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Health Effects of Diets (High-Fiber Diet and Intermittent Fasting) on Experimental Animals

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***Abstract:** The study was conducted on 24 males of sexually mature white mice at the age of 7-8 weeks and weighing 20-30 ± 2 g, four treatments were used in this study, where 6 mice were isolated for first treatment (without any treatment), mice in the second group were fattened with animal fat by 30% until the end of the experiment for a period of (40) days, while the third and fourth groups were fattened in the same way as the second group for a period of 10 days, then the third group was treated with the fasting system, and the fourth group was treated with the fiber system for a month. This study aimed to determine the effect of treatment with a fasting regimen and fiber regimen on body weight and to know the effect on Intestinal tissue bioavailability. The results showed that there is a significant decrease in weight after fasting and after eating fiber (±2.57, ±3.29) respectively, compared to treatment after fattening (±3.81). It is clear from the results of the current study that fasting and fiber have a positive effect on weight reduction in male mice and intestinal bioavailability.*

***Keywords:** Obese, Fasting, Intestinal Microbes, Fiber.*

1. INTRODUCTION

Recently, The World Health Organization estimated that approximately 1.9 billion people are overweight and 600 million adults are overweight worldwide (WHO 2020). Obesity may lead to metabolic deficiencies in the human body, which leads to type 2 diabetes, fatty liver, cardiovascular disease, and cancer (Vucenik and Stains 2012). The importance of microbes is evident in individuals with large weights, as they have a key role in the exacerbation of diseases, poor digestion and cholesterol metabolism, glucose metabolism, and increased chronic inflammation (Cox et al 2015), leading to unhealthy weight gain (Han et al 2017). Obese individuals have a decrease in beneficial bacteri**a,** coupled with a relative increase in pathogenic bacteria, leading to increased intestinal permeability. (Lee et al 2017). Dietary fiber (DF), a complex of plant-derived polysaccharides, can pass through the small intestine without

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absorption, modifying the gut biotope by providing support for microbial growth (Deehan and Walter2016) .Recent studies have shown that it is possible to follow a high-HDF diet to prevent obesity (Anderson et al 2009). First, the physical and chemical properties of DF (viscosity, fermentation) had a protective effect against obesity, since viscous fibers can prolong gastric emptying transport time, thereby converting the nutrients into absorbable components (Chen et al 2018) . Second, DF can improve energy balance by increasing the abundance and diversity of beneficial intestinal microbes associated with obesity (Makki et al 2018), Third, DF can be fermented by gut microbes to produce short-chain fatty acids (SCFAs), which play important roles in maintaining health (Wang et al 2018), preventing certain diseases by lowering the pH of intestine, and inhibiting pathogens in the gut such as harmful bacteria and harmful metabolic compounds (Alexander et al 2019). Also, the inclusion of fiber in HFD has been shown to reduce inflammation levels in peripheral blood and promote lipolysis in mice (Kimura et al 2020).Restricted nutrition has become a major system affecting the immune system, metabolism, nervous system signals, and compensatory processes, collectively constituting most of the essential actors in maintaining the balance of the body's processes, which, when lost, results in a gradual exacerbation of chronic diseases. This is evident for numerous indications when fasting is applied including hypertension (Dong et al 2020), dyslipidemia (Kunduraci and Ozbek 2020), (Grundler et al 2021) ,diabetes (Ali et al 2021), vascular disease (Maugeri and Vinciguerra.2020), endocrine disorders (LiC et al 2021), as well as autoimmune diseases and infections (Morales et al 2021) including arthritis, neurological diseases (Phillips 2019), and sometimes cancer (Sadeghian et al 2021). Most human deaths today could be caused by the long-term persistence of unhealthy food (Wastyk et al 2021).

Fasting reflects an evolution of increased specialization in the context of multicellular microbes. Blood and immune cells circulating in the body are characterized by their branching properties that move throughout the human body, in particular those that dwell between surfaces, and frequently perform functions, defending foreign cells. To allow selective absorption through these surfaces, mucosal barriers incorporate an integral expression of proteins and sugars by neighboring cells, and therefore requires careful regulation of signal generation. Both microbial metabolites and microbial surface components activate a variety of signaling pathways that can strengthen or weaken this barrier (Ghosh et al 2021) along with host cell proliferation (Parada et al 2019) and through bacterial decomposition of the myosin layer above the epithelium. This process has been described as an improvement and reorganization for greater sustainability (Collins et al 2019) through the body's immunity. They form significant improvements in autoimmune diseases such as rheumatoid arthritis through the ketogenic or intermittent fasting regimen also through a diet that simulates both fasting.

Objective: The purpose of this study was to evaluate the effect of fasting and the effects of a dietary fiber diet on obese mice and to examine the histological intestinal and gut microbiota in obese mice.

