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Ali Sabah M. Saleh Al-
Tikriti*

Feryal Farooq Husain

Bayan Y. Al – Abdulla

Food Sciences Dept., College
of Agriculture, University of
Tikrit, Tikrit, Iraq.

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The Effect of Prepared Therapeutic Food Mixtures on the Intestinal Tissues of Malnourished Rats

ABSTRACT

The current study was conducted in the laboratories of the College of Agriculture and Animal House of the College of Veterinary Medicine at Tikrit University for the period from 5/5/2020 to 29/10/2020. And it aimed to produce therapeutic food mixtures to treat people with malnutrition and to know their impact on the health of the small intestine and large intestine. Where three ready-to-use therapeutic food mixtures have been prepared (do not need to be prepared or cooked before eating) consisting of milk, sprouted oats, non-sprouted oats, Sesame, Rice, chickpeas, Dates, bananas, apples, potatoes and oil, provided that the moisture and fat content does not exceed 2.5% and 32%, the percentage of protein and carbohydrates is not less than 14% and 40% respectively, and they were given to Thirty healthy rats and divided into six groups equally, a control group (+) natural, control group (-) (malnourished for a period of time 48 days), 1st, 2nd, 3d and 4th. The studied groups were affected with malnutrition for a period of 24 days, then the first food mixture was given to the 1st, the second food mixture for the 2nd, the third food mixture for 3d, the standard food mixture F-100 for the 4th. After 24 days of consuming the prepared nutritional mixtures, the tissues of the small and large intestine were examined under a microscope. The histological analysis of the small intestine showed that the 1st and the 2nd gave completely healthy tissues to the small intestine similar to the control group (+) and the 4th, while the 3d showed congestion in the blood vessels with hyperplasia of intestinal epithelial cells compared to the control group (-) which showed High-intensity cellular degeneration in the lining cells of the intestine with chronic and acute inflammations. As for the large intestine, the 1st gave a complete recovery of colon tissue, similar to the control group (+) and the 4th, while the 2nd and 3d showed pathological changes that included infiltration and local grouping of inflammatory cells in the submucosal layer of the colon and flattening of the villi and their union with acute inflammation compared to a control group (-), Which showed pathological changes represented by infiltration of inflammatory cells with an overgrowth of mesenteric lymph tissue and multiple lymph sacs. It was found that the first nutritional mixture prepared on which the 1st of rats fed give similar results to the results of the control group (+) and the 4th

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INTRODUCTION

Nutrition is the science that deal with food, nutrients and other substances included in its composition, action, interaction, balance, and its relationship to health and disease, and nutritional needs are the least amount of nutrients through which the normal functions of the body of the

* Corresponding author: E-mail: ali.sabahmsa@st.tu.edu.iq

organism can be preserved. Nutrition early in life is one of the main important variables directly related to the formation, growth and functional organization of the organism (Rolfes et al., 2020).

Malnutrition is a dangerous disease that affects children in the first year of life. It comes under three names: Marasmus, Kwashiorkor, and both types (Marasmus- Kwashiorkor). Severe malnutrition affects more than 6 million children around the world annually and takes poor countries and the countries in which crises are the largest proportion of this deadly disease (Sobotka and Forbes., 2019). In the latest statistics for the year 2020, UNICEF indicated that 6.7 million children under the age of five may suffer from wasting (which will afflict them with serious undernourishment) as a result of the socio-economic impacts of the Covid-19 pandemic, and clarified that the largest proportion will be of the share of African and southern African countries. Asia, UNICEF also indicated in its 2020 report on Iraq that the Corona crisis has increased the spread of malnutrition among Iraqi children to two for every five children, these percentages are terrifying and lethal numbers, as they will leave devastating health effects on future generations (UNICEF., 2020).

Recent research has indicated a link between malnutrition, pathological changes in the intestinal tissues and cells, and an imbalance in the bacteria present in it. It was found that malnutrition leads to infections and degeneration in the goblet cells in the intestine, as its severity varies with the severity of malnutrition, as well as an increase in intestinal permeability and death Villi, which serve to absorb the elements into the blood (Attia et al., 2017).

