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Morphological and histological study of large intestine in adult local rabbits subjected to starvation

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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 large intestine in adult females 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 cecum weight in all the starved groups was observed to show significant decrease (P≤0.05) as compared with the control group. The proximal colon weight in the S3, S6 and S12 groups decreased significantly (P≤0.05) as compared with the control group. The weight of the distal colon was found to show significant decrease (P≤0.05) in all the starved groups as compared with the control group. The vermiform appendix weight in the S3, S9 and S12 group was observed to show significant decrease (P≤0.05) as compared with the control group. The weight of the rectum in the S6 and S9 groups was observed to show a significant decrease (P≤0.05) as compared with the control group. The macroscopic observation revealed changes due to starvation stress mostly in the proximal colon,The proximal colon first segment in the S6, S9 and S12 groups contains protrusions which rised to the surface as tiny bubbles, in all the starved groups the space between these protrusions which almost disappeared and the protrusions sticked to each other. The histological results of the present study in the the cecum included congestion in the mucosa of the S3, S6 and S9 starved groups and severe congestion in the mucosa of S12 group, a significant decrease (P≤0.05) in the thickness of the mucosa, submucosa and muscularis layers in all the starved groups and a significant decrease (P≤0.05) in

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the S9, S9 and S12 serosa thickness. The appendix showed a reduced lymphoid tissue in the S6, S9 and S12 groups, congestion in the mucosa and submucosa of S3, S6 and S12 and congestion in the mucosa and muscularis externa in the S9 group, a significant decrease (P≤0.05) in mucosa of all the starved groups, while the submucosa only in the S12 group, the serosa in the S6, S9 and S12 groups showed a significant decrease (P≤0.05) as compared with the control group. The proximal colon changes included congestion in the mucosa of all the starved groups and congestion in the muscularis of the S12 group, a significant decrease (P≤0.05) in the mucosa and serosa thickness in the S6, S9 and S12 groups, the submucosa in the S3, S6 and S12 groups as compared with the control group. The distal colon showed congestion in the mucosa of S9 group and congested serosal vessel in the S12, a significant decrease (P≤0.05) in the mucosa submucosa and muscularis in the S6, S9 and S12 groups, the serosa showed a significant decrease (P≤0.05) in the S9 and S12 groups. The rectum showed no pathological changes but a significant decrease (P≤0.05) in the mucosa and submucosa of the S6, S9 and S12 groups, the muscularis in the S9 and S12, while the adventitia showed a significant decrease (P≤0.05) in the S12 group only as compared with the control group.

***Keywords*---**rabbit, starvation, large intestine, histology, morphology.

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).

Material and Methods

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.

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

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.

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 cecum,colon,appendix and rectum of the rabbit were removed carefully. The rabbits selected for the present study were slaughtered at approximately similar weights. The weight of organs were measured by using electronic sensitive balance and the length measured by the using of calibrated ruler and thread .

Histological study and morphometry

The cecum, appendix, proximal colon , distal colon and rectum of the rabbit were 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) .

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 ($P \leq 0.05$).

Results

Morphological study

The present study results showed that all starved groups showed a significant decrease ($P \leq 0.05$) in their body weight when compared with control group 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).

As shown in Table (2) a significant decrease (P≤0.05) was observed in the weight of the cecum in all the starved groups as compared with the control group.While the relative weight of the cecum there is a significant increase (P≤0.05) in the S9 group and a significant decrease (P≤0.05) in the S12 group as compared with the control group.The proximal colon weight in the S3, S6 and S12 in the present study was found to show a significant decrease (P≤0.05) as compared with the control group .The weight of the distal colon was found to show significant decrease (P≤0.05) in the four groups as compared with the control group, but no significant change (P>0.05) in the relative weight was observed in all the starved groups as compared with the control group .The vermiform appendix weight in the S3, S9 and S12 group was observed to show significant decrease (P≤0.05) as compared with the control group. While the relative weight of the appendix in the S3 group also showed significant decrease (P≤0.05) as compared with the control group.The weight of the rectum in the S6 and S9 groups was observed to show a significant decrease (P≤0.05) as compared with the control group but the relative weight was found to show no significant change (P>0.05).