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2. MATERIALS AND METHODS

The animals were purchased from the State Company for Pharmaceutical Industry in Samarra, the animal house, and the study was conducted in the same place for the period from the beginning of April to June 2022, after which the samples were collected and prepared.

Animals used in the study: 24 males who were placed for two weeks to cope and to ensure their safety. They were also examined by a specialist veterinarian at the center to ensure that they were free of diseases.

The design of the experiment: The animals were divided into four groups and each group contained six animals and numbered the transactions cages, and water was provided continuously, and they were fed the diet allocated to each treatment. The duration of the experiment lasted 50 days, a light period of 12 hours, darkness of 12 hours, and the temperature was set to 24 ± 2 degrees Celsius. It was confirmed that obesity occurred in animals after checking their weight before the experiment and after fattening. The totals were as follows:

First treatment (A1): The control group included six mice that were fed on a normal balanced diet and consisted of 25% wheat, 25% yellow corn, 24% soybeans, 24% barley, and 2% vitamins and minerals. (Santos and Macedo 2018) The second treatment (A2): a group that was obese and was not treated, fed 30% animal fat with equal amounts of diet (17% wheat, 17% yellow corn, 17% soybeans, 17% barley), 30% animal fats added (fattening system). And 2% vitamins and minerals. (Urmila, et al 2019). Third treatment (A3): A group of obese was treated with fiber (20% fiber, 20% corn, 20% barley, 20% wheat, 18% soybeans, and 2% vitamins and minerals). Fourth treatment (A4): a group of obese was treated with a fasting regimen, fed for 8 hours, and then cut off food for 16 hours (fasting regimen).

Body weight: the Body weights of all male white mice used were measured before the beginning of the experiment to calculate the initial weight, after the introduction of obesity, and after the end of the experiment period.

Histological analysis: After the end of the experiment period, the animals fasted for 12 hours and were killed for histological study (anesthesia and dissection). Histological sections were prepared based on the method mentioned in (Al-Hajj, 2010) and according to the steps.

Preparation of histological section: At the end of each experiment, the animals were starved for (12) hours and then drugged using ether. Placing the animal inside a glass container with a tight lid Dissectors, and after anesthesia, the animal was transferred to the anatomy dish, as the front and back limbs of the animal were fixed with pins, then the opening in the abdomen was made and the opening was completed to the sternum, the abdominal wall was raised to the sides to extract and remove (large intestine). Then placed in a petri dish and fixed immediately after eradication from the animal in a 10% diluted formalin solution. The models were fixed for a period ranging between (18-24) hours, after which the models were washed with alcohol at a concentration of 50% for half an hour, and using alcohols with gradual concentrations of :(70%, 90%, and 100%) (45 minutes) for each concentration. The models were then bathed with xylene for a period ranging from two hours to three hours, then saturated with molten paraffin wax in a convection oven at a degree (55) º for three hours, and then they were poured with special molds using paraffin wax, and left to cool until the cutting process, where they were cut with a thickness of 5 microns and were dyed with hematoxylin and eosin dye. The textile sections were prepared based on the mentioned method.

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Microscopic and Photographic Examination of Histological Section: The examination of the histological sections prepared from the samples prepared for the study was carried out using a light microscope from the Japanese-made company Olympus in the anatomy laboratory / Faculty of Science -the University of Tikrit.
Statistical analysis of data: The results were analyzed statistically using SAS 2010, according to one-way variance analysis. The average coefficients were tested using the Duncan multi-term test at a significant level (0.05) to determine the important differences between the totals.

3. RESULTS AND DISCUSSION

In Table 1, the weights were measured before fattening, after fattening, after fasting treatment, and after fiber treatment. Shows a significant decrease in weight after fasting and after eating fiber (±3.06, ±3.71) respectively, compared to the treatment after fattening (±3.62, ±4.07) fasting led to weight loss in clear proportions. Some studies that combined fasting and physical activity (Cho et al 2019). Different types of fasting refer to the fasting system as an effective lifestyle modification to reduce the risk of cardiovascular disease, reduce body weight (Trepanowski et al 2017). According to studies whose results were that there are different types of fasting it is useful and can be adopted in daily life without any financial burden, (Hutchison et al 2019). It was also found that there are different types of fasting such as fasting Ramadan and fasting between one day and another during the week, which reduces body weight and fat levels, and intermittent fasting from 12 to 36 hours leads to the breakdown of fats and the conversion of their products into ketone bodies in the liver. On the other hand, fasting leads to a successful strategy, leading to weight loss and improved blood sugar control, as well as a daily 16-hour fast that led to calorie restriction leading to a significant decrease in fasting glucose levels and thus weight loss (Karolina and Monika 2021).