Material and Methods

Raw materials

Full fat milk of the brand Altunsa (Turkey), Skimmed milk of the brand Regilait (France), Sesame (India), Rice (Iraq), oil brand Altunsa (Turkey), Chickpeas, Potatoes, Banana, Apples, date. From the local markets of Tikrit - Salah al-Din - Iraq, Oats (Anatolia) were obtained from the Department of Field Crops of Tikrit University.

Preparing the raw materials

Sprouted oats: The oats were cleaned and washed under tap water for 30 min (Krapf et al., 2019) to remove impurities and the grains were completely submerged in water for 8 hours, after which removed the soaking water and spread the grains on strainers that allow the water to evaporate, and moisten the grains 3-4 times a day for 72 hours at a room temperature (25-27) ° C, to perform the sprouting process by elongating the fetal axes with flipping every 8 hours, and the stimulation process took 120 hours, After the completion of the sprouting, the germinated grains were washed and then cooked for 15 minutes, then cooled and prepared for drying by oven drying at a temperature of 60 °C for a period of 18 hours. The grains were spread on the trays of the oven with continuous stirring of the grains, after that, the outer crusts, roots, and sheaths were removed by hand, then milled by an electric grinder, sifted and packed with polyethylene bags.

Chickpeas and non-sprouted Oats: were sorted, method (Sharoba, 2014), cleaned with water, the outer crusts were removed from them, then washed again well, then soaked for 12 hours overnight and cooked after soaking at a temperature of 100 °C for 15 minutes, then was placed on a grate to allow the water to escape, Then it was entered into a drying oven at a temperature of 60 °C for 16 hours with continuous stirring to ensure completion of drying, then it was ground with an electric grinder, screened and preserved until use.

Sesame: method (Elhardallou et al., 2015), as it sorted by hand, washed with water, then exposed to a low heat for 15 minutes until it turned light yellow, then cooled to room temperature, then it was ground with an electric grinder, screened and preserved until use.

Rice: method (Borg et al., 2019) as it cleaned from western materials by hand, washed with running water well, soaked in water for 30 minutes and cooked for 20 minutes, then entered a drying oven at 60 ° C for 7 hours, after which it cooled to room temperature, then it was ground with an electric grinder, screened and preserved until use.

Dates: method (Nadeem and others, 2017), as sorted, washed, and cut into small pieces 3 mm, then dried at a temperature of 60 m for a period of 24 hours with stirring every 4-6 hours to ensure it dried and cooled, then it was ground with an electric grinder, screened and preserved until use.

Apples and Bananas: The method (Singh et al., 2018) was used to dry bananas and apples with some modifications, where used bananas and apples of approximately equal size and length, washed with running water, and then peeled into pieces in equal slices with a thickness of 6 mm, then was treated with ascorbic and stearic acid to stop the work. Polyphenol Oxidase enzyme, after which the slices were placed on a clamp for filtering to speed up the drying process, after which the slices were inserted into the drying oven at 60 ° C for 24 hours with stirring every 3 hours to ensure even moisture removal. And save until use.

Potatoes: were washed well with Tap water, peeled and cut into strips 3-4 mm thick and cooked the potatoes for 5 minutes at a temperature of 90 °C, in order to inhibit the enzyme polyphenol oxidase, prevent its darkening and eliminate pathogenic microorganisms, And it was placed on a filter for a filter, after which it was dried at a temperature of 60 °C for 16 hours, with continuous stirring, to ensure that it was completely dried, Then it was cooled at room temperature, then ground with an electric grinder, and packed and preserved until use.

Food used for comparison: The standard food F-100 obtained from the Nutritional Rehabilitation Center at Salah al-Din General Hospital was used as food in comparison with the prepared food mixtures.

Formation of mixtures

Table (1) shows that 3 ready-to-use therapeutic food mixtures for children were prepared using different materials that included: milk, vegetables, fruits and nuts with different mixing ratios, where the type of protein used in the mixtures was taken into consideration to know the best in treating malnutrition, and the mixes were designed to be Protein content should not be less than 14%, fat should not be more than 32%, and carbohydrates should not be less than 40%.

