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EFFECT OF STARVATION ON STOMACH IN FEMALE LOCAL RABBITS

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ABSTRACT

Starvation is a very serious form of malnutrition characterized by a deficiency in nutrients, vitamins and caloric intake. The present study attempts to detect the effect of starvation on the morphology and histology of stomach in adult female rabbits. the present study included 25 rabbits,their weight ranged between(1500-1800 gm), divided into one control group and four starved groups (S3, S6, S9 and S12) each contained 5 rabbits. The morphological results of stomach showed significant decrease ($P \leq 0.05$) in weight and length.The macroscopic observation revealed changes due to starvation stress ,these changes included a shrinkage in the shape of the stomach,separation of the stomach wall from the lining with an increases in the presence and numbers of the longitudinal folds especially in the fundic part as compared with the control group.The.histological changes in stomach body was limited to congestion in the mucosa in the S6 group, in the S9 group the congestion only in the muscularis externa, while the mucosa and muscularis externa showed congestion in the S12 group as compared with the control group. While in the case of the changes in the layers thickness the stomach, submucosa showed a significant decrease ($P \leq 0.05$) in S6, S9 and S12 groups and the muscularis layer showed a significant decrease ($P \leq 0.05$) in S3 and S12 groups.The fundic part of the stomach revealed congestion in the submucosa layer of all the starved groups, a significant decrease ($P \leq 0.05$) was observed in the muscularis thickness of the S6 and S9 groups, while the pyloric part revealed congestion in the submucosa and muscularis layers of the S3 and S12 groups, congestion in the serosa in the S6 group. The muscle thickness in S6, S9 and S12 groups showed a significant decrease ($P \leq 0.05$) as compared with the control group.

Keywords: Rabbit, Starvation, Stomach, Histology, Morphology.

I. INTRODUCTION

Starvation can be defined as a post absorptive condition derived from a limitation on food resources by external factors (Serrano-Contreras *et al*.,2016) . During long-term periods of starvation, animals increase their ability to survive by changing the activity of the digestive system (Włodarczyk *et al*., 2017). Feed restriction can result in several metabolic changes that lead to lower body weight, immunodepression and modified function of the digestive system, especially the liver and small intestine. These changes affect the enzyme activity in the brush border, mucosa cell mass, protein content and mucosa integrity (Oliveira *et al*., 2013).

Starvation either produced experimentally, psychologically, or pathologically in conditions such as anorexia nervosa or certain obstructive tumors of esophagus, or non-availability of food due to any other means is likely to alter the physiology of the individual. In general, it would be reflected in weight loss, sluggish activity, and even apathy. The alimentary tract mucosa which has the onus of physiological adaptation to incoming food, digestion, absorption, and onward transmission is expected to react and get adjusted to the changed physiological state (Guha and Tiwari, 2016). There is evidence that the length of starvation induces changes in villus height and crypt depth (Chappell *et al*., 2003; Song *et al*., 2009).This has already been demonstrated in other animal species, although, to the best of our knowledge, not in adult rabbit models. Throughout starvation animals display morphological changes in the gastrointestinal tract. These include a decrease in the length and mass of the small intestine, a decrease in mucosal weight, changes in villus length and thickness, and phenotypic changes in the enterocytes (Dunel-Erb *et al*., 2001; Karasov *et al*., 2004; Zeng *et al*., 2012).

Conservation of energy and body tissues is the essential metabolic response to starvation. The body will, however, mobilize its own tissues as a source of energy which leads to the destruction of visceral organs and muscles and extreme reduction of adipose tissues (Mai-siyama *et al.*,2017). Several earlier studies documented the impact 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 (Ali, 2011) and pancreas (Kitagawa and Ono, 1986).The risk of food limitation and, ultimately, starvation dates back to the dawn of heterotrophy in animals, yet starvation remains a major factor in the regulation of modern animal populations. Researchers studying starvation more than a century ago suggested that animals subjected to sublethal periods of food limitation are somehow more tolerant of subsequent starvation events (McCue *et al*., 2017).