Table 2
Changes in large intestine weight (gm) (Mean ± SD) in females rabbits subjected to different starvation periods (3,6,9 and 12) days

organs	Weight / Relative weight	control	S3	S6	S9	S12
Cecum	Weight (gm)	19.84±1.61 d	16.96±0.96 c	12.96±1.79 a	14.92±0.88 b	12.72±1.49 a
	Relative weight %	22.89±2.15 bc	22.99±1.79 bc	20.62±1.77 ab	23.59±0.97 c	20.20±1.84 a
Appendix	Weight (gm)	3.04±0.48 b	1.88±0.51 a	2.48±0.37 ab	1.82±0.24 a	1.88±0.64 a
	Relative weight %	3.50±0.54 bc	2.54±0.67 a	3.94±0.35 c	2.89±0.45 ab	2.98±0.97 ab

Proximal colon	Weight (gm)	8.24±1.45 b	6.76±0.82 a	6.40±1.14 a	6.94±1.04 ab	6.62±0.48 a
	Relative weight %	9.46±1.34 ab	9.13±0.92 a	10.16±1.23 ab	10.94±1.23 b	10.52±0.38 ab
Distal colon	Weight (gm)	7.96±1.07 c	6.90±0.48 b	5.80±0.69 a	6.28±0.88 ab	6.28±0.41 ab
	Relative weight %	9.20±1.43 a	9.33±0.40 a	9.23±0.59 a	9.90±1.06 a	10.00±0.76 a
Rectum	Weight (gm)	1.24±0.43 c	1.08±0.08 bc	0.92±0.08 ab	0.74±0.21 a	1.08±0.08 bc
	Relative weight %	1.41±0.43 ab	1.46±0.11 ab	1.47±0.16 ab	1.18±0.37 a	1.72±0.18 b

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

As shown in Table (3) no significant decrease (P≤0.05) was observed in the length and relative length of the cecum in all the starved groups as compared with the control group..The appendix and rectum length and relative length in all the starved groups was found to show no significant change (P>0.05) as compared with the control group.The proximal colon length in the four groups was found to show no significant difference as compared with the control group. The relative length of the proximal colon in all the starved groups was found to show significance decrease (P≤0.05) as compared with the control group .The distal colon length and relative length in all the starved groups was found to show no significant change (P>0.05) as compared with the control group.

Table 3
Changes in large intestine length (cm) (Mean ± SD) in females rabbits subjected to different starvation periods (3,6,9 and 12) days

Organs	Length / Relative length	Control	S3	S6	S9	S12
Cecum	Length (cm)	33.60±2.88 a	36.20±2.59 a	34.00±1.00 a	33.00±3.08 a	34.20±1.30 a
	Relative length %	13.46±0.72 a	14.12±1.29 a	14.14±0.52 a	13.97±0.88 a	14.65±0.59 a
Appendix	Length (cm)	6.30±0.67 ab	5.90±0.42 ab	6.20±0.57 ab	5.60±0.65 a	6.60±0.55 b
	Relative length %	2.54±0.34 ab	2.30±0.17 a	2.58±0.20 ab	2.37±0.26 a	2.82±0.18 b
Proximal colon	Length (cm)	22.60±1.95 ab	22.60±1.14 ab	21.20±1.64 a	23.80±0.84 b	23.20±1.79 ab
	Relative length %	9.09±0.94 ab	8.80±0.34 a	8.81±0.55 a	10.11±0.78 c	9.94±0.79 bc

Distal colon	Length (cm)	35.40±0.89 ab	36.00±4.30 b	35.20±1.92 ab	36.40±1.82 b	32.40±1.14 a
	Relative length %	14.22±0.65 ab	14.02±1.64 a	14.63±0.75 ab	15.44±1.03 b	13.87±0.25 a
Rectum	Length (cm)	3.60±0.89 a	4.20±0.84 a	3.60±0.89 a	3.20±0.45 a	3.40±0.55 a
	Relative weight %	1.45±0.40 a	1.64±0.37 a	1.49±0.34 a	1.36±0.23 a	1.46±0.24 a