Table (1) Raw materials used in preparing ready-to-use food mixes

Formula	full-fat milk(%)	skimmed milk(%)	Sesam(%)	Sprouted oats(%)	Oats are not sprouted(%)	Chickpea(%)	Appl(%)	Banan(%)	Ric(%)	Potat(%)	Date(%)	Oil(%)
1	55						10	10		10		15
2		15		30		25		10				20
3			65		20				7.5		7.5	

Preparation of laboratory rats

This study was conducted in the Animal House of the College of Veterinary Medicine / University of Tikrit for the period from 23 / August to 9 / October / 2020, with the aim of knowing the effect of malnutrition on the intestines of rats and the effectiveness of therapeutic food mixtures on it, and used to complete the experiment Thirty healthy adult female Western Albino Rat, and make sure that there was no disease by the competent veterinarian. The ages of the animals ranged between (50-55) days, weighing (130-135g). The experimental animals were placed in metal cages of appropriate dimensions to the extent that they were free to move, with the use of sawdust to cover the cage floor in order to ensure the greatest amount of moisture absorption and the mulch was changed. On a periodic basis every day to maintain the cleanliness of the cage, and it was emphasized to provide drinking water preserved in sterilized plastic bottles. The animals were subjected throughout the duration of the experiment to standard laboratory conditions of ventilation, temperature 20-25 °C, and the light cycle was 14 hours of light and 10 hours of darkness. The animals were divided into 6 groups for each group of 5 animals, and the animals were killed after 9 weeks, and the groups were as follows:

- 1- Control group (+): the prepared standard feed was fed completely throughout the duration of the experiment.
- 2- The control group (-): suffered from malnutrition throughout the duration of the experiment.

3- The first group: she suffered from malnutrition and was treated with the first therapeutic nutritional mixture.

4- The second group: she suffered from malnutrition and was treated with the second therapeutic nutritional mixture.

5- The third group: suffered from malnutrition and was treated with the third therapeutic nutritional mixture.

6- The fourth group: was malnourished and treated with the F-100 standard food mixture.

The animals were given direct feeding for 7 days and the amount consumed was calculated every 24 hours and the results were recorded, and at the beginning of the experiment, the studied rats suffered from malnutrition by giving them half of the daily needs of each rat while monitoring its weight, and this procedure reduced the percentage of calories, the amount of nutrients and the proportion of protein and fats consumed and your carbohydrates are almost halved (NRC, 1995)

Histological Studies

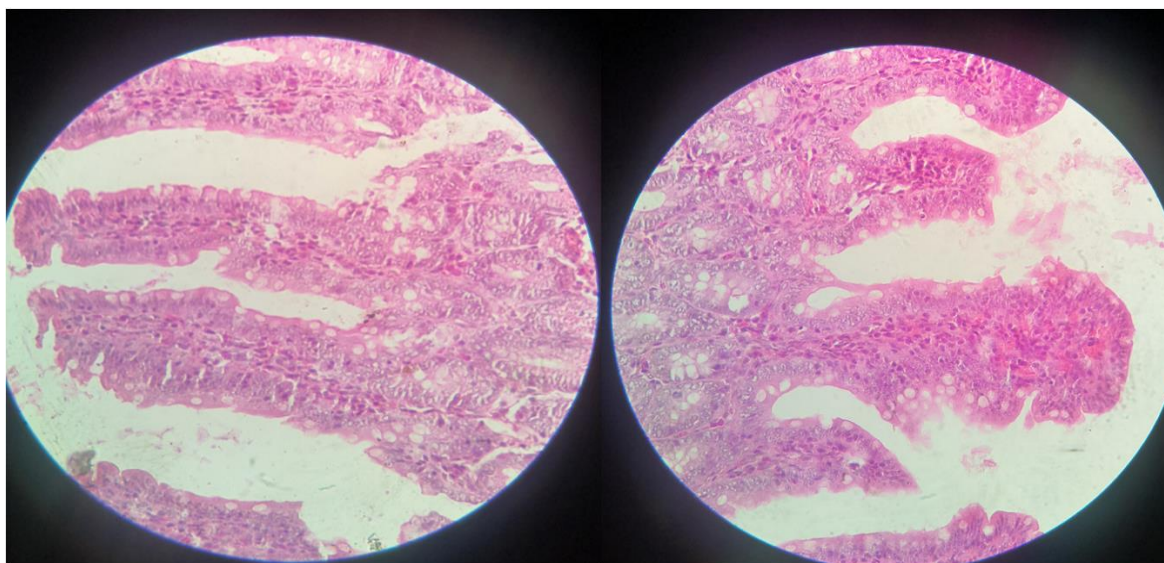
A physiological histological study was conducted to find out the effect of therapeutic nutritional mixtures on the small and large intestine of rats, and the preparation method was followed before (Bancroft and Steven, 1982).

