Original Article

Effect of Ketogenic Diet on Colonic Changes of Stress Induced Irritable Bowel Syndrome of Adult Male Albino Rat Model (Histological and Morphometric Study)

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ABSTRACT

Background: Irritable bowel syndrome (IBS) is a chronic gastrointestinal disorder. Psychological stress is identified as a trigger for IBS. Research on the effectiveness of the ketogenic diet for treating IBS remains limited.

Aim of the work: This work aimed to evaluate the effectiveness of ketogenic diet in mitigating colonic changes associated with experimentally induced irritable bowel syndrome in adult male albino rats.

Material and methods: Thirty adult male albino rats were used, divided equally into three groups:

- Control Group (Group I): Rats were fed a standard diet for ten days and then continued the same diet for an additional ten weeks without being exposed to water avoidance stress (WAS).
- IBS Group (Group II): Rats were fed a standard diet and subjected to WAS for ten days and then continued a standard diet for ten weeks.
- IBS-KD Group (Group III): Rats were fed a standard diet initially and exposed to WAS for ten days and then switched to ketogenic diet (KD) for ten weeks.

Results: Rats of the IBS group, which were fed a standard diet, showed several histological alterations of the descending colon, such as an irregular surface mucosal epithelium with detached cells in the lumen of the colon, degenerated colonocytes, a few goblet cells and large mast cells. In contrast, rats of the IBS-KD Group that were fed a ketogenic diet showed an almost regular histological structure of the descending colon.

Conclusion: Ketogenic diet could be regarded as a potentially beneficial approach for irritable bowel syndrome.

Key Words: Colon, Irritable bowel syndrome, Ketogenic diet.

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INTRODUCTION

Irritable bowel syndrome (IBS) is a chronic gastrointestinal disorder which impacts between 9% and 23% of people worldwide. Those with IBS often experience recurring abdominal pain, bloating and alterations in their bowel habits. The precise cause of IBS remains unidentified, but research is ongoing into various contributing factors such as disturbances in the gut-brain axis, dietary intolerance, imbalances in gut microbiota and genetic predispositions[1-3].

Disturbance of the gut-brain axis can cause hypersensitivity and inflammation of the intestinal mucosa[4]. Psychological stress is a precipitating factor for IBS due to its influence on the gut-brain axis[5].

As a chronic condition, irritable bowel syndrome requires long-term management. Current treatments are primarily aimed at relieving symptoms, leaving the quest for a definitive cure still ongoing[6].

Previous studies have highlighted the potential advantages of nutritional approaches, such as low carbohydrate diet for managing IBS[7].

The ketogenic diet is defined by its high fat, low carbohydrate and moderate protein composition. This dietary approach drives the body to rely on fat for energy via a process known as ketogenesis. During ketogenesis, fatty acids are converted into ketone bodies in the liver mitochondria and subsequently transported through the bloodstream to be utilized by other organs[8].

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Although studies on the effectiveness of the ketogenic diet for IBS are still limited, it is thought to be promising in the treatment for disorders related to the gut-brain axis. The ketogenic diet may aid in remodeling intestinal microbiota and modulating neurotransmitters and neurotrophins in the intestinal mucosa. Elevated levels of these factors are associated with IBS and contribute to increased intestinal hypersensitivity[9-11].

The majority of prior studies have examined the biochemical impacts of the ketogenic diet on IBS, while only a few have described the histological changes in experimental animal models.

AIM OF WORK

This work aimed to evaluate the effectiveness of ketogenic diet in mitigating colonic changes associated with experimentally induced irritable bowel syndrome in adult male albino rats.

MATERIAL AND METHODS

Experimental Animals:

Thirty adult male albino rats, each weighing 200 to 250 gm and aged 3 to 6 months, were used in the study. These rats were obtained and housed at the Animal House of the Ain Shams Medical Research Centre, Faculty of Medicine, Ain Shams University. They were kept in wire mesh cages (three rats per cage) with proper ventilation and were subjected to standard light/dark cycles. All groups had unrestricted access to water.

Study design:

The rats were assigned to three groups, with ten rats per group:

- Group I (Control Group): Rats were fed a standard diet for ten days and then continued the same diet for an additional ten weeks without being exposed to water avoidance stress (WAS).

- Group II (IBS Group): Rats in this group were fed a standard diet and subjected to WAS for ten days and then continued a standard diet for ten weeks.

- Group III (IBS-KD Group): Rats in this group were fed a standard diet initially and exposed to WAS for ten days and then switched to ketogenic diet (KD) for ten weeks.

Rats were monitored for signs of irritability, including loss of appetite, weight loss and loose or watery stools. The weight and stool condition of rats were assessed at the beginning of the study and after ten days of water avoidance stress. Rats displaying at least two of these symptoms were considered positive for IBS.

Experimental Methods:

Water avoidance stress (WAS) protocol for ten days:

It is a potent psychological stressor for inducing IBS. It leads to heightened colonic sensitivity, increased motility and inflammation in rats[12,13].

The apparatus for the water avoidance stress test comprised a rectangular plastic container (40x20x20 cm) with a plastic block (8x6x6 cm) positioned at its center. The container was loaded with fresh water (25°C), rising to 1 cm below the upper surface of the plastic block. Rats were placed on the block for 1h each day (from 9 a.m. to 10 a.m.) for ten consecutive days[14].

Diet regimens:

The standard diet was in the form of 30gm pellet of 52% carbohydrates, 15% protein, 7% fat ,6% fibers and more than 10% water[12,15].

The ketogenic diet was in the form of 30 gm pellet of 86% fat, 8.5% protein, 5.5% carbohydrates, and less than 10% water[12,15].

All the pellets were purchased from Meladco for Animal Food, Egypt.

Collection of specimens:

Upon completion of the experimental period, rats were sedated with an intraperitoneal injection of ketamine at a dosage of 60 mg/kg body weight. For each rat, the large intestine was extracted by a longitudinal abdominal

incision. The weight and length of the large intestine "from cecum to anus" were measured after emptying the fecal content[16]. A 5cm segment of the descending colon (located 10 cm from the anus)[17] was then excised and immediately rinsed with buffered saline. After collecting the specimens, the rats were euthanized by decapitation.

Processing of specimens for light microscope examination:

The proximal segment of the descending colon was fixed in 10% neutral formalin and then processed into paraffin blocks. The distal segment was cut into smaller pieces, fixed in 2.5% glutaraldehyde and processed into epon blocks. The paraffin blocks were sectioned into 5µm slices and stained for histological and immunohistochemical analysis. Hematoxylin and Eosin (H&E) stain[18] was used for general histological examination, while Alcian blue-PAS stain[19] was employed for detecting mucin and mucin producing cells.

For immunohistochemical examination, caspase3 "apoptosis indicator" was used. Sections were incubated with rabbit cleaved caspase3 polyclonal antibody to cleave caspase3 at a dilution of 1:200 (Invitrogen, Sweden AB Stockholm Sweden). The secondary antibody was anti-mouse antibody diluted of 1:500 (Invitrogen, Molecular Probes, Eugene, Oregon, USA)[20].

In the preparation of semithin sections, 1µm sections from epon blocks were cut and stained with toluidine blue[21].

Examination of the stained sections was carried out by a light microscope with automatic photomicrographic camera (BX3M series, Olympus, Tokyo, Japan) at Anatomy department, Faculty of Medicine, Ain Shams University.

Morphometric analysis:

Macroscopic analysis:

The length of large intestine (in cm) and its weight (in gm) were measured for each rat in all groups.

Image analysis:

Image analyzes software (Leica Q Win V.3. Wetzlar, Germany) was used at Histology Department, Faculty of Medicine, Ain Shams University. For each group, six non-overlapping fields (x200) of six different rats were assessed for the following parameters:

- The mean crypt length/one crypt in µm (H&E stained sections).
- Goblet cells number/one crypt (Alcian blue-PAS stained sections).
- The mean area % of caspase 3 expression in relation to a square of 100 um^2 area (Immunohistochemically stained sections).

Statistical analysis:

SPSS software (version 20, IBM Corp., Armonk, NY, USA), one-way ANOVA and Bonferroni Post Hoc test were used. Differences between rats' groups (I, II and III) were compared. The mean value ± standard deviation and *P-value* were presented in tables and bar charts. *P-value* was considered "significant" ≤ 0.05 and "non-significant" > 0.05.

Ethical consideration:

The approval number from the Research Ethics Committee is FMASL MS 100/2022, Faculty of Medicine, Ain Shams University.