II. MATERIAL AND METHODS

2.1. Experimental Animals

Adult female rabbits of the genus *Oryctolagus cuniculus* were the experimental animals used for this study with an average weight (1,250-1,800 gm) collected from different locations in the province of Babylon. They were housed in special rabbit cages in the Biology Department / College of Science's animal house at the University of Babylon, with proper hygiene and sterilization maintenance. The animals were housed with controlled conditions of temperature (25 ± 3^{0}C), and 12 hours of light /dark cycles. The Rabbits were given the opportunity to adapt for about two weeks before the start of the experiment, while they were given food and water *ad libitum* during this period.

2.2. Experimental design

Twenty-five adult rabbits were divided into control group of five rabbits and four groups (starved) of five rabbits each. The first, is the control (n = 5) was maintained on standard animal food and tap water *ad libitum*. The experimental animals formed the second, third, fourth and fifth groups and each of the groups contained 5 rabbits, starved for different periods of times (3,6,9 and 12) days.

Control group: The animals have free access to standard animal food and tap water ad libitum.

Group S3: All animals of this group were deprived of food and water for 3 days.

Group S6: All animals of this group were deprived of food and water for 6 days.

Group S9: All animals of this group were deprived of food and water for 9 days.

Group S12: All animals of this group were deprived of food and water for 12 days.

2.3. Animal body weight

Body weights loss of the animals subjected to starvation for 3,6,9 and 12 days were measured by using electrical balance at the end of the experimental periods to estimate the body weight loss due to food and water deprivation as compared with the control group.

2.4. Animal anesthesia, dissection and measurements

The studied rabbits were anesthetized by using chloroform. The animals after the anesthesia were put on a dissecting board, the fore and hind limbs were fixed to the board, and then the animal was dissected by making incision along the abdominal side with a sharp scissors after the removing of the hair on the abdomen. The stomach of the rabbit were removed carefully.

The rabbits selected for the present study were slaughtered at approximately similar weights. The weight of stomach was measured by using electronic sensitive balance and the length measured by the using of calibrated ruler and thread.

2.5. Histological study and Morphometry

The stomach of the rabbit was removed and washed with normal saline, fixed in boun's fixative for 24 hour and then dehydrated in ascending grades of alcohol, cleared in xylene and embedded in paraffin. Serial sections of 5µm thick were cut and stained with haematoxylin and eosin (Bancroft *et al.*, 2013) for histopathological examination. Images were examined and photographed under a microscope digital camera. Sections were studied using light microscope with ocular micrometer and micrometer stage. Multiple measurements were done, from each section we measured the thickness of the mucosa , submucosa , muscularis externa and serosa . We took tissue samples from all individuals in the study and measured 10 sections per tissue sample (Karasov *et al*., 2004).

2.6. Statistical analysis

Statistical Package for Social Science (SPSS) version 23.0 (SPSS, Chicago, USA) was used for statistical analysis of the data. Data was given in the form of arithmetical mean values and standard deviation. One way analysis of variance (ANOVA) was performed. The means were separated using Duncan Multiple Test. The level of significance was accepted under (P≤0.05).

III. RESULTS AND DISCUSSION

3.1. Morphological results

The present study results showed that all the groups subjected to starvation, regardless of the duration of food restriction, showed a significant decrease (P≤0.05) in their body weight when compared with the control group at the end of starvation session, and the degree of weight loss correlated (Liu *et al*., 2018) to the length of food restriction as shown in Table (1).

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

Groups	Mean ±SD of body weight in (gm)
Control group	**1633.2 ± 141.6 b**
Starved for 3 days (S3 group)	**1087.4 ± 21.5 a**
Starved for 6 days (S6 group)	**1031.8 ± 28.1 a**
Starved for 9 days (S9 group)	**1024.4 ± 28.5 a**
Starved for 12 days (S12 group)	**1004.4 ± 15.1 a**

*Different symbols mean significant differences (P≤0.05).

Animals under conditions of food restriction and hunger will show a decline in their body weight, and the severity of the weight loss depends mainly on the duration of the starvation session. The weight decrease can be attributed to the absence of food and water consumption. The weight parameter considered as important factor for the progress of biological activities and its affected by external effectors (Guyton and Hall, 2006).