As for dietary fiber in the diet, it prevents obesity. By reducing the calories in the meal. Clinical studies indicate a significant reduction in the risk of cardiovascular disease in people who consume a lot of fiber and whole grains compared to those on a low-fiber diet. The results of the study apply exclusively to dietary fiber consumption, not dietary supplements (Santos and Macedo 2018). Other substances contained in cereals and plant products have a good effect on human health. Studies have shown that an increase of 10 g/day reduces the risk of chronic disease (by 14%) and death (by 27%). It has been found that some fiber may affect the lowering of blood lipids mainly by limiting the intestinal absorption of dietary fats. In some analyses, oat bran, fruits, and vegetables are good sources, reducing the risk of heart disease (Wing et al 2011).

Table 1 the statistical analysis of the weights before the experiment, after fattening, and after treatment

Treatmens	Weight before fattening	Weight after fattening	Weight after treatments
T1	28.7±3.34 B a	29.0±3.51 B a	28.5±2.22 AB a

T2	36.1±4.51 A a	35.0±4.15 A a	28.0±3.28 AB a
T3	24.0±2.59 B a	30.0±3.62 B a	25.5±3.06 B a
T4	26.2±3.65 B a	36.5±4.07 A a	30.8±3.71 A a

In Table 2, pathological microorganisms and non-pathological microorganisms were measured before fattening, after fattening, after fasting treatment and fiber treatment. To a large extent, the gut microbiota is influenced by influences of the host as it reflects the nutrients provided in the host's variable diet. Another potentially related mechanism, supported by animal studies, is the effect of fasting on host health and balance (e.g., concerning blood pressure or diabetes (Okawa et al 2021) by altering the pool of secondary bile acids produced by gut bacteria during hepatic entero-circulation. Increased gut permeability leads to the release of a heterogeneous class of compounds into the circulatory system that activates the immune response called prognosis, some of which act as an antidote. Directly to microbes, many of which are beneficial for intestinal permeability (Beli et al 2018). Several studies indicate an increased abundance of probiotics in the intestine in the case of fasting or nutrition. In general, this refers to a classification associated with health improvements in fasting (Jordan et al 2019). Pattern enrichment of common probiotic genera has been observed across multiple studies regarding the role of fasting and refeeding phases. In other studies it refers to the protective role, in contrast, it was seen in three studies that bacteroids enriched during the same fast, and then exhausted during refeeding; its specific effect appears to be strong. Being the genus Bacteroidetes, it is central to the ratio of Bacteroidetes - Firmicutes phylum which is often suggested as a biomarker of gut eubiosis and is reflected in the high-level summary of gut microbiome composition patterns such as intestinal patterns. Numerous studies indicate the depletion of the classification of Lachnospiraceae (which are fermenting bacteria of sugars). Alpha diversity increases in the gut, well known as a sign of health and balance (Wolter et al 2021). In summary, there is a good basis to conclude that a variety of fasting interventions enrich many basic anti-inflammatory measures, especially SCFA producers, although variable, which may reflect some form and times of fasting.

HFD fiber has a role in regulating energy and metabolism in the body through its fermentation, physical, and chemical properties (Li et al 2022). Researchers have noted that oat fiber improved impaired liver function caused by HFD through the work products of gut microbes. From what we found that the effect of FF on improving liver function and promoting thermogenesis was dependent on the intestinal microflora. The interaction between nutritional components and the formation of microorganisms in the intestine turns out to have a significant impact on the metabolism of the host. In this study, HFD caused an increased abundance of beneficial bacteria, and a decrease in the abundance of non-beneficial bacteria, which is consistent with previous studies (Canfora et al 2019). This abundance is inversely associated with obesity and insulin resistance. It is considered a beneficial organ for microbial presence

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due to its ability to promote effective body weight loss. Studies have proven that beneficial bacteria can improve insulin in blood serum, increase colon mucus, and reduce liver fat accumulation, thereby mitigating the development of obesity in rats. It is also found to be abundant in the colon and contributes to the production of energy for the colon significantly which helps protect gut health, enhances digestive function, and reduces inflammation levels (Xu et al 2020). As well as stimulate the regulation of secretion and production of mucus, to improve the function of the intestinal barrier. Studies indicate it stimulates oxidative stress in the mitochondria and thus prevents obesity. Pathogenic intestinal microflora can lead to increased permeability of the intestinal mucosa and damage to vascular endothelial cells (Riva et al 2017).