RESULTS

I) Microscopic results:

Control group (Group I):

Examination of H&E stained longitudinal sections of the descending colons from Group I rats revealed the regular layers of the colonic wall; mucosa, submucosa, muscularis externa and serosa. The mucosal surface showed intact surface epithelium and narrow glands' pits. The full thickness of the mucosa was mostly occupied by closely packed simple tubular glands (crypts of Lieberkühn) arranged in parallel rows and separated by thin connective tissue of lamina propria with a few scattered eosinophils. The muscularis mucosa was formed of a regular thin layer of smooth muscle below the lamina propria separating it from the underlying vascular submucosa (Figures 1,2).

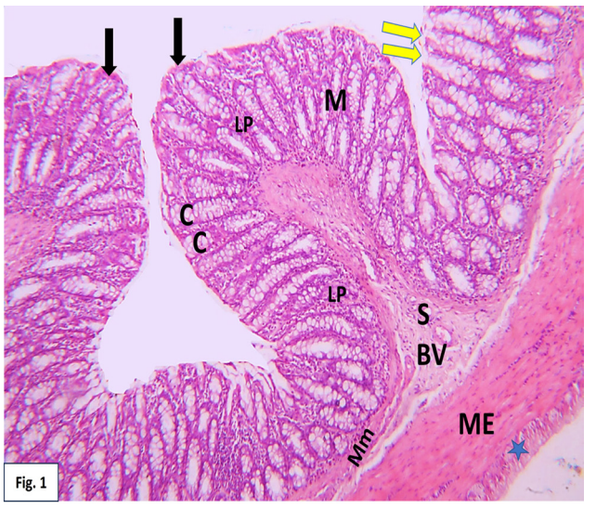


Fig. 1: Microphotograph of a longitudinal section of rat's descending colon "control group" showing regular structure of colon: mucosa (M), submucosa (S), muscularis externa (ME) and serosa (blue star). The mucosa showed intact surface epithelium (black arrow), crypts' pits (yellow arrow) and closely packed crypts (C) arranged in parallel rows. Notice, the thin lamina propria (LP) in between the crypts, the regular muscularis mucosa (Mm) and the small submucosal blood vessels (BV). (H&E, x100)

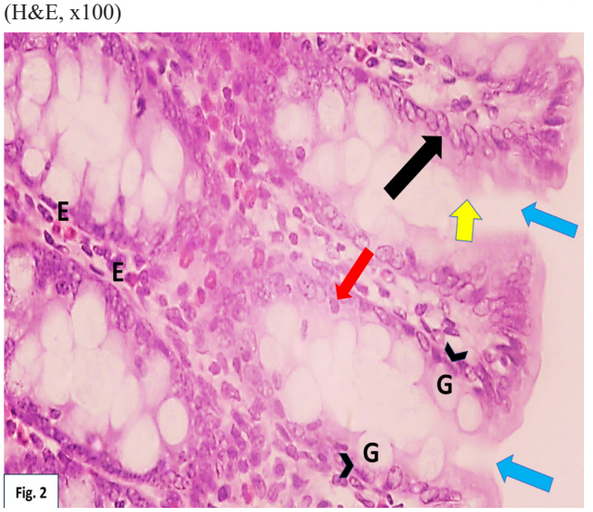


Fig. 2: Microphotograph of a longitudinal section of the mucosa of rat's descending colon "control group" showing the columnar cells lining the crypts having basal nuclei (black arrow) and apical microvilli (yellow arrow), plenty of goblet cells (G) with basal oval nuclei (black arrowhead) and a few scattered small rounded intraepithelial lymphocytes (red arrow). Notice, the crypts' pits (blue arrow) and a few scattered eosinophils in the laminal propria (E). (H&E, x400)

In longitudinal and transverse sections, the colonic mucosa showed the mucosal crypts lined with columnar cells (colonocytes) that had basal vesicular nuclei, acidophilic cytoplasm and apical microvilli giving them the brush border, in addition to plenty of cells having clear cytoplasm and basal oval nuclei (goblet cells). A few scattered small, rounded cells with apical rounded nuclei were detected (intraepithelial lymphocytes). In transverse sections, the peri-cryptal myofibroblasts with their characteristic elongated nuclei were noticed (Figures. 2,3).

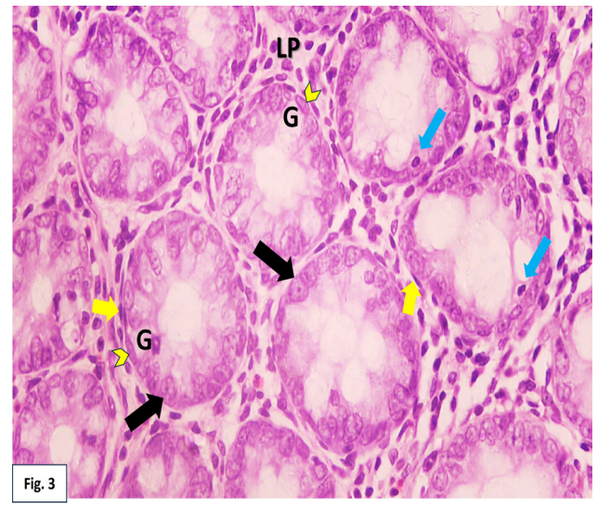


Fig. 3: Microphotograph of a transverse section of the mucosa of rat's descending colon "control group" showing the colonocytes (black arrow) lining the crypts having basal vesicular nuclei, goblet cells with clear cytoplasm (G) and oval basal nuclei (yellow arrowhead) and a few scattered intraepithelial lymphocytes (blue arrow). Notice the thin lamina propria (LP) in between the crypts and the peri-cryptal myofibroblasts with their elongated nuclei (yellow arrow). (H&E, x400)

Additionally, in longitudinal sections the muscularis externa was formed of thick inner circular and thin outer longitudinal smooth muscle layers. In between the two layers, the myenteric nerve plexus was observed (Figure. 4).

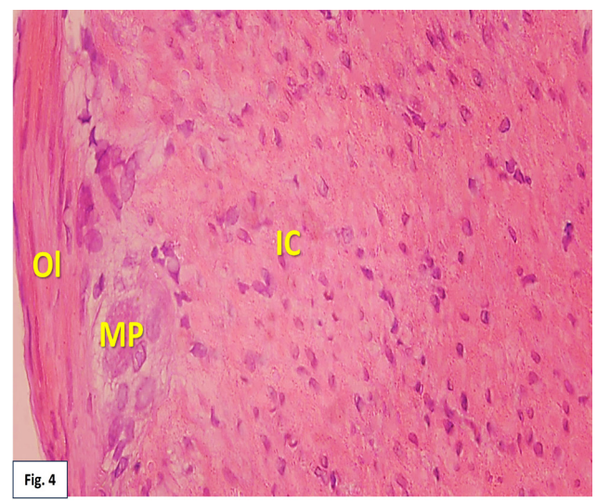


Fig. 4: Microphotograph of a longitudinal section of the muscularis externa of rat's descending colon "control group" showing thick inner circular (IC), thin outer longitudinal (OL) smooth muscle layers and the myenteric plexus (MP) in between. (H&E, x400)

Examination of semithin sections showed that the colonocytes lining the crypts were lying on an intact regular basement membrane and had basal nuclei with prominent nucleoli. Goblet cells appeared darkly stained and extended into the crypt lumen (Figure. 5).