RESULTS AND DISCUSSION

The effect of therapeutic mixtures on the Small intestine:

Control group (+)

The histological part of the gut of rats (duodenum) shows normal tissues and without noticing any pathological changes. The diagnosis was made using a stain (hematoxylin and eosin) for the control group (+) as they were fed a standard diet containing all the necessary nutritional elements throughout the study period. The results were consistent with what indicated (Salameh et al., 2019) that rats who ate a balanced, complete diet had a healthy small intestine.

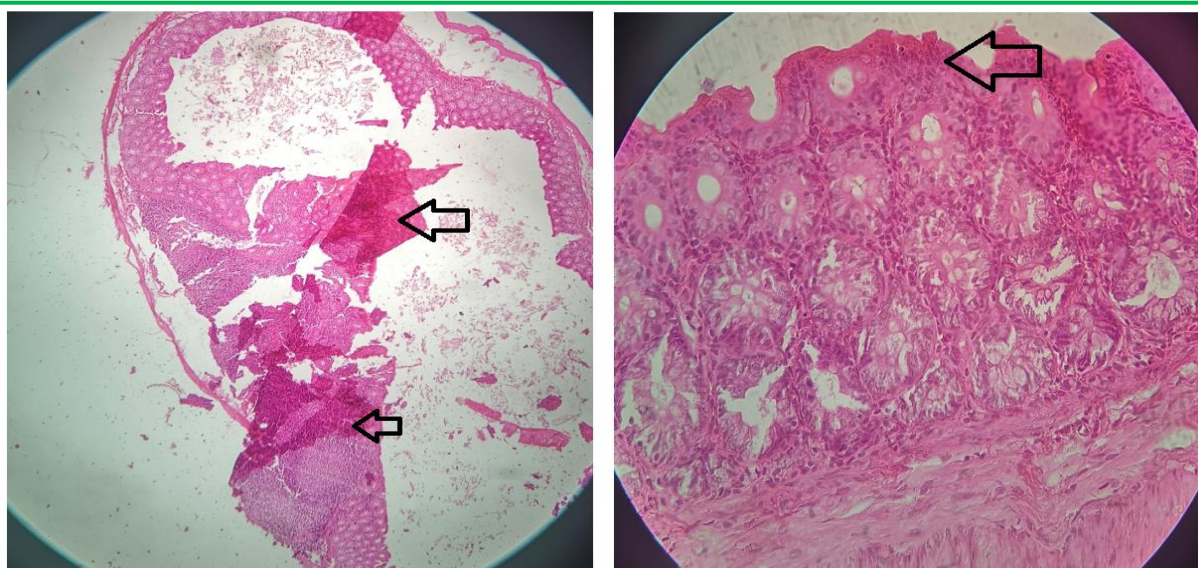


Picture (1) Cross section of rats (duodenum) control group (+) at magnification power x 40

Control group (-)

The histological part of the gut of rats (duodenum) diagnosed using (hematoxylin and eosin) stain, for a control group (-) shows the presence of severe cellular degeneration in the cells of the intestine lining with the presence of chronic and acute infections (arrow).

(Salameh et al. 2019) reported that malnutrition has led to infections in the intestine and a decrease in the villi, which leads to poor absorption of nutrients into the blood, which eventually leads to imbalance in the various body processes and affecting its functions. Also (Das et al., 2004) reported that malnutrition in laboratory animals led to cellular degeneration in the intestinal tissue.