Table 2 Bioavailability

Treatments	
A1	non Pathogenic
A2	Pathogenic
A3	non Pathogenic
A4	non Pathogenic

Histological studies: The effect of fasting system and fiber on intestinal tissues
The effect of the fasting: 1- Control group

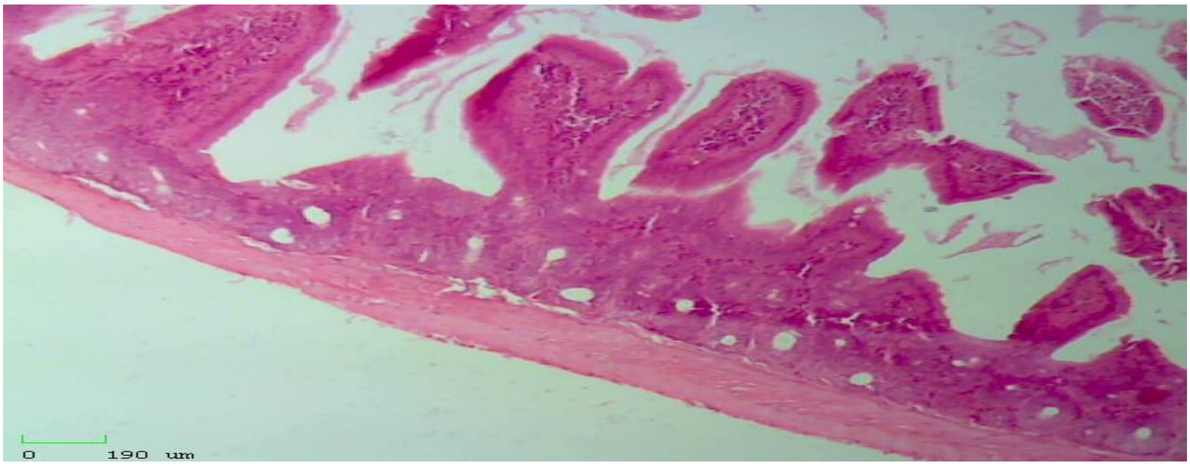

Figure (1-4) shows a cross-section of 10x in the large intestine in a group of healthy male mice with normal nutrition

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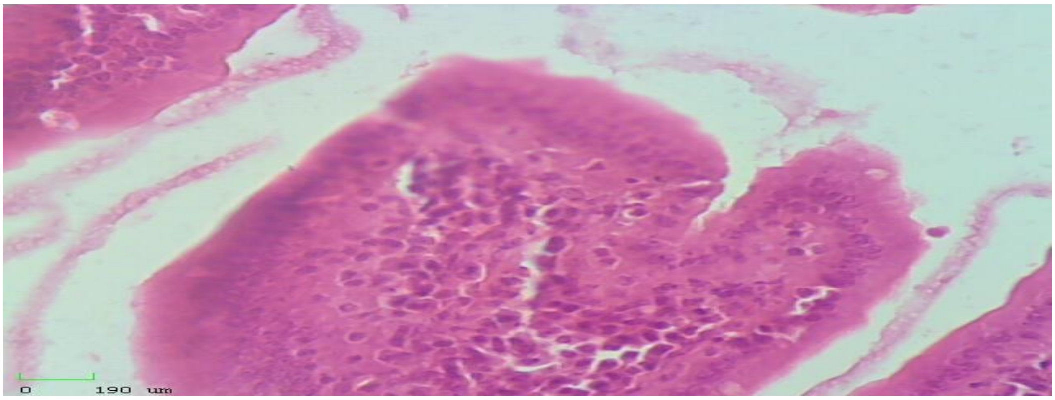

Figure (2-4) 400x mucous layer in the large intestine in the male group of healthy mice with normal nutrition

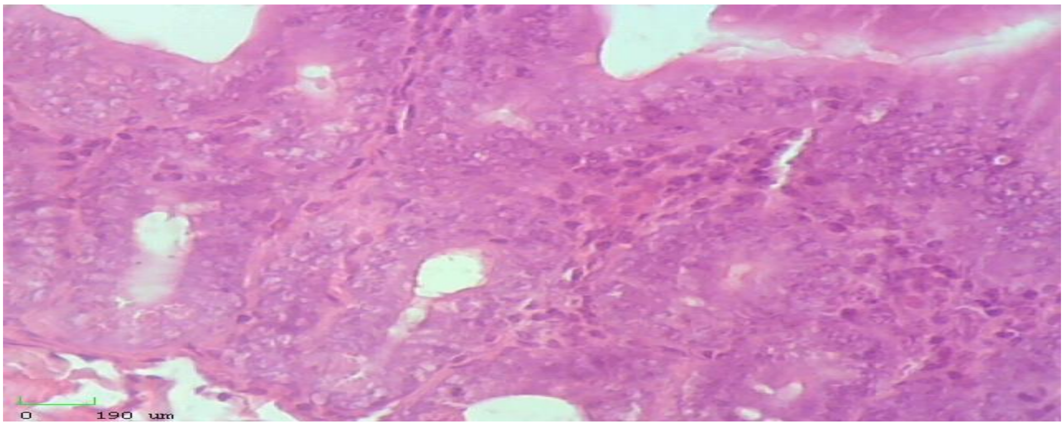

Figure (3-4) Submucosal layer 400x in the large intestine in the male group of healthy mice with normal nutrition