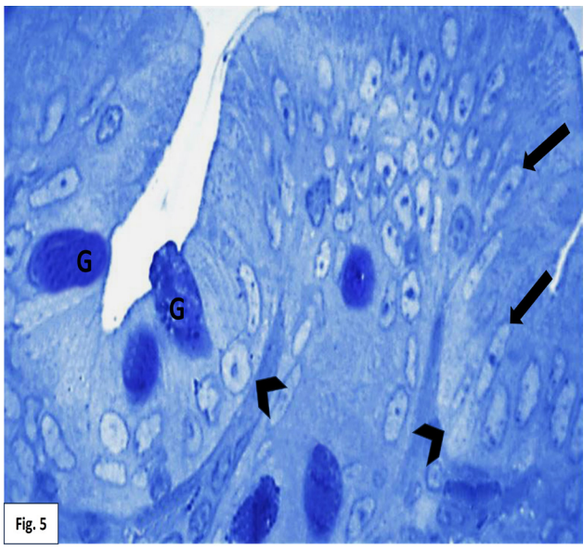


Fig. 5: Microphotograph of a semithin section of the mucosa of rat's descending colon "control group" showing columnar cells lining the crypts having basal vesicular nuclei (black arrow) and lying on intact basement membrane (black arrowhead). The goblet cells appeared darkly stained and extending into the crypt's lumen (G). (Toluidine blue, x1000)

Alcian blue-PAS stained sections showed strong positive reaction of numerous large goblet cells, as well as of the mucus inside the lumen of the colon (Figures. 6,7)

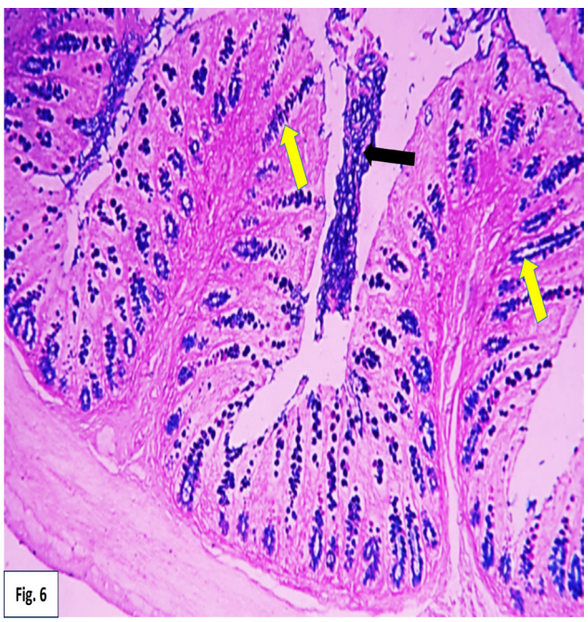


Fig. 6: Microphotograph of a longitudinal section of rat's descending colon "control group" showing strong positive reaction (blue stain) of many cells lining the crypts (yellow arrow) and strong positive reaction of the luminal mucus (black arrow). (Alcian blue-PAS, x100)

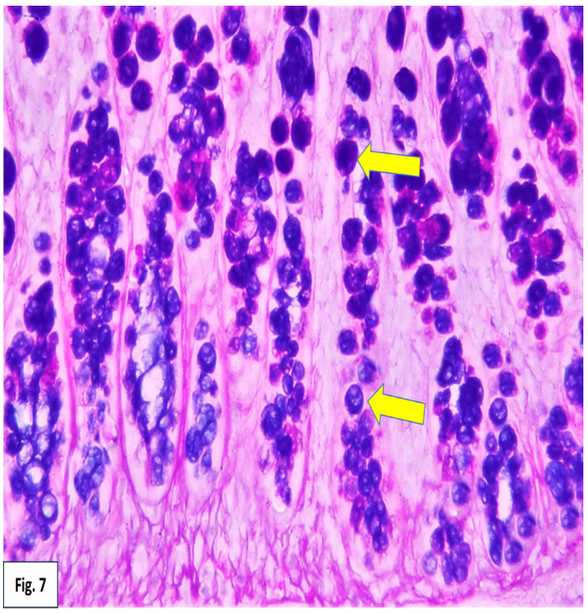


Fig. 7: Microphotograph of a longitudinal section of the mucosa of rat's descending colon "control group" showing strong positive reaction (blue stain) of numerous large goblet cells (yellow arrow). (Alcian blue-PAS, x400)

Immunohistochemically stained sections with caspase 3 showed negative immune reaction of most colonocytes and goblet cells, with only a few cells exhibiting a positive reaction, mainly located at the base of the crypts (Figure. 8).

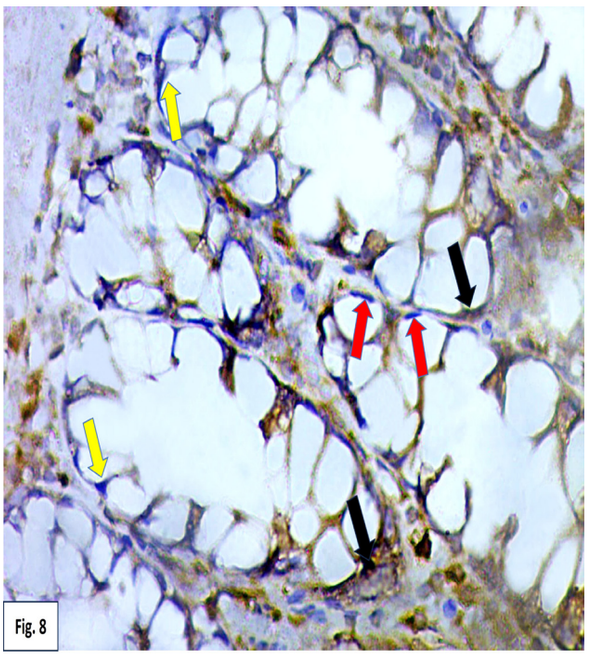


Fig. 8: Microphotograph of a longitudinal section of the mucosa of rat's descending colon "control group" showing negative immune reaction (blue stain) of most colonocytes (yellow arrow) and goblet cells (red arrow), with a few cells showing positive immune reaction mainly at the base of the crypts (black arrow). (Caspase 3, x400)

Stress induced IBS (Group II):

Examination of H&E stained longitudinal sections of descending colons from Group II rats showed irregular surface mucosal epithelium with detached cells in the lumen of the colon. The crypts were widely spaced and the submucosa exhibited dilated congested blood vessels (Figure. 9).

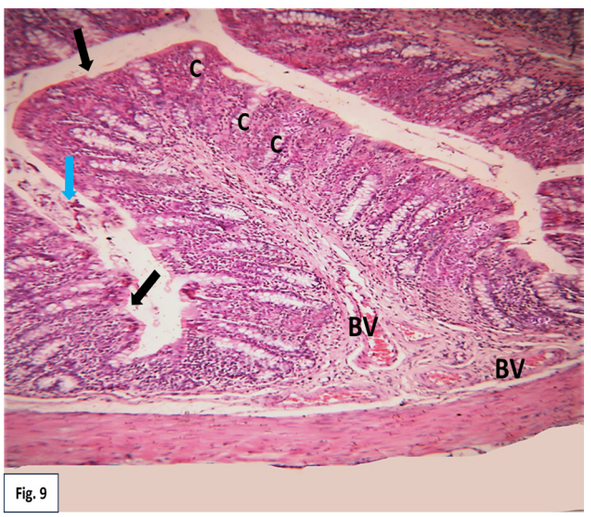


Fig. 9: Microphotograph of a longitudinal section of rat's descending colon "IBS group" showing irregular surface mucosal epithelium (black arrow), widely spaced crypts (C), dilated congested submucosal blood vessels (BV). Notice, the detached cells in the lumen of the colon (blue arrow). (H&E, x100)

In transverse and longitudinal sections, the colonic mucosa demonstrated that most of colonocytes lining the crypts had dark stained nuclei and the goblet cells being few and scarcely detectable. Obliterated crypts' pits and detached cells inside the crypts lumina were observed. The crypts were mostly irregular and separated by wide lamina propria heavily infiltrated with eosinophils (Figures. 10,11).

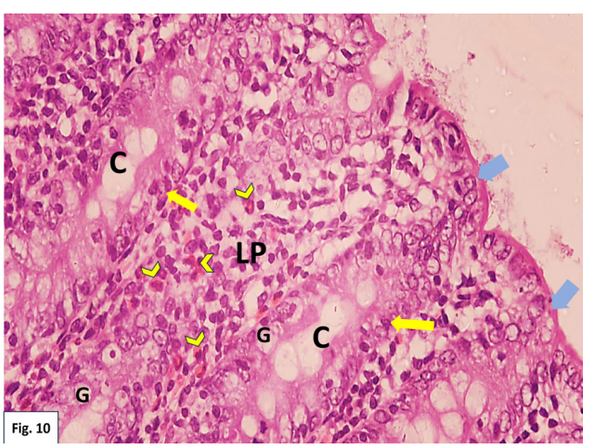


Fig. 10: Microphotograph of a longitudinal section of the mucosa of rat's descending colon "IBS group" showing most of the crypts (C) lined with colonocytes having darkly stained nuclei (yellow arrow) with obliterated crypts' pits (blue arrow), a few and scarcely detected goblet cells (G) and wide lamina propria (LP) heavily infiltrated with eosinophils (yellow arrowhead). (H&E, x400)