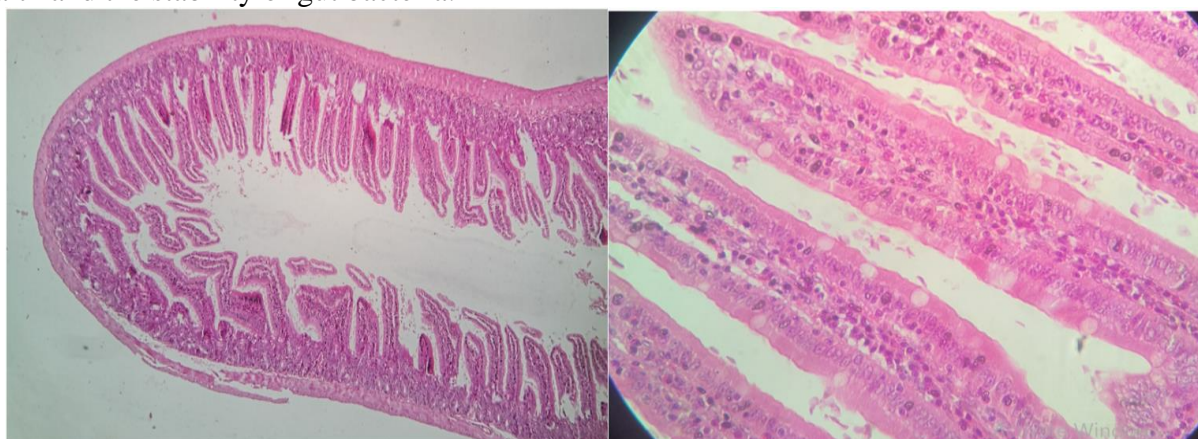


Picture (2) cross section of rat (duodenum) control group (-) at magnification power x 40

First group

The histological part of the gut of rats (duodenum) shows normal tissues and without observing any pathological changes. The diagnosis was made using a stain (hematoxylin and eosin) for the first group, as the first therapeutic mixture contained whole milk, bananas, apples, and potatoes.

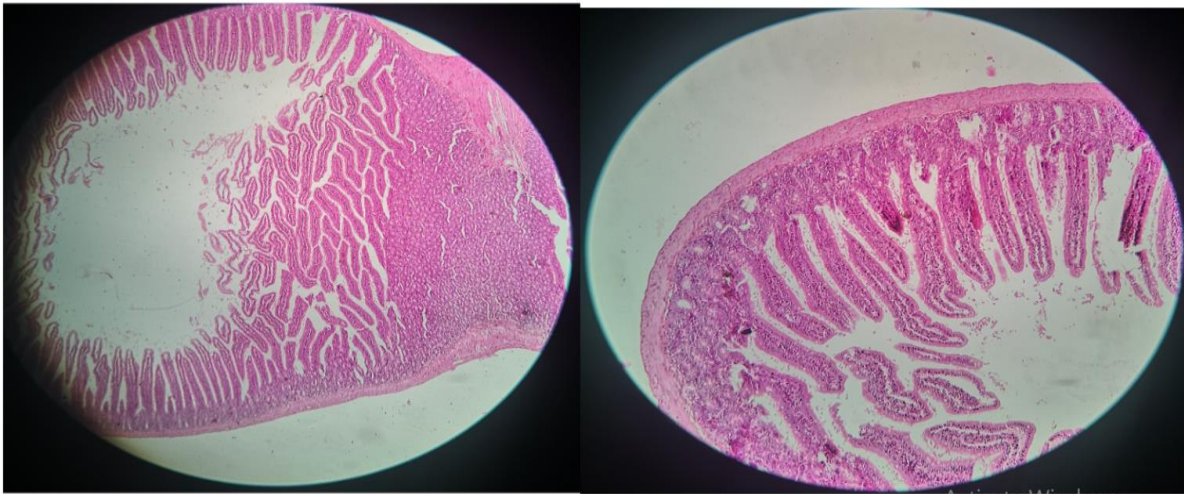
(Chatterton et al., 2013) stated that the advantages of breast milk and cow's milk are that they have anti-inflammatory proteins, and thus it acts as a preventive, therapeutic food for intestinal infections. (Bibi et al., 2019) showed that potatoes have a great role in improving intestinal health and promoting healthy microbes that break down large compounds into small ones, thus improving nutrient absorption into the blood. (Tian et al., 2020) stated that bananas have a role in digestive health and the stability of gut bacteria.