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

The present study showed that the large intestine parts are the cecum , colon and rectum , on the entire length of the cecum internal lining there is a numerous longitudinal folds (spiral folds) sometimes referred to as (spiral valve) that maintain the external feature of haustra , the width of these folds varies along the cecum length and in the distal portion of the cecum the spiral folds thins out and disappear into the cecal wall at the entrance of the appendix which showed no observable change in the starved groups as compared with the control group (Figure 1 a & b). The appendix was also found to be normal in the starved groups as compared with the control group (Figure 2 a & b). In the control group the proximal colon gross anatomically contains three segments, the first segment contains three taeniae (longitudinal bundles of muscular tissues) forming three rows of sacculations referred to as haustra and displays on the mucosal surface a pattern of protrusions. In the first few cm neighboring the cecocolical orifice this pattern takes the form of a series of elevated folds or looped configurations. The former protrusions have been termed "Warzen" (Figure 3 a). The second segment of the proximal colon, possessing a single tenia and one raw of haustra, macroscopically displays protrusions similar to the latter segment, which, however, are somewhat smaller and less regularly arranged.

The third segment called Fusus coli, the mucosal surface of the fusus coli is distinguished by prominent longitudinally running folds which are permanent structures in contrast to folds that transiently appear in other segments of the colon. While the distal colon is composed of one raw of haustra each haustra is separated by short longitudinal folds. The protrusions in the first segment of the proximal colon in the S6,S9 and S12 groups rised to the surface as tiny bubbles (Figure 3 b) in the S3 group the protrusions were observed to be similar to that of the control group and there was a slight change in the space between these protrusions (Figure 3c). The space between these protrusions in the four groups subjected to different starvation periods almost disappeared and sticked to each other. In the S9 group these changes were observed to be more noticeable than the other groups subjected to starvation (Figure 3 b). In the distal colon there were no observable changes .The last part of the large intestine is the rectum which is a simple tubular form with a thicker walls ,it was found to contain multiple longitudinal folds of variable length and scattered throughout the tissue in the control group and no distinguishable change was observed in the rectum of all the starved groups (Figure 4 a & b).

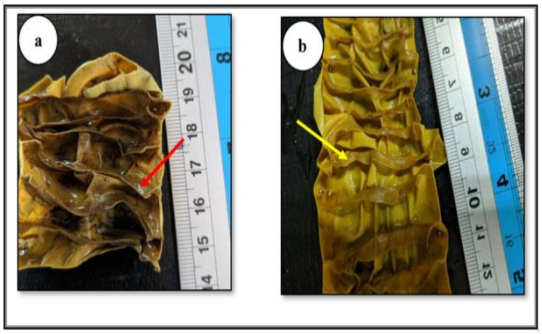

Figure 1. a: Internal lining of the cecum in the control group showing the normal shape of the spiral folds (red arrow). b: Internal lining of the cecum in the starved groups (S3) showing the spiral folds (yellow arrow) which is similar to that of the control group

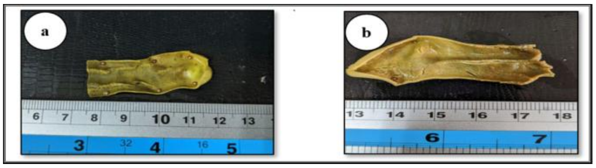

Figure 2. a: Internal lining of the appendix in the control group. b: Internal lining of the appendix in the starved groups (S3)

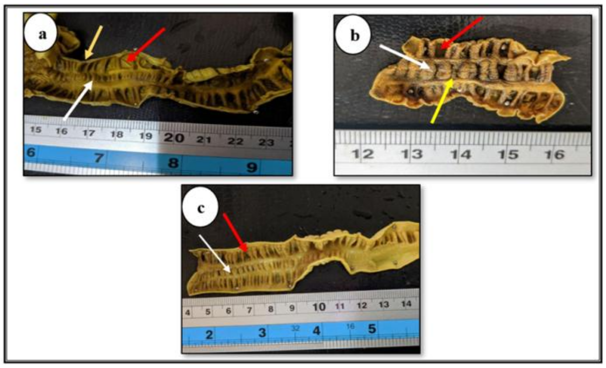

Figure 3. a: Internal lining of the proximal colon in the control group showing the first segment which contain three taeniae (yellow arrow), haustra (red arrow) and the protrusions (white arrow). b: Internal lining of the first segment in the proximal colon in the S9 starved group showing the haustra (red arrow), the change in the protrusions shape (white arrow) and the change in the space between these protrusions (yellow arrow). c: Internal lining of the proximal colon in the S3 starved group showing the slight change in the space between protrusions (white arrow) and haustra (red arrow)