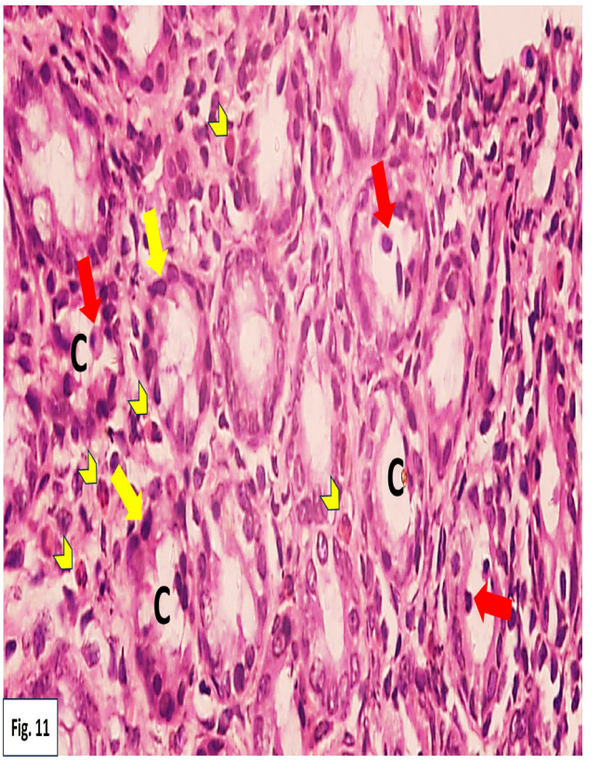


Fig. 11: Microphotograph of a transverse section of the mucosa of rat's descending colon "IBS group" showing irregular crypts (C) lined mostly with colonocytes having darkly stained nuclei (yellow arrow), detached cells in some crypts' lumina (red arrow) and infiltrated lamina propria with many eosinophils (yellow arrowhead). (H&E, x400)

Additionally, in longitudinal sections, the smooth muscles' cells of the inner circular layer of muscularis externa showed irregular nuclei with perinuclear vacuolations. Apparent regular outer longitudinal smooth muscle layer and myenteric nerve plexus were observed (Figure. 12).

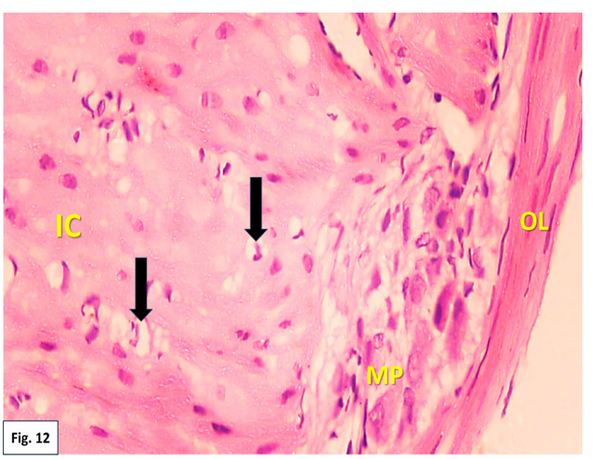


Fig. 12: Microphotograph of a longitudinal section of the muscularis externa of rat's descending colon "IBS group" showing the cells of the inner circular smooth muscle layer (IC) having irregular nuclei with perinuclear vacuolations (black arrow), regular outer longitudinal (OL) smooth muscle layer and myenteric nerve plexus (MP). (H&E, x400)

Examination of semithin sections revealed that some of the colonocytes had irregular nuclei, the goblet cells were scarcely detected and large mast cells were present. The cells were lying on an irregular basement membrane (Figure. 13).

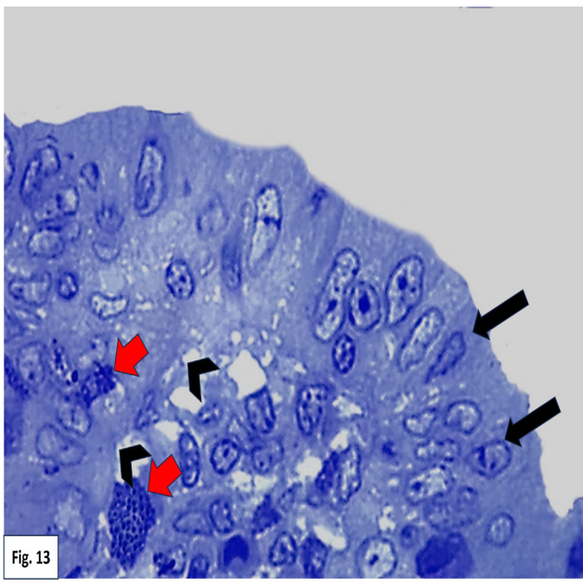


Fig. 13: Microphotograph of a semithin section of the mucosa of rat's descending colon "IBS group" showing some colonocytes having irregular nuclei (black arrow), large mast cells (red arrow). Notice, the irregular basement membrane (arrowhead) and the deficiency of goblet cells. (Toluidine blue, x1000)

Alcian blue-PAS stained sections showed weak positive reaction of a few small goblet cells and of scanty luminal mucus (Figures. 14,15).

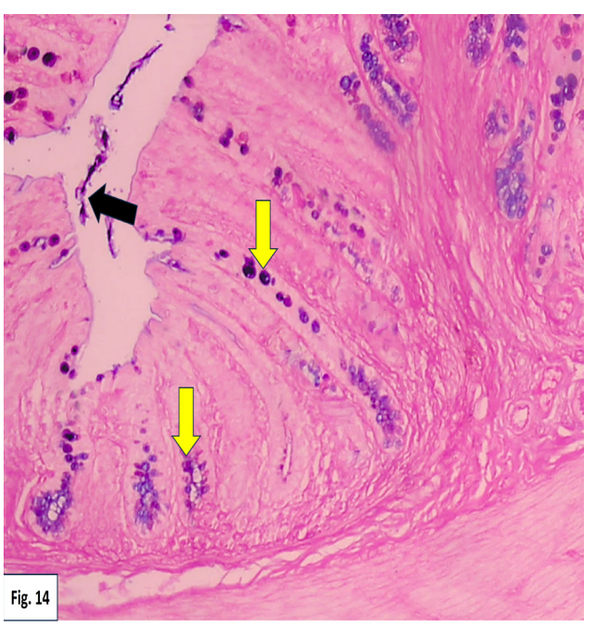


Fig. 14: Microphotograph of a longitudinal section of rat's descending colon "IBS group" showing week positive reaction (blue stain) of a few cells lining the crypts (yellow arrow) and of scanty luminal mucus (black arrow). (Alcian blue-PAS, x100)

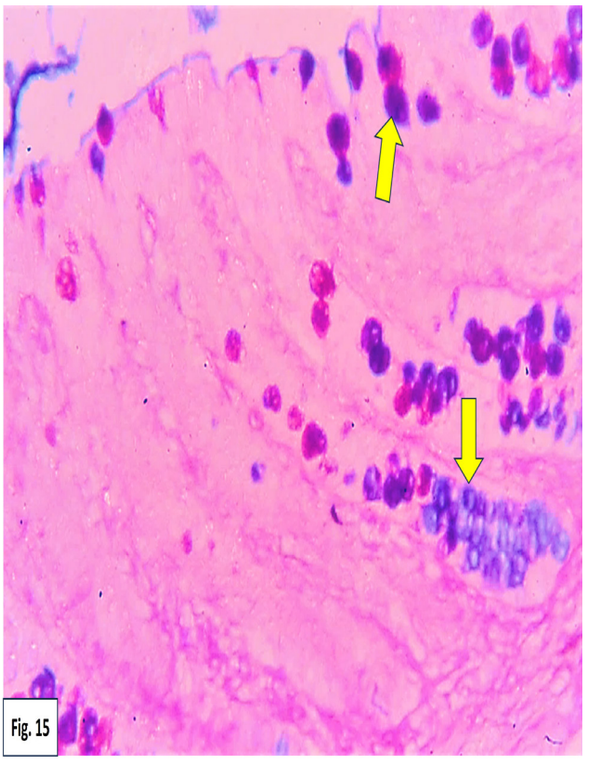


Fig. 15: Microphotograph of a longitudinal section of the mucosa of rat's descending colon "IBS group" showing weak positive reaction (blue stain) of a few small goblet cells (yellow arrow). (Alcian blue-PAS, x400)

Immunohistochemically stained sections with caspase 3 showed positive immune reaction of most of the crypts' lining cells (Figure. 16).