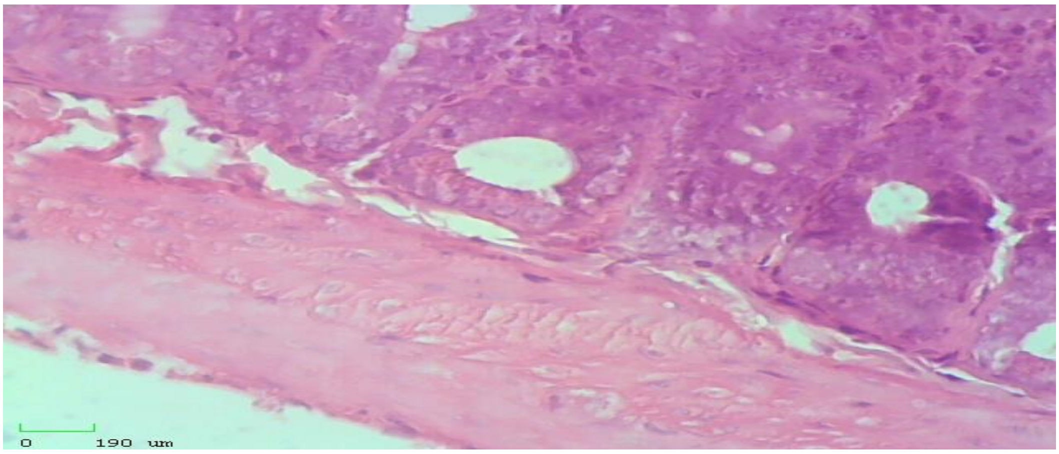

Figure (4-4) 400x serous and muscular layer in the large intestine in the male group of healthy mice with normal nutrition.

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Figure 4, 3, 2, 1, shows the histological sections of the large intestine in mice of the control group, ahealthy structural body of the intestine, as the four layers (mucosa, submucosa, muscular and serous) appear in their clear and proper form.

2- Obesity Group:

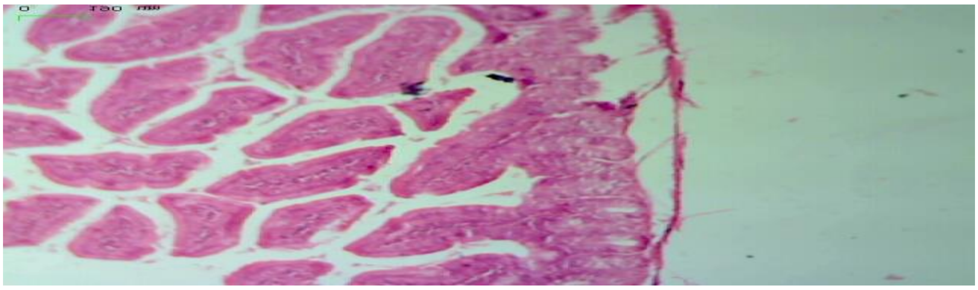

Figure (5-4) shows a cross-section of 10x in the large intestine in the group of obese male mice

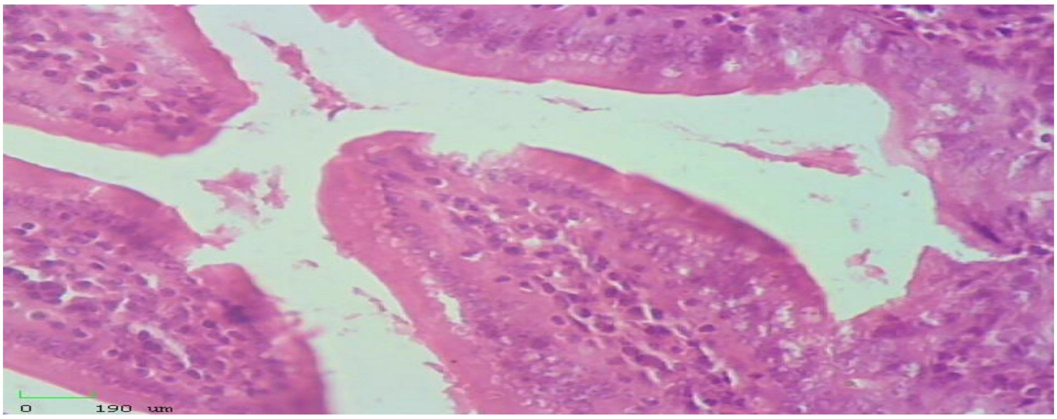

Figure (6-4) shows the 400x mucous layer in the large intestine in the group of obese male mice