Picture (3) a cross section of rats (duodenum), first group at magnification power x 40

Second group

The histological part of the gut of rats (duodenum) shows normal tissues and without noticing any pathological changes. The diagnosis was made using a stain (hematoxylin and eosin) for the second group as it contained sprouted oats, skimmed milk and chickpeas mainly, whose effectiveness in treating the intestine was indicated by scientific studies. (HE et al., 2018) stated that oat and fiber protein has a role in improving intestinal health by restoring its tissues and promoting the beneficial bacteria in it.



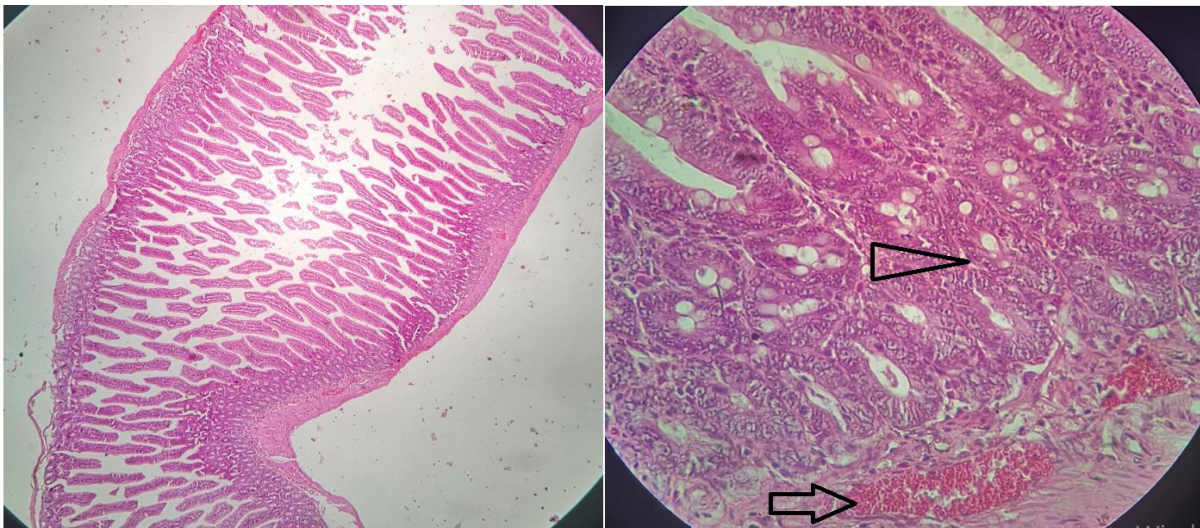
Picture (4) cross section of rats (duodenum), second group, at magnification power x 40

Third group

The histological part of the gut of rats (duodenum) of the third group, which was diagnosed using a stain (hematoxylin and eosin), shows congestion of blood vessels (arrow) with hyperplasia of intestinal epithelial cells (arrowhead).

It was found that it obtained a partial cure compared to a control group (-). This is due to the effectiveness of the nutrients in the third mixture, and it may require a longer time to achieve full recovery, as the third food mixture contained sesame, non-sprouted oats, rice and dates.