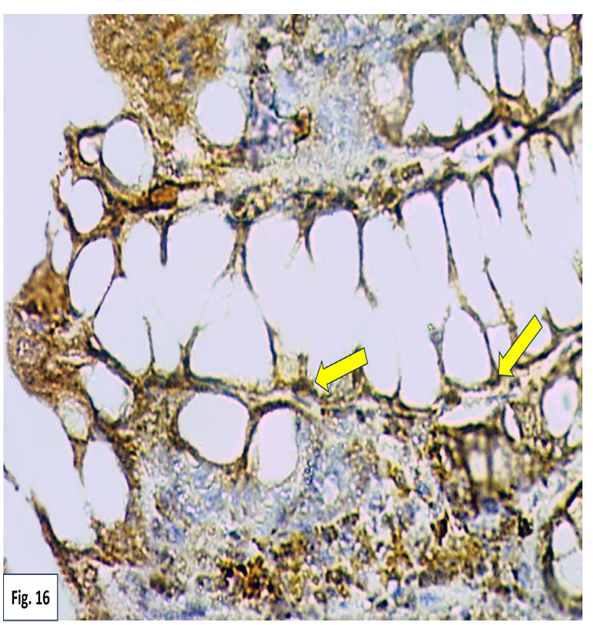


Fig. 16: Microphotograph of a longitudinal section of the mucosa of rat's descending colon "IBS group" showing positive immune reaction (brown stain) of most of the crypt's lining cells (yellow arrow). (Caspase 3, x400)

Ketogenic diet treated group (Group III):

Light microscopic examination of H&E stained longitudinal sections of the descending colons from Group III rats displayed nearly regular colonic architecture. The mucosa showed an intact surface epithelium, with regular crypts separated by a thin lamina propria. The submucosa contained small blood vessels (Figure. 17).

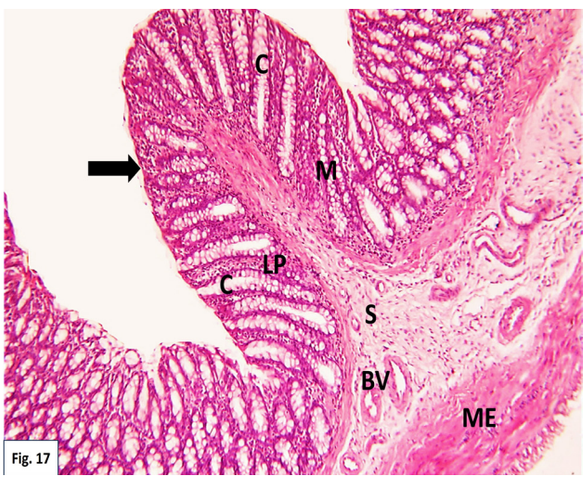


Fig. 17: Microphotograph of a longitudinal section of rat's descending colon "keto treated group" showing regular colonic wall layers: mucosa (M), submucosa (S) and muscularis externa (ME). The mucosa showed intact surface epithelium (black arrow) and parallel regular crypts (C). Notice, the thin lamina propria (LP) and the small submucosal blood vessels (BV). (H&E, x100)

In transverse and longitudinal sections, the colonic mucosa displayed regularly aligned crypts. The crypts were lined with colonocytes featuring basal vesicular nuclei and numerous goblet cells. The intervening lamina propria was thin and contained a few scattered eosinophils (Figures. 18,19).

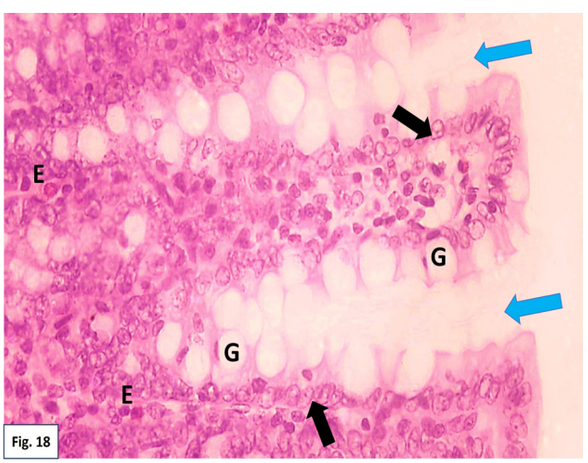


Fig. 18: Microphotograph of a longitudinal section of the mucosa of rat's descending colon "keto treated group" showing the mucosal crypts lined with regular colonocytes (black arrow) and plenty of goblet cells (G). Notice, the opened crypts' pits (blue arrow) and the few scattered eosinophils in the laminal propria (E). (H&E, x400)

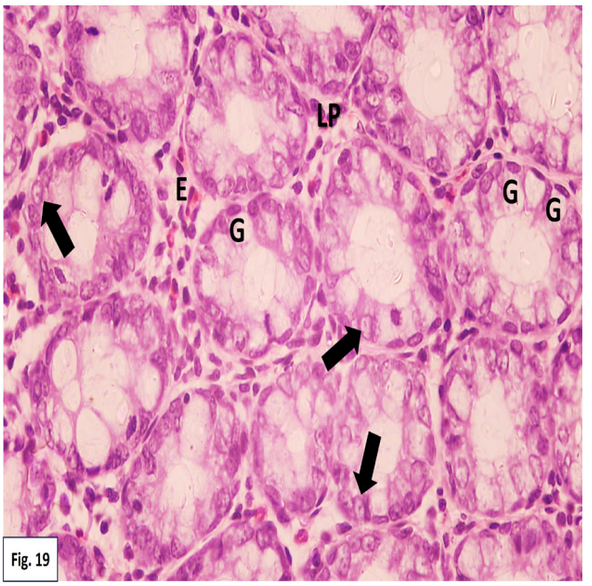


Fig. 19: Microphotograph of a transverse section of the mucosa of rat's descending colon "keto treated group" showing regular crypts with their lining colonocytes having basal vesicular nuclei (black arrow), plenty of goblet cells (G) and thin intervening lamina propria (LP) with a few scattered eosinophils (E). (H&E, x400)

Additionally, in longitudinal sections, the muscularis externa showed a regular structure with an inner circular layer and an outer longitudinal layer of smooth muscle, with the myenteric plexus in between (Figure. 20).

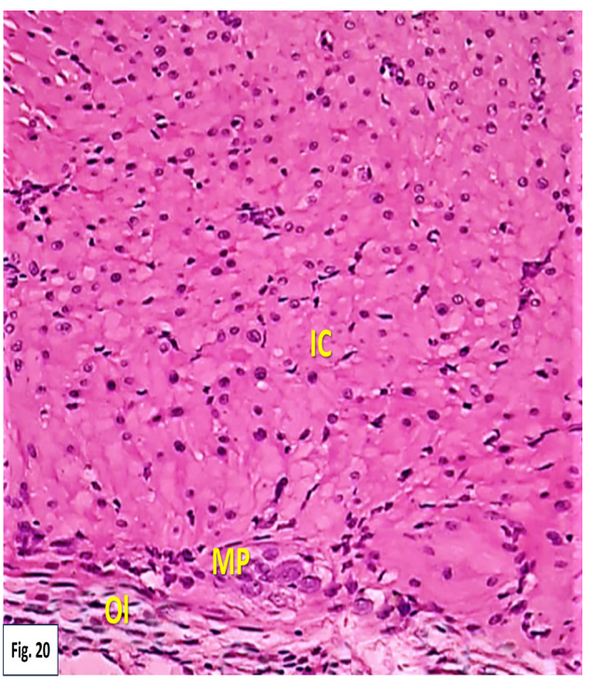


Fig. 20: Microphotograph of a longitudinal section of the muscularis externa of rat's descending colon "keto treated group" showing regular structure of the inner circular (IC) and the outer longitudinal (OL) smooth muscle layers with the myenteric plexus in between (MP). (H&E, x400)

Examination of semithin sections revealed regular colonocytes lining the crypts, characterized by oval basal nuclei and prominent nucleoli. Goblet cells appeared darkly stained and extended into the crypt lumen. The cells were lying on a regular basement membrane (Figure. 21).

