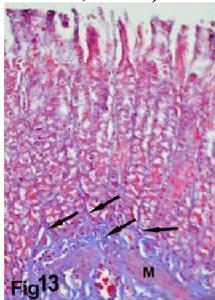


Fig. 13. A photomicrograph of a section in fundic mucosa of adult female rat following starvation for two days showing increased thick strands of collagen fibers (→) between the fundic glands in the lamina propria in comparison with that of the control group. Note: Muscularis mucosa can be seen (M). (M.T, X 400).

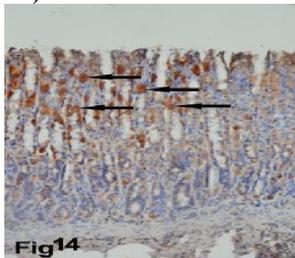


Fig. 14. A photomicrograph of a section in fundic mucosa of adult female rat following starvation for two days showing strong positive caspase 3 immune reaction in the parietal cells (→). Weak to moderate positive caspase 3 immune reaction in the base of the fundic glands in comparison with that of control. (Caspas 3 immunostain, X400)

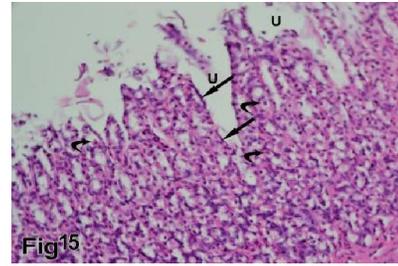


Fig. 15. A photomicrograph of a section in fundic mucosa of adult female rat following starvation for four days showing gastric ulcer (U) with atrophy of the lining epithelium and flattened nuclei (→). Vacuolated parietal cells (curved arrows) with pyknotic nucleus. (H&E, X 400)

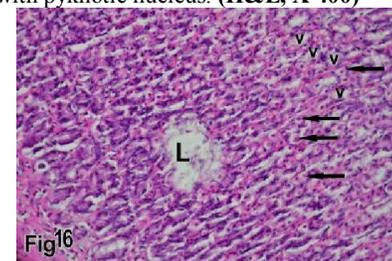


Fig. 16. A photomicrograph of a section in fundic mucosa of adult female rat following starvation for four days showing vacuolation of fundic gland cells (V), degeneration, loss of some cells (L) and distortion of the gland. Some parietal cells appeared with pale cytoplasm and pyknotic nuclei (→). (H&E, X 400)

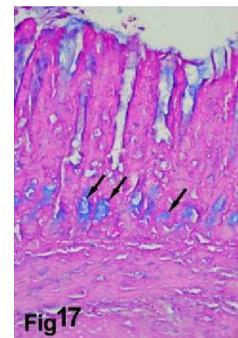


Fig. 17. A photomicrograph of a section in fundic mucosa of adult female rat following starvation for four days showing moderate PAS-AB positive reaction in the apical part of the gland. Many patches of blue staining of acid mucin (→) appear in the basal part of the gland. (PAS-AB, X 400)

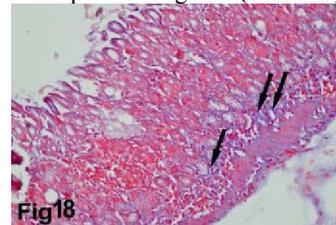


Fig. 18. A photomicrograph of a section in fundic mucosa of adult female rat following starvation for four days showing thick strands of collagen fibers which increased between the fundic glands (→) in the lamina propria in comparison to that of control group. (M.T, X 400).

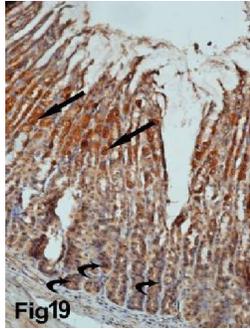


Fig. 19. A photomicrograph of a section in fundic mucosa of adult female rat following starvation for four days showing strong positive immune reaction in the cytoplasm of most of glandular cells specially parietal cells (→) and the basal part of the gland (curved arrows). (Caspas 3 immunostain, X400)



Fig. 20. A photomicrograph of a section in fundic mucosa of adult female rat following starvation for six days showing multiple ulcers (U) and loss of gastric mucosa. Exfoliation of the superficial epithelium (→) (H&E, X 100)

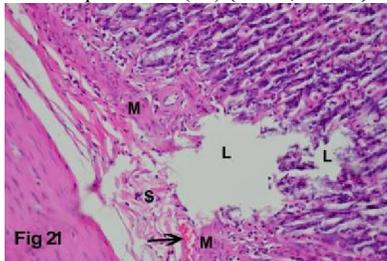


Fig. 21. A photomicrograph of a section in fundic mucosa of adult female rat following starvation for six days showing loss of some cells in the basal part of the fundic gland (L) with destruction of the muscularis mucosa (M) and submuco (S). Note: the dilated congested capillaries in the lamina propria (→) (H&E, X 400)