An effective way to minimize energy expenditure during food scarcity is a decrease in organ mass. All organs weighted after starvation showed a decrease in mass compared to control values. Our data show that weight loss in response to starvation is more pronounced in the small intestine than in the total body and this agree with (Chediack *et al*., 2012).

Table (2): Effect of starvation on morphological parameters in rabbits starved for (3,6,9 and 12) days

Organ	Weight / Length	Control	S3	S6	S9	S12
stomach	**Weight (gm)**	17.7±2.4 a	15.3±1.8 b	14.4±0.5 b	13.5±1.2 b	14.4±0.9 b
	Length (cm)	20.70±2.44 b	17.80±0.84 a	19.20±1.30 ab	18.80±2.28 ab	18.40±2.41 ab

*Different symbols mean significant differences (P≤0.05).

As illustrated in Table (2) and comparing with control group the weight of the stomach showed a significant decrease ($P \leq 0.05$) in all the starved groups as compared with the control group. a similar observation in stomach weights was obtained by (Funes *et al*., 2014). The length of the stomach in the S3 group was observed to show a significant decrease ($P \leq 0.05$) as compared with the control group this result disagree with the result obtained by (Rios et al., 2004). The decrease in the stomach length could be attributed to the fact that the stomach showed a shrinkage in shape as a result of starvation which lead to the stomach emptiness as mentioned by (Guha and Tiwari, 2016).

The stomach part that were studied in the present study included (fundic part, stomach body and pyloric part), the present study showed that there was a shrinkage in the shape of the stomach organ as a whole as compared with control group (Figure 1 a & b) and this result agree with (Guha and Tiwari,2016) who found that when the abdomen was opened, the stomach was found shrivelled up and reduced in size and also revealed that the stomach on its surface showed wrinkles. The present study also showed that there is a longitudinal folds spreaded along the internal lining of the stomach especially in the fundic part with normal shape of the stomach wall in the control group, while in the all the starved groups there was an increase in the presence and numbers of the folds mostly in the fundus , and the stomach wall in these groups was observed to be separated from the lining of the stomach especially in the S12 group as a result of stomach emptiness(Figure 1 c & d)

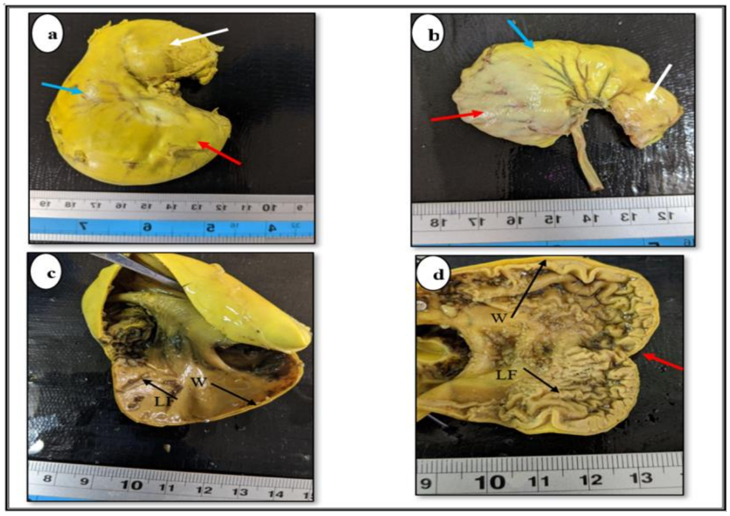

Figure (1): a: External shape of the stomach in the control group showing the fundic part (red arrow), stomach body (blue arrow) and pyloric part (black arrow). b: External shape of the stomach in the starved groups showing the fundic part (red arrow), stomach body (blue arrow), pyloric part (black arrow) and shrinkage of the stomach as a whole. c: Internal lining of the stomach in the control group showing the longitudinal folds (LF) and normal wall (W). d: Internal lining of the stomach in the starved groups showing the abundance in longitudinal folds especially in the fundic part (red arrow) and the separation of the wall.