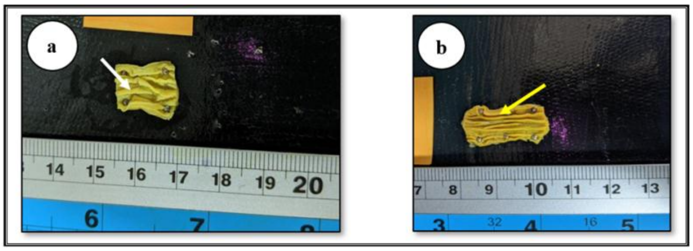

Figure 4. a: Internal lining of the rectum in the control group showing the longitudinal folds (white arrow). b: Internal lining of the rectum in the starved groups (S12) showing the longitudinal folds (yellow arrow)

Histological study

In the present study ,which was oriented on the histological aspects of starvation,the pathological changes that were observed in the cecum were included congestion in the mucosa of the S3, S6 and S9 starved groups (Figure 1 b & c) and severe congestion in the mucosa of S12 group (Figure 1 d). While the changes in the tunics thickness revealed that there was a significant decrease ($P \le 0.05$) in the thickness of the mucosa, submucosa and muscularis layers in all the starved groups as compared with the control group. The last layer which is the serosa showed a significant decrease ($P \le 0.05$) in the S6, S9 and S12 groups (Table 4). The appendix histological results included reduced lymphoid tissue in S6, S9 and S12 groups (Figure 2 c & d), S3, S6 and S12 showed congestion in the mucosa and submucosa as compared with the control group (Figure 2 b & d), congestion in the mucosa and muscularis externa in the S9 group (Figure 2 c).The results of the layers thickness revealed that there was a significant decrease ($P \le 0.05$) in the appendix mucosa of all the starved groups , the submucosa showed a significant decrease ($P \le 0.05$) only in the S12 group, while the serosa in the S6, S9 and S12 groups showed a significant decrease ($P \le 0.05$) as compared with the control group (Table 4-4).

The histological changes of the rabbit proximal colon included congestion in the mucosa of all the starved groups and congestion in the muscularis of the S12 group as compared with the control group (Figure 3 b & c).The changes in the thickness of the layers represented by a significant decrease ($P \le 0.05$) in the mucosa thickness in the S6, S9 and S12 groups, the submucosa showed a significant decrease ($P \le 0.05$) in the S3, S6 and S12 , while the serosa in the S6, S9 and S12 groups as compared with the control group (Table 4). The distal colon histological changes included congestion in the mucosa of S9 group and congested serosal vessel in the S12 group as compared with the control group (Figure 4 b & c). The changes in the layers thickness included a significant decrease ($P \le 0.05$) in the mucosa, submucosa and muscularis externa in the S6, S9 and S12 groups, while the serosa layer showed a significant decrease ($P \le 0.05$) in the S9 and S12 groups (Table 4). The histological results of the rectum showed no pathological changes in the starved group as compared with the control group (Figure 5 a & b). As for the changes in the tunics thickness, a significant decrease ($P \le 0.05$) in the thickness of mucosa and submucosa of the S6, S9 and S12 groups, the muscularis externa showed a significant decrease ($P \le 0.05$) in the S9 and S12, while the adventitia showed a significant decrease (P≤0.05) in the S12 group only (Table 4).

Table 4
Histological comparison between the large intestine parts (Mean ± SD) in rabbits subjected to different starvation periods (3,6,9 and 12) days