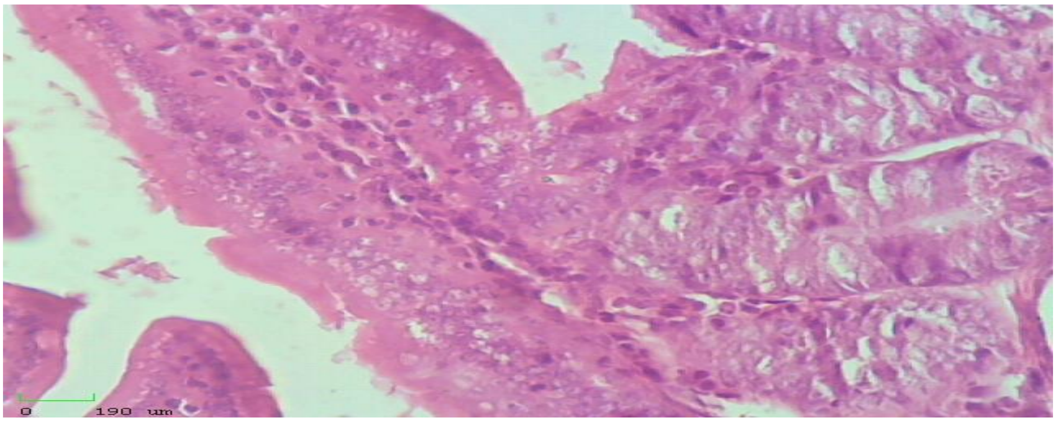

Figure (7-4) shows the submucosal layer 400x in the large intestine in the group of obese male mice

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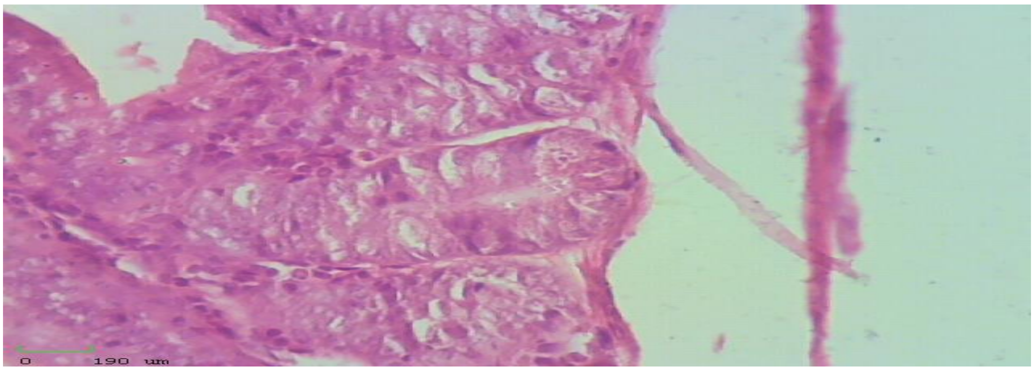

Figure (8-4) shows the 400x muscular and serous layer in the large intestine in the group of obese male mice

The mucous layer is eroded on the surface and disjointed in some aspects with the presence of enlarged cells and inflammatory cells and bleeding and the core of this layer is disjointed, while the muscle layer is decayed and disjointed with the presence of dissections in the muscle fibers.

3-Fasting group:

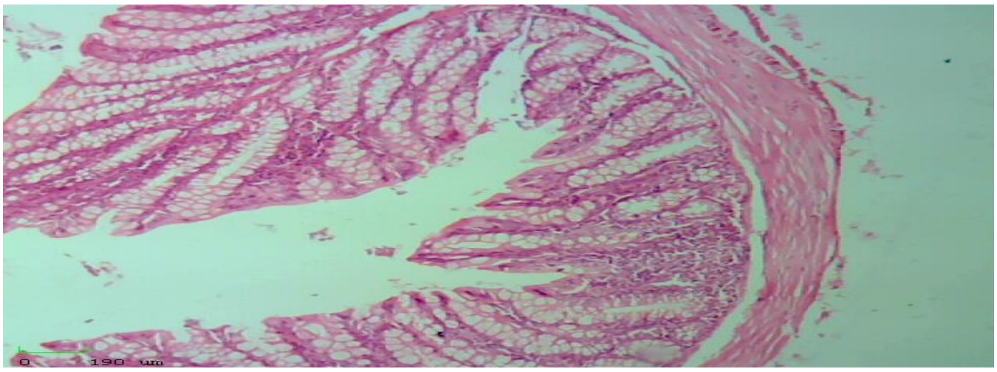

Figure (9-4) shows a cross-section of 10x in the large intestine in the male group of mice when fasting is applied