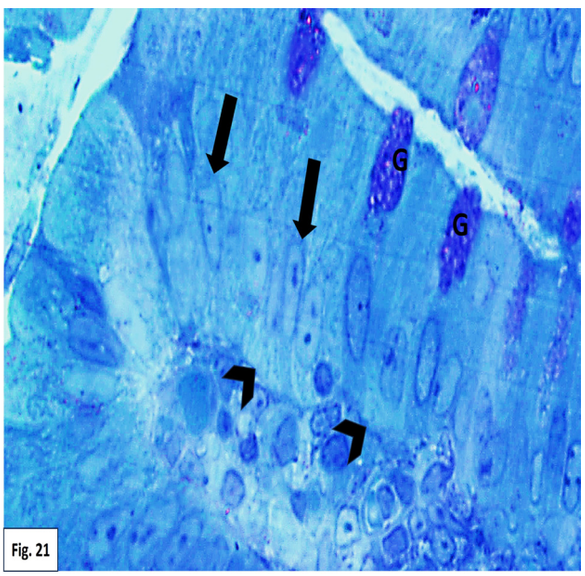


Fig. 21: Microphotograph of a semithin section of the mucosa of rat's descending colon "keto treated group" showing regular columnar cells lining the crypts having oval vesicular nuclei (black arrow), the goblet cells appeared darkly stained and extending into the crypt's lumen (G). Notice, the regular basement membrane (black arrowhead). (Toluidine blue, x1000)

Alcian blue-PAS stained sections showed strong to moderate positive reaction of numerous large goblet cells, as well as of the luminal mucus (Figures. 22, 23)

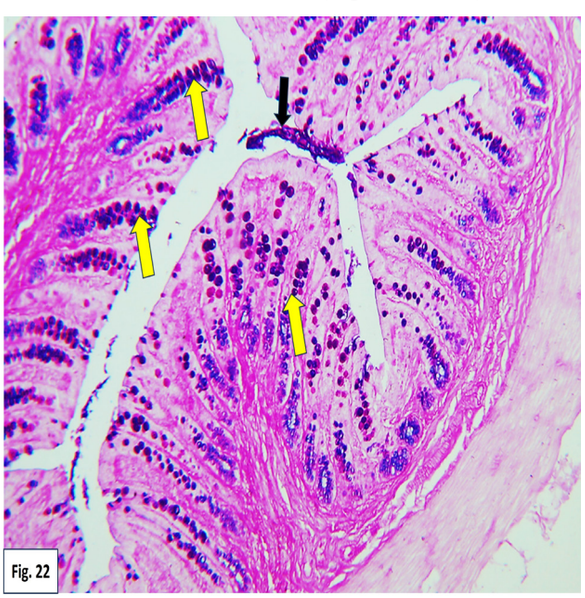


Fig. 22: Microphotograph of a longitudinal section of rat's descending colon "keto treated group" showing strong positive reaction (blue stain) of many cells lining the crypts (yellow arrow) and of luminal mucous (black arrow). (Alcian blue-PAS, x100)

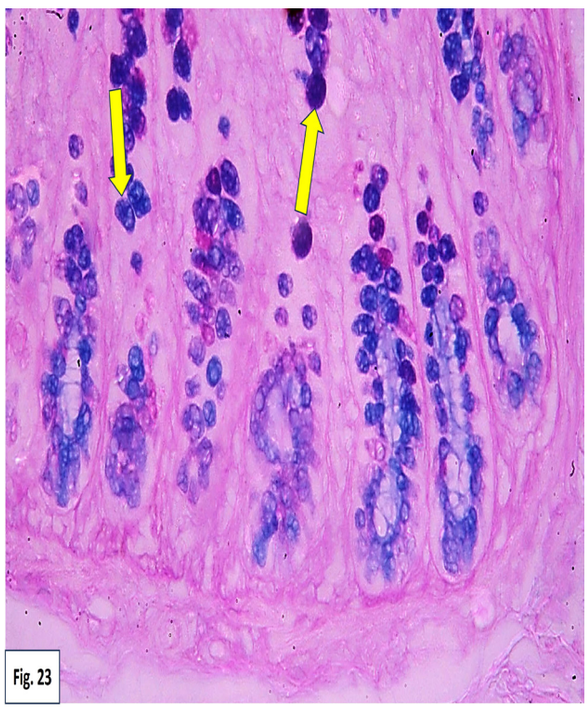


Fig. 23: Microphotograph of a longitudinal section of the mucosa of rat's descending colon "keto treated group" showing strong to moderate positive reaction (blue stain) of numerous large goblet cells (yellow arrow). (Alcian blue-PAS, x400)

Immunohistochemically stained sections with caspase 3 showed negative immune reaction of most colonocytes and goblet cells (Figure. 24).

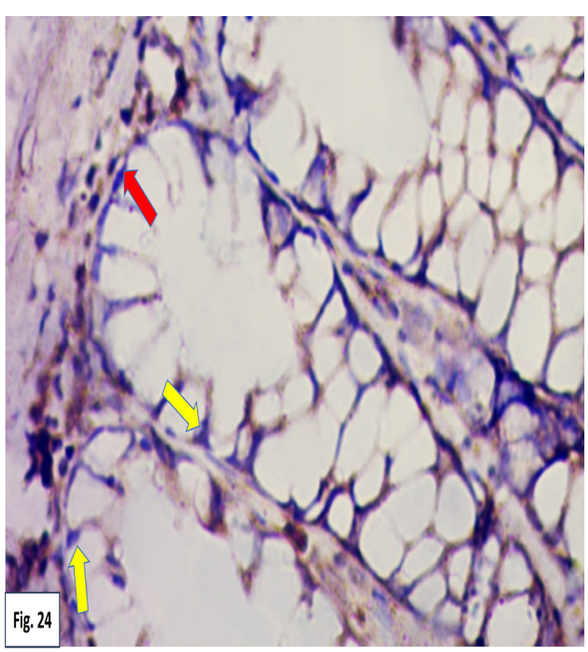


Fig. 24: Microphotograph of a longitudinal section of the mucosa of rat's descending colon "keto treated group" showing negative immune reaction (blue stain) of most colonocytes (yellow arrow) and goblet cells (red arrow). (Caspase 3, x400)

Morphometric results and statistical analysis:

I-Macroscopic analysis:

1- Weight of large intestine:

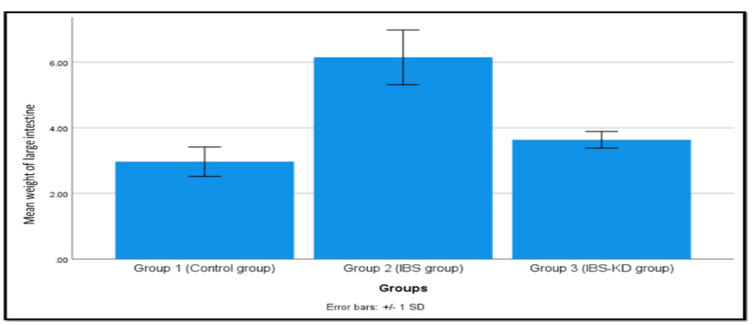
The results revealed significant difference between Group I and II, as well as between Group II and III with no significant difference between Group I and III as regards the mean weight of large intestine (in gm), the mean length of large intestine (in cm,) the mean crypt length (in µm), the mean number of goblet cells and the mean area % of caspase 3 expression in the mucosa (Tables (1-5) and Bar charts (1-5)).

Table 1: Comparison between the three groups as regards weight of large intestine in gm.

	Weight of large intestine (gm)									
	Group I (Control group)			Group II (IBS group)			Group III (IBS-KD group)			*P-value*
Min.-Max.	2.50	¬	3.75	5.12		7.20	3.30		3.97	*P1 < 0.001
Mean ±SD	2.97	±	0.45	6.15	±	0.83	3.64	±	0.25	**P2 = 0.061 *P3 < 0.001

Group I versus Group II (P1).
Group I versus Group III (P2).
Group II versus Group III (P3).

Significant *P-value* (*) ≤ 0.05.
Non-significant *P-value* (**) > 0.05.



Bar chart (1): Comparison between the three groups as regards weight of large intestine (in gm).

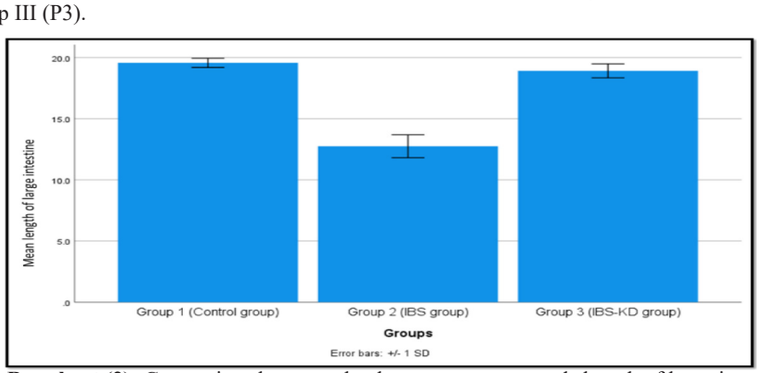
2- Length of large intestine:

Table 2: Comparison between the three groups as regards the length of large intestine in cm.