Groups / Layers		Control	S3	S6	S9	S12
Cecum	Mucosa (µm)	284.6±47.93 c	118.7±18.58 ab	116.7±5.03 ab	129.3±3.05 b	76.7±11.54 a
	Submucosa (µm)	83.3±11.01 b	23±7.55 a	30.3±7.09 a	22±6.08 a	20.7±7.02 a
	Muscularis externa(µm)	176±24.33 c	142.7±13.31 b	112±10.39 a	114.7±7.02 a	93.3±14.04 a
	Serosa (µm)	48±17.43 b	45±18.68 b	21±7.21 a	22±5.56 a	17.3±4.16 a
Appendix	Mucosa (µm)	1150.6±50.81 c	1152±65.20 c	804.7±105.19 b	643.3±46.31 a	531.3±30.35 a
	Submucosa (µm)	20.6±6.42 b	18.7±3.05 b	16.7±6.42 b	13.3±1.15 ab	6±1 a
	Muscularis externa(µm)	105.3±4.61 a	102±6 a	90.7±21.38 a	86.7±6.11 a	95.3±7.02 a
	Serosa (µm)	23.3±4.16 b	22±2 b	15.3±1.15 a	13±1.73 a	17.3±1.15 a
Proximal colon	Mucosa (µm)	247.3±16.77 d	202.7±29.14 cd	114±36.71 ab	144.7±51.78 bc	78.7±14.46 a
	Submucosa (µm)	48±6.92 d	30±6 bc	24.7±4.16 ab	38.7±8.08 cd	16.3±6.81 a
	Muscularis externa(µm)	202±25.53 b	152±52.57 ab	108±35.16 a	124.7±6.11 a	112±18.33 a
	Serosa (µm)	38±3.46 c	32.7±11.54 bc	15±8.54 a	17±4.58 ab	17.3±12.74 ab
Distal colon	Mucosa (µm)	342.7±61.49 c	272.7±9.45 bc	254±74.48 b	152.7±7.02 a	132.7±11.37 a
	Submucosa (µm)	66±20.88 b	41±13.23 ab	34.3±18.77 a	29±15.71 a	16±1.73 a
	Musculari	282±35.16	228.7±46.14	167.3±23.6	124±20.78	103.3±6.42

	s externa(µm)	c	c	9 b	ab	a
	Serosa (µm)	31.3±11.01 b	24.7±15.14 ab	15.7±5.68 ab	13.3±4.50 a	8.3±2.30 a
Rectum	Mucosa (µm)	665.3±126.11 c	620±91.65 bc	477.3±59.13 ab	408.6±99.28 a	400±36 a
	Submucosa (µm)	145.3±27.15 b	138±14.42 b	93.3±19.21 a	106.7±11.01 a	82.7±6.11 a
	Muscularis externa(µm)	589.3±71.67 c	597.3±43.65 c	504.7±92 bc	406±27.49 ab	340.7±46.57 a
	Serosa (µm)	118±42.57 b	106.7±4.61 b	104.7±10.06 b	82.7±3.05 b	18±2.65 a

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

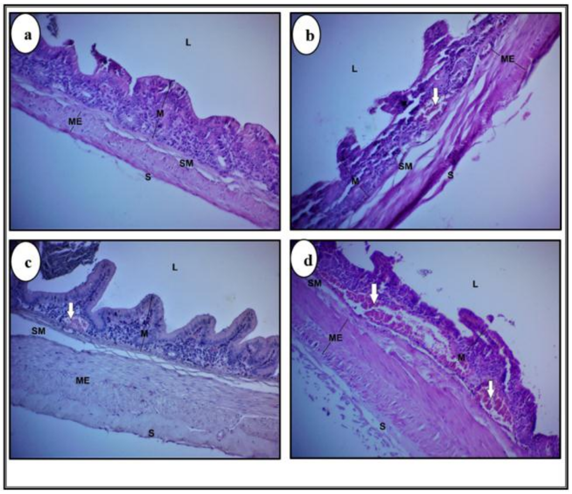

Figure 1. a: Cross section in the cecum of the control group showed the four layers: mucosa M, submucosa SM, muscularis externa ME, serosa S and lumen L. H&E, 200x. b: Cross section in the cecum of the S3 group showed the four layers: mucosa M, submucosa SM, muscularis externa ME, serosa S, lumen L and congestion in the mucosa (white arrow). H&E, 200x. c: Cross section in the cecum of the S9 group showed the four layers: mucosa M, submucosa SM, muscularis externa ME, serosa S and congestion in the mucosa (white arrow). H&E, 200x. d: Cross section in the cecum of the S12 group showed the four layers: mucosa M, submucosa SM, muscularis externa ME, serosa S and severe congestion in the mucosa (white arrows). H&E, 200x