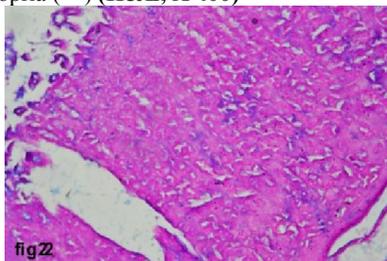


Fig. 22. A photomicrograph of a section in fundic mucosa of adult female rat following starvation for six days showing very weak PAS-AB positive reaction in the apical part of the gland. Weak to moderate PAS positive reaction appear in the base of the gland. (PAS-AB, X 400)

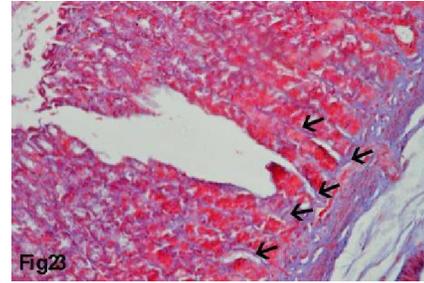


Fig. 23. A photomicrograph of a section in fundic mucosa of adult female rat following starvation for six days showing very thick collagen fibers between the fundic glands (→) in comparison to that of control group (M.T, X 400).

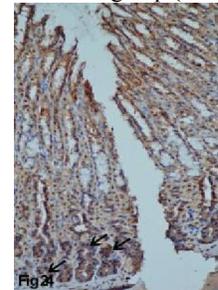


Fig. 24. A photomicrograph of a section in fundic mucosa of adult female rat following starvation for six days showing weak to moderate positive caspase 3 immune reaction in apical part of the fundic mucosa and very strong positive caspas 3 immune reaction in the basal part of the gland (→) (Caspas 3 immunostain, X400)

4. Discussion

Starvation is a situation affecting the metabolism and structure of living organisms. Secretions of the cells located in stomach glands enable the nutrients in the stomach to be decomposed and the digestion process to be started (Al-Qudah, 2011). There are different studies which focus on the effect of short and long term hunger on various cells of stomach (Koc and Muslu, 2007; Al-Qudah, 2011). In these studies, histological findings varied according to the genus, age and the hunger level of the experimental animals (Sonmez and Ozan, 2005).

The aim of the present study is to throw more light on the effect of both hunger and thirst on the histopathology of gastric fundus mucosa of female albino rats.

Mucosa of fundus of stomach of control group shows normal general structure as findings reported by Amer *et al.* (2013) who described normal structure of fundus of stomach during their experiment and this proved that, studied animals were healthy.

In the current study, there was highly significant reduction in the rat body weight after exposure to starvation when compared to control group. The rats weight markedly decreased with increased duration of starvation. This is in agreement with Vermeulen *et al.* (1997) who recorded reduction

of approximately 10% after 10 hours of food deprivation. Others, have reported weight loss up to 48 gm in male rats weighed 264 gm which represent over 18% of their body weight (**Dohm et al., 1983**). On the other hand, **Claassen, (1994)** observed weight loss in rats varied between 3.3% and 18% after 24 hours of fasting. He concluded that, Large changes in rat body weight have been reported following fasting.

In the present study, examination of the fundic mucosa sections of rats exposed to starvation varies according to the duration of starvation. The first changes in fundic mucosa resulting from hunger were observed at the end of the first day of last feeding and water intake in the form of desquamation and shedding of superficial epithelium with dilatation of some glands and congestion of some capillaries. These changes increased in severity in the second day as the superficial mucosal cells appeared with pyknotic and fading nuclei. The glands showed more cystic dilatation with flattening of their lining epithelium with loss of glandular epithelium in some areas. These findings can be explained by surface epithelium form a physical barrier between the lumen and underlying mucosa. An increase in the loss of epithelial cells or a decrease in cell renewal may lead to mucosal damage (**Asar et al., 2000**). This agree with **Al-qudah, (2011)** who reported same findings for first day of starvation, with parietal cells degeneration in the second day following food and water deprivation while **Colakolu et al. (1999)** observed widening in gland lumen after 3 days of hunger. On the other hand, **Alvares (1992)** detected no change in stomach mucosa of mice after 18 hours of hunger. More over, **Zaviacic et al. (1977)** reported that, there were some pouring in the surface mucus cells and shrinkage in the parietal cells after 3 days of hunger.