	Length of large intestine (cm)									
	Group I (Control group)			Group II (IBS group)			Group III (IBS-KD group)			*P-value*
Min.-Max.	19.0		20.0	11.5		14.0	18.0		19.50	*P1 < 0.001
Mean ±SD	19.57	±	0.38	12.75	±	0.94	18.92	±	0.57	**P2 = 0.113 *P3 < 0.001

Group I versus Group II (P1).
Group I versus Group III (P2).
Group II versus Group III (P3).

Significant *P-value* (*) ≤ 0.05.
Non-significant *P-value* (**) > 0.05.



Bar chart (2): Comparison between the three groups as regards length of large intestine (in cm).

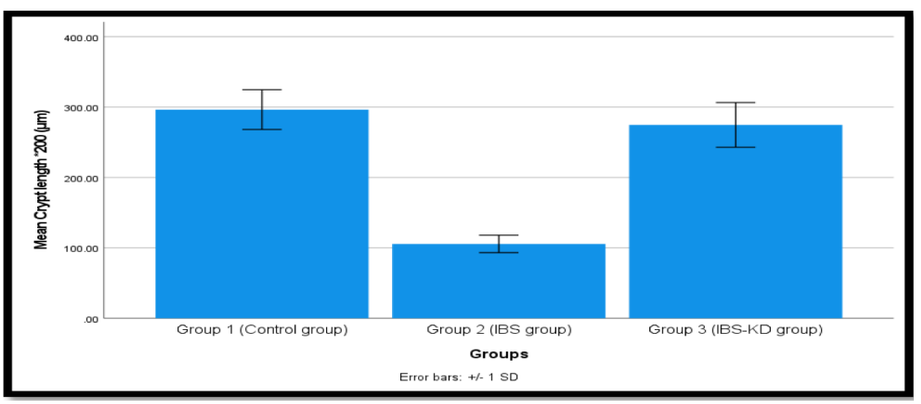
II-Image analysis:
1-Intestinal crypt length

Table 3: Comparison between the three groups as regards crypt length in µm.

	Crypt length (µm)									
	Group I (Control group)			Group II (IBS group)			Group III (IBS-KD group)			*P-value*
Min.-Max.	275.50		350.47	87.48		122.95	210.90		295.40	*P1 < 0.001
Mean ±SD	296.46	±	28.23	105.65	±	12.43	274.72	±	31.73	**P2 = 0.161 *P3 < 0.001

Group I versus Group II (P1).
Group I versus Group III (P2).
Group II versus Group III (P3).

Significant *P-value* (*) ≤ 0.05.
Non-significant *P-value* (**) > 0.05.



Bar chart (3): Comparison between the three groups as regards crypt length in µm.

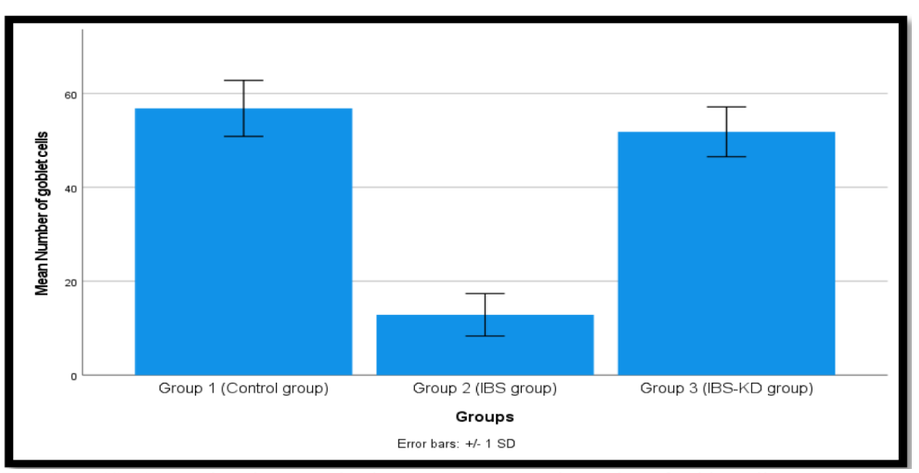
2- Mean number of goblet cells:

Table 4: Comparison between the three groups as regards number of goblet cells.

	Number of goblet cells									
	Group I (Control group)			Group II (IBS group)			Group III (IBS-KD group)			*P-value*
Min.-Max.	50		67	8		19	46		60	*P1 < 0.001
Mean ±SD	56.83	±	5.98	12.83	±	4.54	51.83	±	5.31	**P2 = 0.124 *P3 < 0.001

Group I versus Group II (P1).
Group I versus Group III (P2).
Group II versus Group III (P3).

Significant *P-value* (*) ≤ 0.05.
Non-significant *P-value* (**) > 0.05.



Bar chart (4): Comparison between the three groups as regards number of goblet cells.

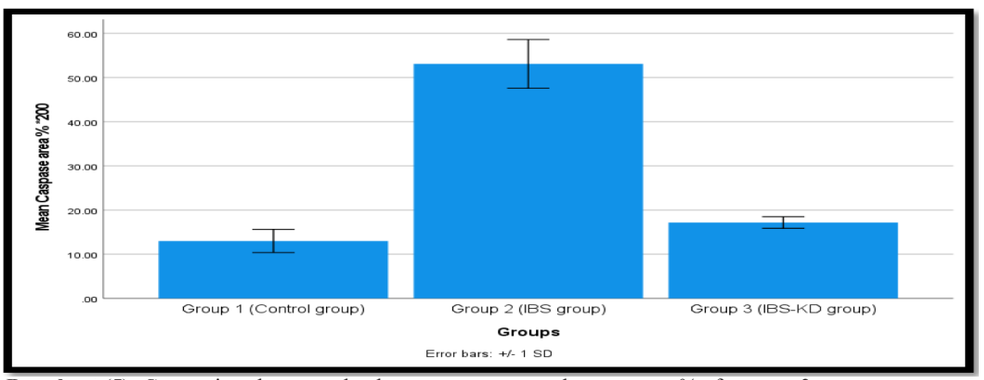
3-Mean area % of caspase 3 expression in the mucosa:

Table 5: Comparison between the three groups as regards mean area % of caspase 3 expression.

	Caspase 3 area %									
	Group I (Control group)			Group II (IBS group)			Group III (IBS-KD group)			*P-value*
Min.-Max.	9.67		16.05	47.16		59.40	15.64		18.79	*P1 < 0.001
Mean ±SD	13.01	±	2.65	53.09	±	5.52	17.19	±	1.32	**P2 = 0.064 *P3 < 0.001

Group I versus Group II (P1).
Group I versus Group III (P2).
Group II versus Group III (P3).

Significant *P-value* (*) ≤ 0.05.
Non-significant *P-value* (**) > 0.05.



Bar chart (5): Comparison between the three groups as regards mean area % of caspase 3.

DISCUSSION

Irritable bowel syndrome (IBS) can affect individuals across all ages. Given its chronic nature and effect on quality of life, IBS poses a considerable financial strain. Individuals with IBS are more prone to seek medical care and require time away from work[22].

Currently, IBS lacks a definitive cure. Nevertheless, specific dietary approaches can enhance the quality of life for individuals with IBS. Among these, the ketogenic diet is regarded as a promising option[23].

The current study was structure to evaluate the effectiveness of ketogenic diet in mitigating colonic changes associated with experimentally induced irritable bowel syndrome in adult male albino through water avoidance stress (WAS) protocol.

Bradesi et al.[24] stated that a ten-day WAS is highly effective in rodents for inducing colonic hyperalgesia, enhancing colonic motility and causing gut inflammation. Water avoidance stress offers several advantages over other stressors, including the lack of tolerance with repeated exposure and the continued presence of chronic hyperalgesia, hypermotility and inflammation even after the stressor is removed.

Male rats were selected in the current study as female rats exhibit a delay in stress induced IBS, likely due to the protective effects of estrogen on the colonic mucosa[25]. The descending colon was chosen to assess the effect of IBS, as previous studies on rat IBS have predominantly focused on this segment due to its prominent structural changes[26-28].

In the present work, application of water avoidance stress protocol for ten consecutive days on rats fed a standard diet (IBS group) revealed several histological alterations of the descending colon, such as an irregular surface mucosal epithelium with detached cells in the lumen of the colon, some degenerated colonocytes, a few goblet cells and large mast cells with underlying an irregular basement membrane. ***Lee et al.***[29] and ***Gigante et al.***[12] attributed these colonic changes associated with IBS to the increase in the mucosal mast cell counts. Mast cells secrete histamine, tryptase, and TNF-alpha, which compromise the integrity of intestinal barrier tight junctions and provoke hypersensitivity[30,31].