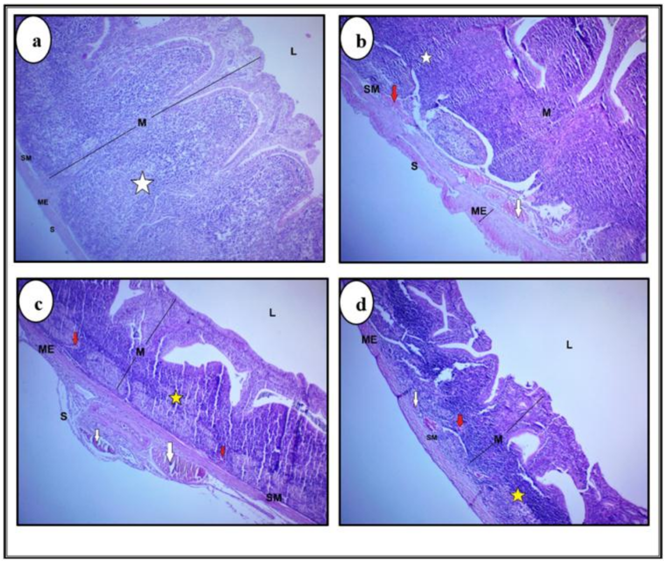

Figure 2. a: Cross section in the appendix of the control group showed the four layers: mucosa M, submucosa SM, muscularis externa ME, serosa S, lumen L and mucosal lymphoid tissue (white star). H&E, 100x. b: Cross section in the appendix of the S3 group showed mucosal lymphoid tissue (white star), congestion in the mucosa (red arrow) and submucosa congestion (white arrow). H&E, 100x. c: Cross section in the appendix of the S9 group showed reduced lymphoid tissue (yellow star), congestion in the mucosa (red arrow) and muscularis congestion (white arrows). H&E, 100x. d: Cross section in the appendix of the S12 group showed reduced lymphoid tissue (yellow star), congestion in the mucosa (red arrow) and submucosa congestion (white arrows). H&E, 100x

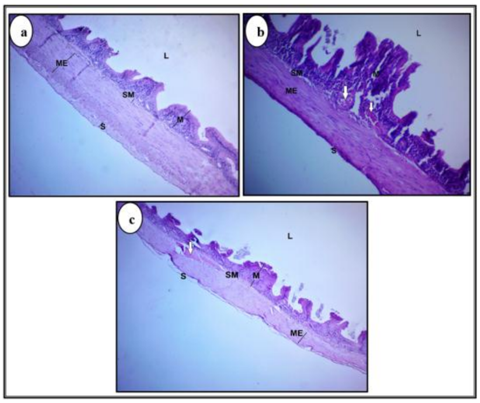

Figure 3. a: Cross section in the proximal colon of the control group showed the four layers: mucosa M, submucosa SM, muscularis externa ME, serosa S and lumen L. H&E, 100x. b: Cross section in the proximal colon of the S3 group showed congestion in the mucosa (white arrows). H&E, 200x. c: Cross section in the proximal colon of the S12 group showed muscularis congestion (white arrow). H&E, 100x

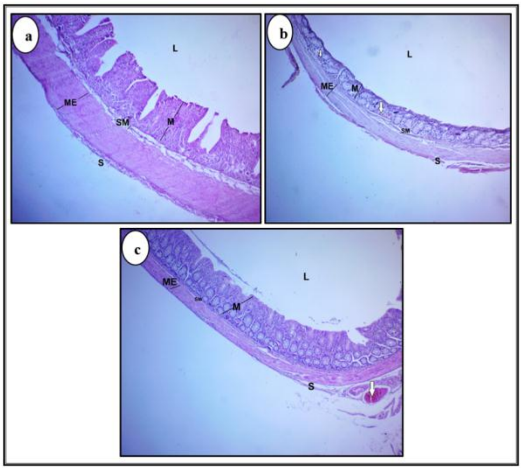

Figure 4. a: Cross section in the distal colon of the control group showed the four layers: mucosa M, submucosa SM, muscularis externa ME, serosa S and lumen L. H&E, 100x. b: Cross section in the distal colon of the S9 group showed congestion in the mucosa (white arrow). H&E, 100x. c: Cross section in the distal colon of the S12 group showed congested serosal vessels (white arrow). H&E, 100x