3.2. Histopathological results

The histopathological alteration of the fundic part of the stomach was limited to congestion in the submucosa layer of all the starved groups (Figure 2 b & c).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. A significant decrease ($P \leq 0.05$) was observed in the muscle thickness of the S6 and S9 starved groups (Table 3). Guha and Tiwari (2016) stated that after 7 days of starvation no appreciable changes are observed in all the three constituents of mucous membrane in the fundic part of the stomach only at the 14 days of starvation the fundic part of stomach showed that the epithelial lining appears to be thrown into folds. The lamina propria is flooded with cellular elements, predominantly the eosinophils.

The stomach body revealed congestion in the mucosa in the S6 group (Figure 3), in the S9 group the congestion only in the muscularis externa (Figure 2 c), while the mucosa and muscularis externa showed congestion in the S12 group as compared with the control group (Figure 2 d). In the case of the changes in the layers thickness the stomach, submucosa showed a significant decrease ($P \leq 0.05$) in S6, S9 and S12 groups, the muscularis layer showed a significant decrease ($P \leq 0.05$) in S3 and S12 groups (Table 3), this agree with (Liu *et al*., 2018).

On the other hand, the pyloric part revealed congestion in the submucosa and muscularis layers of the S3 and S12 groups (Figure 4 b), congestion in the serosa in the S6 group (Figure2 b& c).The muscle thickness in S6, S9 and S12 groups showed a significant decrease (P≤0.05) as compared with the control group (Table 3). Liu *et al*.(2018) also mentioned that the thickness of the muscular layer is reduced with the prolongation of hunger.

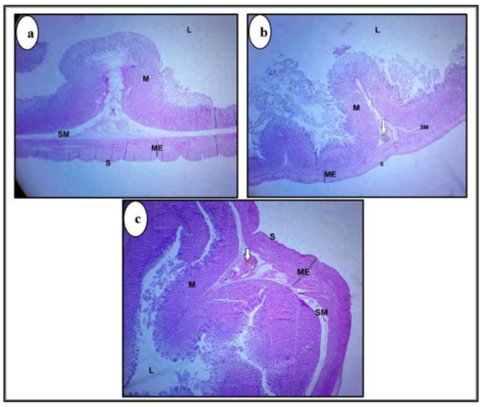

Figure (2) a: Cross section in the fundic part of the stomach of the control group showed the four layers: mucosa M, submucosa SM, muscularis externa ME, serosa S and lumen L. H&E, 40x. b: Cross section in the fundic part of the stomach of the S9 group showed the four layers: mucosa M, submucosa SM, muscularis externa ME, serosa S, lumen L, congestion in the mucosa (white arrow). H&E, 40x. c: Cross section in the fundic part of the stomach of the S12 group showed the four layers: mucosa M, submucosa SM, muscularis externa ME, serosa S, lumen L, congestion in the mucosa (white arrow). H&E, 40x.

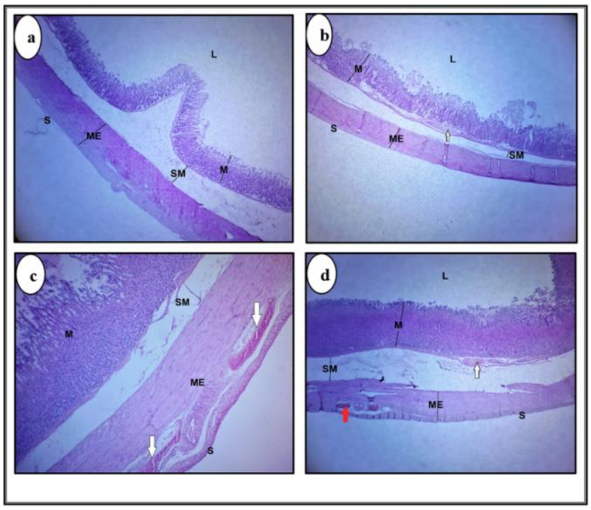

Figure (3) a: Cross section in the stomach body of the control group showed the four layers: mucosa M, submucosa SM, muscularis externa ME, serosa S and lumen L. H&E, 40x. b: Cross section in the stomach body of the S6 group showed the four layers: mucosa M, submucosa SM, muscularis externa ME, serosa S, lumen L, congestion in the mucosa (white arrow). H&E, 40x. c: Cross section in the stomach body of the S9 group showed congestion muscularis congestion (white arrow). H&E, 100x. d: Cross section in the stomach body of the S12 group showed congestion in the mucosa (white arrow) and muscularis congestion (red arrow). H&E, 40x.