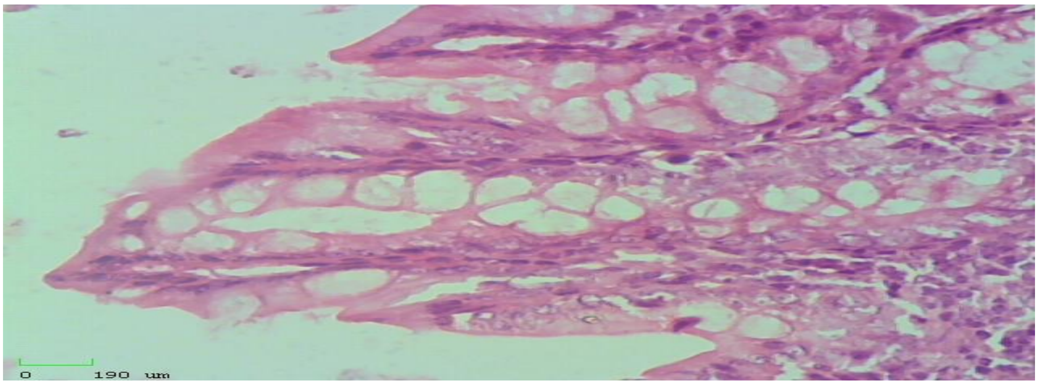

Figure (10-4) shows the 400x mucous layer in the large intestine in the male rat group when fasting is applied

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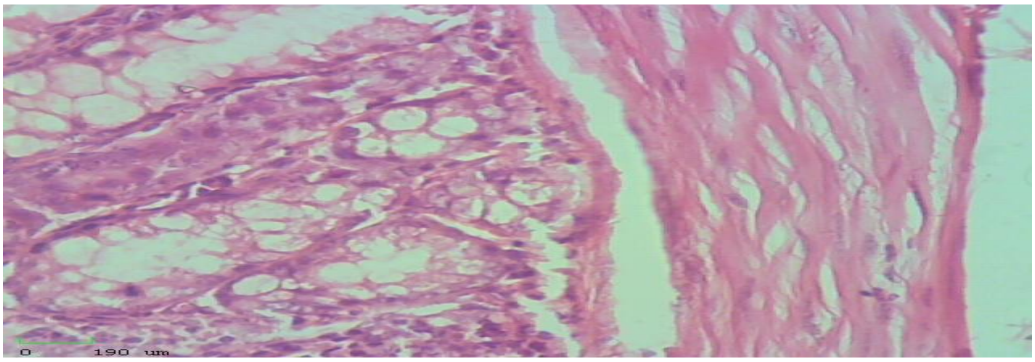

Figure (11-4) shows the submucosa, serous, and muscular layer 400x in the male rat group when fasting is applied

The results of the histological study shown in Figure (4-11) showed that in the mucous layer, the intestinal units appear thin with an enlargement in the size of the goblet cells, while the submucosal layer appears normal and unaffected. The muscle layer is disjointed with the dissolution of part of it, while the serous layer is fairly normal. Deep regeneration of the intestinal epithelium during fasting was thought to be the main cause of changes in the gut microflora, but analyses showed that fasting in vertebrates suggests that a decrease in nutrient availability rather than structural reorganization is the cause of the change in the gut microbiota. Some studies have suggested that fasting that is limited to water only increases intestinal permeability in the model of mice with colitis. Increased intestinal permeability is offset by lower calorie intake in humans and animals Conversely, some research suggests that when calorie restriction in health conditions can improve intestinal barrier permeability in rats and in humans who follow a low-calorie diet for a month. (Littlewood et al 2016) Sometimes when consumed by certain beneficial intestinal bacteria may adversely affect the integrity of the intestinal barrier, increase inflammation, and increase the risk of infection (Rangan et al 2019).

4- Fiber Group:

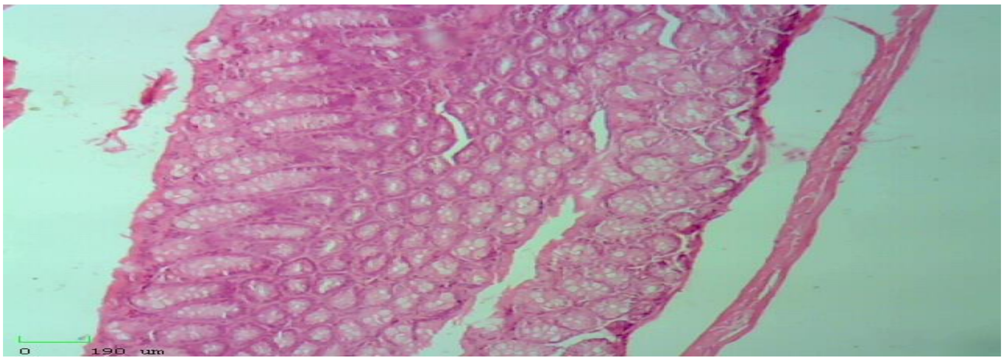

Figure (12-4) shows a cross-section of 10x in the large intestine in the male group of mice when applying the fiber system