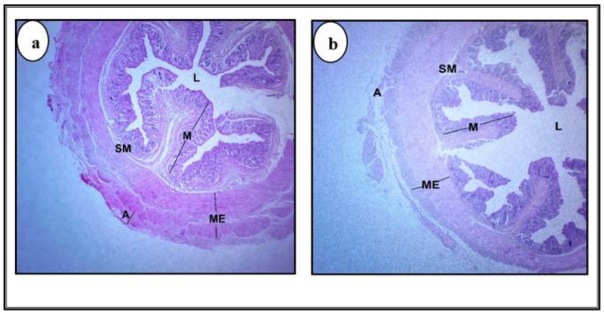

Figure 5. a: Cross section in the rectum of the control group showing the four layers: mucosa M, submucosa SM, muscularis externa ME, adventitia A and lumen L. H&E, 40x. b: Cross section in the rectum of the S12 group showing the four layers: mucosa M, submucosa SM, muscularis externa ME, adventitia A, lumen L, reduced mucosa and muscle thickness .H&E, 40x.

Discussion

The results of this current study revealed that all the groups subjected to starvation, regardless of the duration of food restriction, showed a significant decrease ($P \leq 0.05$) in their body weight when compared with the control group at the end of starvation session, and the degree of weight loss correlated to the length of food restriction (Liu et al., 2018). 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).

The survival of an organism during food deprivation (generally known as starvation for the purpose of this study) depends in part on the internal hormonal and biochemical adjustments such as the maintenance of optimal blood sugar by the mobilization or breakdown of stored glycogen when the glucose level of the body drops (Wu and Cederbaum, 2003). Starvation induces a wide range of responses that may also alter the gene expression, biochemical activities, physiological and behavioral responses of the starved organism especially if the period of starvation and/or malnutrition is not halved, and food taken to assuage the hunger. In such scenarios, starvation therefore results in a reduction of body weight and during starvation essential metabolic processes are maintained at the expense of accumulated endogenous energy reserves, which sometimes results in a loss of weight in the starved animals (Wang *et al.*, 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 and liver than in the total body and this agree with (Chediack *et al.*, 2012). As illustrated in Table (4-2) and comparing with control group, the GIT weight means showed a significant decrease ($P \leq 0.05$) in all the starved groups as compared with the control group this result agree with the results obtained by (Zeng *et al.*, 2012). The results of our current study showed that weight means of the proximal colon in the S3, S6 and S12 groups showed a significant decrease ($P \leq 0.05$) as compared with the control group and that's agree with (Song *et al.*,2009).

The large intestine parts are the cecum , colon and rectum , in the macroscopic observation of the present study it was noticed that on the entire length of the cecum internal surface there is a numerous longitudinal folds (spiral folds) sometimes referred to as (spiral valve) that maintain the external feature of haustra and increasing the absorption surface of the mucosa, the width of these folds varies along the cecum length and in the distal portion of the cecum the spiral folds thins out and disappear into the cecal wall at the entrance of the appendix as mentioned by Stan (2014), the cecum showed no observable change in the starved groups as compared with the control group. The appendix was also found to be normal in the starved groups as compared with the control group.

As for the macroscopic observation of the proximal colon in the control group it was found that it contains three segments, the first segment contains three taeniae (longitudinal bundles of muscular tissues) forming three rows of sacculations referred to as haustra and displays on the mucosal surface a pattern of protrusions. In the first few cm neighboring the cecocolical orifice this pattern takes the form of a series of elevated folds or looped configurations. The former protrusions have been termed "Warzen". The second segment of the proximal colon, possessing a single tenia and one raw of haustra, macroscopically displays protrusions similar to the latter segment, which, however, are somewhat smaller and less regularly arranged. The third segment called Fusus coli, the mucosal surface of the fusus coli is distinguished by prominent longitudinally running folds which are permanent structures in contrast to folds that transiently appear in other segments of the colon. While the distal colon is composed of one raw of haustra each haustra is separated by short longitudinal folds as mentioned by

(Snipes *et al.*, 1982). In the S6, S9 and S12 groups, the protrusions in the proximal colon first segment rised to the surface as tiny bubbles, in the S3 group these protrusions were observed to be similar to that of the control group and there was a slight change in the space between these protrusions. The space between these protrusions in the other three groups subjected to starvation (S6, S9 and S12) almost disappeared and sticked to each other since the colon does not contain food resides in the same amounts of that in the control group.

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