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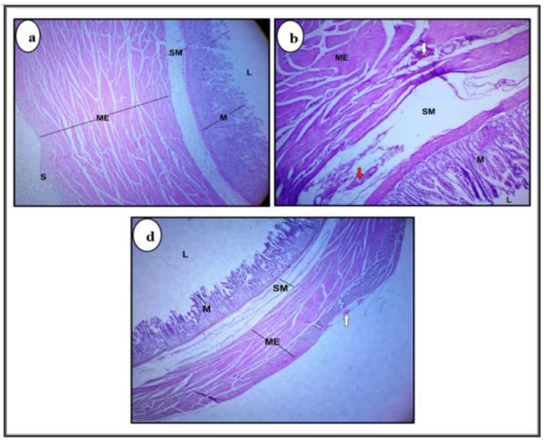

Figure (4) a: Cross section in the pyloric part of the stomach of the control group showed the four layers: mucosa M, submucosa SM, muscularis externa ME serosa S and lumen L. H&E, 40x. b: Cross section in the pyloric part of the stomach of the S3 group showed the four layers: mucosa M, submucosa SM, muscularis externa ME, serosa S, lumen L, congestion in the submucosa (red arrow) and muscularis congestion (white arrow). H&E, 100x. c: Cross section in the pyloric part of the stomach of the S6 group showed the four layers: mucosa M, submucosa SM, muscularis externa ME, serosa S, lumen L and congestion in the serosa (white arrow). H&E, 40x.

Table (3):Effect of starvation on the layers thickness in stomach parts (Mean ± SD) in rabbits starved for (3,6,9 and 12) days.

Groups / Layers		Control	S3	S6	S9	S12
Fundic part	**Mucosa (µm)**	**594.6±23.69 a**	**551.3±57.49 a**	**526.6±65.61 a**	**579.3±20.03 a**	**556±14.42 a**
	Submucosa (µm)	**137.3±23.18 ab**	**70.7±29 a**	**183±108.34 b**	**74.67±20.03 a**	**116.6±6.42 ab**
	Muscularis externa (µm)	**137.3±23.18 ab**	**70.7±29 a**	**183±108.34 b**	**74.67±20.03 a**	**116.6±6.42 ab**
	Serosa (µm)	**89.3±34.01 b**	**58.7±19.21 ab**	**56±24.58 ab**	**50±6.92 ab**	**40.7±5.03 a**
Stomach body	**Mucosa (µm)**	**518±224.13 a**	**576±164.79 a**	**411.3±45.44 a**	**612±51.88 a**	**640±200.11 a**
	Submucosa (µm)	**366±16.37 b**	**274±104.91 ab**	**186±4 a**	**208.6±92.11 a**	**176±77.48 a**
	Muscularis externa (µm)	**351.3±128.94 a**	**604.6±91.66 b**	**240±51.73 a**	**319.3±50.05 a**	**515.3±48.01 b**
	Serosa (µm)	**62.6±10.26 ab**	**96.6±36.35 b**	**46±12.49 a**	**52.7±11.01 a**	**68±7.21 ab**
Pyloric part	**Mucosa (µm)**	**648.3± 64.53 a**	**578±22.54 a**	**632±31.18 a**	**622±42.14 a**	**626.7±80.20 a**
	Submucosa (µm)	**279.3±77.78 a**	**218±19.70 a**	**260±15.87 a**	**204.7±86.30 a**	**185.3±15.27 a**
	Muscularis externa (µm)	**834.7±35.8 b**	**815.3±99.9 b**	**592±58.41 a**	**590±62.45 a**	**532±126.30 a**
	Serosa (µm)	**148.6±71 a**	**130.7±13.31** a	**103.3±13.32 a**	**85.3±44.73 a**	**110±41.03 a**

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*Different symbols mean significant differences (P≤0.05)

IV. CONCLUSIONS

In conclusions the present study has yield some original data regarding the starvation effect on stomach in adults rabbits. Starvation resulted in some morphological and histological changes, indicating that rabbits have the ability to withstand starvation for only short period of time.

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