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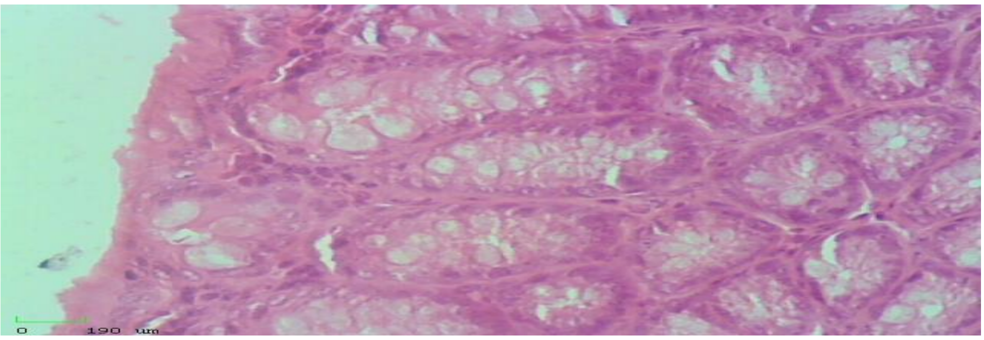

Figure (13-4) shows the 400x mucous layer in the large intestine in the male rat group when applying the fiber system

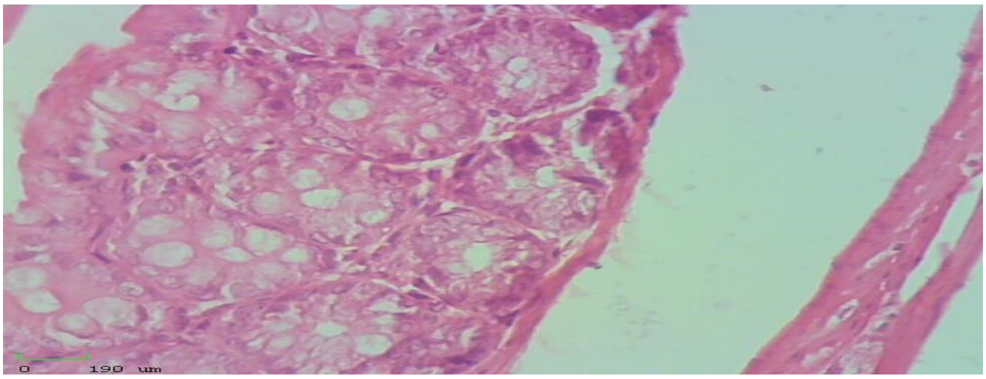

Figure (14-4) shows the mucosa, submucosa, muscular, and serous layer 400x in the large intestine in the male rat group when applying the fiber system

The mucous layer is normal. Goblet cells are normal size. The submucosal layer is normal, and the mucosal layer is normal. The muscle is disjointed and some parts are separated from each other, and the serous layer is not affected. This study emphasizes the importance of dietary fiber for maintaining the bowels and their integrity, and the growth of colon cells in mice. Previously it was believed that the physiological effect of dietary fiber is limited to the intestinal lumen, affecting the rate of gastric emptying (Mesnage et al 2019) , and contributing to the content of feces and moisture. Research has proven that fiber alters the physical properties of rodent intestines and increases the proliferation of intestinal epithelial cells, making fiber an important factor for mitigating intestinal injury. The decrease in colon weight is caused by simple fermentation processes, and this corresponds to previous studies when soluble fiber is given to mice. The absence of fiber in diets leads to lower levels of intestinal tissue, and this matches research showing intestinal cell growth after fiber supplementation in rodents and humans. The maintenance of the intestinal barrier is done through dietary fiber, which leads to the improvement of the gastrointestinal tract and the health of the body in general. When dietary fiber is removed, bacteria convert metabolism from fiber breakdown to mucolytic and thus reduce the thickness of the mucus layer in the colon leading to increased microbial transmission and the occurrence of inflammation. Several studies mention the health benefits of consuming dietary fiber for humans, but in practice, the doses are not in the required quantities due to some harmful effects represented by flatulence. Fiber is essential for

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maintaining gut size, colon cell hormone levels, and maintaining bowel integrity. Patients are advised to eat low-fiber meals after intestinal injury, as in the case of pelvic radiation therapy (Santos and Macedo 2018).

4. CONCLUSIONS

It can be concluded that the inclusion of 20% fiber can effectively alleviate the accumulation of fat in the liver, metabolic syndrome, and promotes weight loss in obese rats. In addition, the proportion of beneficial bacteria can increase. Improved obesity and metabolic syndrome may be associated with a decrease in the abundance of pathogenic bacteria in the colon. Our results point to the great potential of dietary fiber for obesity control in high-fat diets.

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