Additionally, the colonic mucosa of IBS group showed widely spaced crypts by excess lamina propria heavily infiltrated with eosinophils. The crypts also showed obliterated pits. The submucosa showed congested blood vessels. ***Becker et al***.[32] noted that irritable bowel syndrome is characterized by low-grade intestinal inflammation. Consequently, the previous findings may be attributed to the increased intestinal inflammation and histamine

level associated with IBS as detailed by ***Zhang et al***.[30], they further observed that intestinal inflammation leads to vascular congestion, heightened vascular permeability and eosinophil infiltration. Additionally, one theory of IBS inflammation suggests a reduction in the intestinal expression of cannabinoid receptors (CB1R and CB2R). These receptors are believed to have a protective role in motility disorders associated with inflammation[29, 33].

Stress can produce and worsen IBS symptoms through activating mast cells. When activated, mast cells initiate a series of events involving serotonin, mast cell and neuroimmune interactions along with the release of inflammatory mediators like IL-6 and TNF-α, which contribute to IBS symptoms like pain and changes in bowel habit[34,35].

The IBS group also showed shed cells in the crypts lumina and a significant decline in crypt length in comparison to the control group, which is a key indicator of disturbed mucosal barrier's integrity. IBS leads to degradation of intercellular tight junctions, allowing luminal contents to contact immune cells in the lamina propria. This contact initiates immune activation and raises levels of proinflammatory mediators and oxidative species. Thus, cell shedding from the base of the crypt is increased[36].

Regarding the muscularis externa of the same group. The inner circular layer exhibited irregular nuclei with perinuclear vacuolations. The IBS leads to upregulation of specific proliferative and profibrotic mediators, such as connective tissue growth factor. These mediators promote excessive extracellular matrix accumulation and fibrosis, resulting in calcium accumulation and hyperexcitability. Consequently, this process leads to the degeneration and vacuolation of colonic smooth muscle cells[37, 38].

Alcian blue-PAS staining showed a limited number of small goblet cells and minimal luminal mucus in the IBS group. The reduction in the number of goblet cells was confirmed statistically. This observation aligns with ***Chen et al***.[39], who reported a significant decrease in the number of goblet cells in IBS rat model. The IBS activates the production of digestive proteases by the luminal bacteria and intestinal mast cells, such as the serine proteases that accelerate degradation of the mucus[40,41].

Moreover, crypts' lining cells apoptosis was increased in in IBS group, as detected by caspase-3 immunohistochemical stain and confirmed statistically. The detected degeneration of colonocytes in IBS may be attributed to the increase in colonic TNF-α and IL-6, which induce cellular apoptosis with subsequent erosion of intestinal epithelial cells[42,43].

The IBS group also showed a significant decrease in the length and increase weight of the large intestine, indicating colitis[44]. A similar observation was recorded by ***Chun et al***.[45] on stress-induced IBS in a rat model.

In contrast, the present study found that rats in the IBS-KD group, which were fed ketogenic diet displayed regular colonic architecture. They had an intact surface mucosal epithelium and regularly aligned crypts separated by thin lamina propria with few scattered eosinophils. The crypts were lined with regular colonocytes and a plentiful number of goblet cells, also showed nonsignificant difference in crypt length compared to the control group. The cells were resting on a regular basement membrane without submucosal congestion. Additionally, the muscularis externa exhibited a regular structure in both of its layers. The improvement of the colonic structure following a ketogenic diet could be attributed to the increase in ketone body production, which acts as signaling molecules that promotes colonic epithelial cells proliferation, differentiation and overall colonic function[12,46].

The ketogenic diet's anti-inflammatory and antioxidant effects may also contribute to these improvements[47]. ***Massi et al***.[48] reported that ketogenic diet induces upregulation of cannabinoid receptors in IBS rats. Activation of these receptors on immune cells and colonocytes is essential for preventing colonic inflammation. Cannabinoids exert anti-inflammatory effects by diminishing the chemotaxis of activated T cells.

Concerning the antioxidant effect of ketogenic diet, ketone body metabolism protects cells from oxidative damage by lowering the formation of reactive oxygen species and boosting the natural antioxidant defenses[49]. Moreover, research has shown that the ketogenic diet reduces inflammation by boosting the activity and expression of mitochondrial uncoupling proteins. These proteins have a part in regulating cellular metabolism and energy production, as well as protect against oxidative stress[50].

Alcian blue-PAS stain revealed a remarkable presence of plenty of large goblet cells and a notable amount of luminal mucus in the IBS-KD group. The goblet cells number was confirmed statistically. Ketogenic diet increases ketone bodies production like acetoacetate and β-hydroxybutyrate which in turn promotes intestinal cells

proliferation and differentiation[51]. This may account for the observed restoration of goblet cell numbers and mucus production following the use of ketogenic diet. The mucus produced by goblet cells is regarded as a crucial element of gut barrier integrity[52].

Additionally, apoptosis of crypts' lining cells was reduced in IBS-KD group as detected by caspase-3 immunohistochemical stain and confirmed statistically. ***Palermo et al***.[53] noted that ketogenic diet inhibited both immune and apoptotic reactions in mice infected with Covid-19. ***Kong et al***.[54] observed that ketogenic diet reduces the expression of apoptotic mediators in mice model of colitis. Additionally, ***Ciaffi1 et al***.[55] reported that the ketogenic diet decreased several inflammation-related markers, including TNF-α and IL-6 in humans.

The statistical results indicated nonsignificant difference in the weight and length of the large intestine between the IBS-KD group and the control group.

CONCLUSION

Ketogenic diet could be regarded as a potentially beneficial therapy for irritable bowel syndrome.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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تأثير النظام الغذائي الكيتوني على التغيرات القولونية في نموذج ذكورالجرذان البيضاء البالغة المصابة بمتلازمة القولون العصبي المستحثة بالإجهاد (دراسة نسيجية وقياسية)

هالة طه شعلان، رنا اشرف فتحي، مها خالد العشرى و ايناس انور بخيت

قسم التشريح وعلم الأجنة، كلية الطب، جامعة عين شمس

الخلفية: تعتبر متلازمة القولون العصبي حالة مزمنة تصيب الجهاز الهضمي، حيث يُعتبر الإجهاد النفسي عاملًا محفزًا لها. و تظل الدراسات حول فعالية النظام الغذائي الكيتوني في علاج هذه الحالة محدودة.

الهدف: يهدف هذا العمل إلى تقييم فعالية النظام الغذائي الكيتوني في تحسين التغيرات القولونية المرتبطة بمتلازمة القولون العصبي المستحثة تجريبياً في الجرذان البيضاء البالغة.

المواد والطرق: تم استخدام ثلاثين جرذًا أبيض بالغًا، تم تقسيمهم إلى ثلاث مجموعات متساوية:

- المجموعة الضابطة (المجموعة الأولى): تم إطعام الفئران نظامًا غذائيًا قياسيًا لمدة عشرة أيام، ثم استمروا على نفس النظام الغذائي لمدة عشرة أسابيع إضافية دون التعرض للإجهاد الناتج عن تجنب الماء
- مجموعة متلازمة القولون العصبي (المجموعة الثانية): تم تغذية الجرذان بنظام غذائي قياسي وتعرضوا لإجهاد تجنب الماء لمدة عشرة أيام ثم استمروا على النظام الغذائي القياسي لمدة عشرة أسابيع.
- مجموعة متلازمة القولون العصبي-النظام الكيتوني (المجموعة الثالثة): تم تغذية الجرذان بنظام غذائي قياسي في البداية وتعرضوا لإجهاد تجنب الماء لمدة عشرة أيام ثم تحولوا إلى النظام الغذائي الكيتوني لمدة عشرة أسابيع.

النتائج: أظهرت جرذان مجموعة متلازمة القولون العصبي، التي تم تغذيتها بنظام غذائي قياسي، عدة تغييرات نسيجية في القولون النازل، مثل ظهور ظهارة مخاطية غير منتظمة مع خلايا منفصلة في تجويف القولون، تدهور الخلايا القولونية، قلة الخلايا الكأسية، وخلايا بدينة كبيرة. بالمقابل، أظهرت جرذان مجموعة متلازمة القولون العصبي-النظام الكيتوني، التي تم تغذيتها بنظام غذائي كيتوني، هيكلًا نسيجيًا منتظمًا في القولون النازل.

الاستنتاج: يمكن اعتبار النظام الغذائي الكيتوني نهجاً محتملاً مفيداً لمتلازمة القولون العصبى.