Multiple fundic ulcer was detected in this work after starvation for 6 days, with atrophy of surface epithelium, flattening of nuclei, and widening of gastric pits. This run in harmony with **Matsumoto et al.(1989)** who observed that, stomach ulcers occurred in mice due to hunger. The role starvation in the pathogenesis of gastric ulcer is poorly understood. **Abd El-Haleem et al. (2013)** attributed gastric ulcer may be due to abnormalities in gastric micro-circulation regulation which result in mucosal surface hypoxia. Cellular hypoxia usually induces release of lysosomal enzymes into the cells and the surrounding extracellular spaces leading to their damage with increased fragility of surface epithelium.

When the rat gastric mucosa is damaged, its histamine concentration falls and the histamine content of the gastric lumen increases. As histamine causes vasodilatation and increased capillary permeability, its presence in the interstitial fluid would probably result in the flow of a large volume of

plasma-like fluid from capillaries into interstitial spaces and through the mucosa, rendered more permeable by damage, into the gastric lumen (**Zhao et al., 2009**).

In the current study, many congested blood capillaries and extravasated red blood cells were observed in the fundic lamina propria after starvation. These findings may be attributed to alteration in gastric microcirculation with result increased gastric blood flow (**Ohta et al., 1994**). Other authors reported decreased blood flow in the mucosa and increased in the submucoas and serosa (**Gupta et al., 1998**). This could explain the mucosal surface hypoxia (**Cubillas and Rockey, 2010**). This agreed with **Al-qudah, (2011)** who reported congested blood vessels in the gastric mucosa after first day of starvation which progressed to congestion of submucosal blood vessels on the third day of hunger.

Microscopic examination of Periodic Acid Chief- Alcian Blue (PAS-AB) stained sections of the gastric fundic mucosa after starvation showed interrupted, very thin and faint PAS-AB positive reactions of the surface mucous film, weak PAS-AB positive reaction in mucous- secreting cells in the neck region and moderate PAS-AB positive reactions near the base of the gland. The mucus-bicarbonate-phospholipid barrier constitutes the first line of mucosal defense (**Laine et al., 2008**). This barrier is formed of mucus gel, bicarbonate and surfactant phospholipids which cover the mucosal surface and prevent penetration of pepsin and proteolytic digestion of the surface epithelium (**Allen and Flemstrom, 2005**). Starvation may causes a deficiency in oxygenation and reduction in mucus secretion which result in breakdown of the mucosal barrier and makes the mucosa susceptible to injury (**Tomikawa et al., 2000**). Moderate PAS-AB positive reactions in the basal cells of the gland could be explained by loss of acid secreting parietal cells which is associated with trans-differentiation of chief cell lineage to mucous cells (**Goldenring et al., 2011**).

In the present study, masson trichrome stained sections of gastric fundic mucosa after starvation showed thick strands of collagen fibers between the fundic glands in the lamina propria. These findings were correlated with the work carried out by **Majumadar et al. (1990)**; **Colakolu et al. (1999)** who reported that, both aging and starvation are associated with atrophy of fundic mucosal glands with marked deposition of connective tissue in the lamina propria in the base of the gland. These findings could partly attributed to increased suitability of the mucosa to various damaging agents together with impairment of the repair process (**Fligiel et al., 1994**)

In the current study, histopathological examination of sections of the gastric fundic mucosa

after water and food deprivation for four days showed gastric ulcer with atrophy of the lining epithelium with glandular distortion, some parietal cells were vacuolated with pyknotic nuclei, Some fundic gland cells showed vacuolation with degeneration and loss of some other cell. On the other hand, some cells in the basal part of the fundic gland were lost with destruction of the muscularis mucosa and submucosa after six days of starvation. These findings represent a well known effect of starvation as reported by **Al-qudah, (2011)**. Apoptosis and degeneration of parietal cells and cells on the base of fundic gland could be confirmed by strong positive caspase 3 immunoreactivity in cytoplasm of parietal cells and cells on basal part of the gland as reported in the present study. Caspases are important components of the mammalian apoptotic cascade in mammalian cells. Caspase-dependent apoptosis is a well characterized mechanism for removing senescent, defective, or unneeded cells (**Said et al., 2004**). Caspase 3 is a core member of the caspase family that becomes activated during apoptosis in a wide variety of tissues that causes fragmentation of DNA molecules by activated endonucleases (**Lowe and Rode, 1989; Turner and Lysiak, 2008**). Starvation suppressed gastric mucosal cyclo-oxygenase-1 and increased gastric mucosal TNF- α , Fas and Fas ligand level. This increased death signal lead to activation of caspase-3 and caspase-8 (**Sun et al., 2006**).

Conclusion:

In this study, it was noticed that, hunger causes an obvious reduction in the fundic mucosa of stomach surface epithelium with multiple gastric ulcers as well as deposition of collagen fibers in the lamina propria and base of the glands. From this study and other studies, our conclusion is that, starvation induced structural changes in fundic gastric mucosa which were in direct proportion to duration of hunger.

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