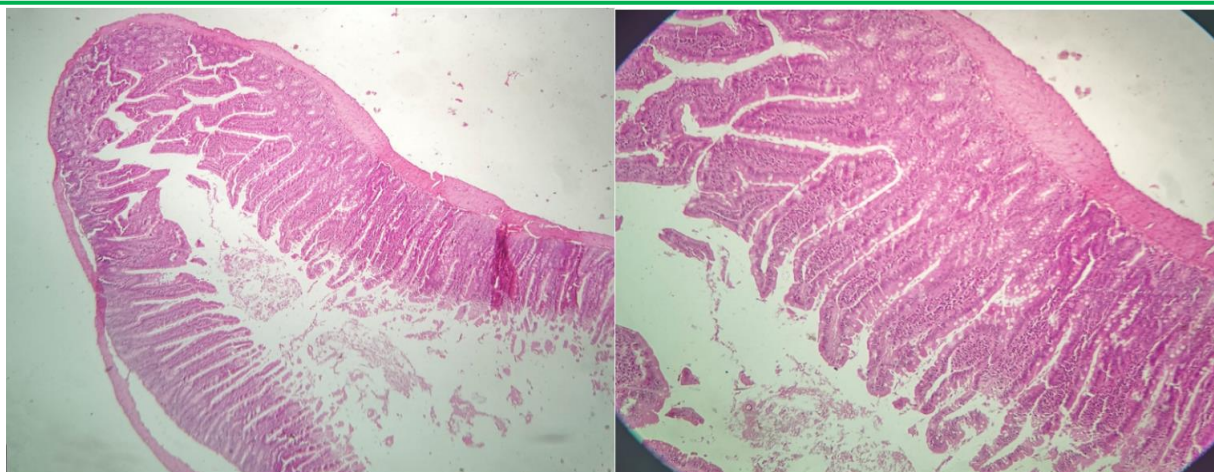
(Hajimohammadi et al. 2020) indicated that sesame flour contributed to an increase in the number of intestinal villi, which increased the efficiency of nutrient absorption and strengthened intestinal microbes during administration to laboratory animals. (HE et al., 2018) stated that oat proteins and fiber have a role in improving gut health by restoring their tissues and promoting beneficial bacteria in them.



Picture (5) cross section of rats (duodenum), third group, at magnification power x 40

Fourth Group

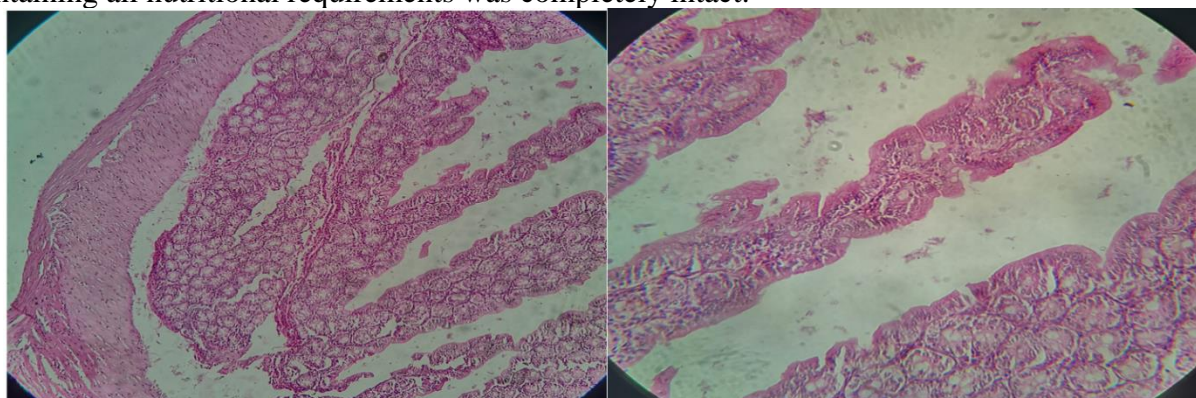
The histological part of the gut of rats (duodenum) of the fourth group shows normal tissues and no pathological changes are observed. The diagnosis was made using a stain (hematoxylin and eosin), as this group was fed the F-100 standard food mixture designed to treat malnutrition, which contained all the essential nutrients.



Picture (6) cross section of rats (duodenum), Fourth group, at magnification power x 40

The effect of therapeutic mixtures on the large intestine control group (+)

The histological part of the gut of rats (colon) shows normal tissues and without noticing any pathological changes. The diagnosis was made using a stain (hematoxylin and eosin) for the control group (+). The reason for this is due to these groups eating nutritional mixtures capable of enhancing immunity and restoring intestinal tissues and cells by providing all the nutrients and requirements that the animal needs to complete its functions to the fullest, these results were in agreement with (Salameh et al., 2019) where the intestine of the control group fed on the standard containing all nutritional requirements was completely intact.

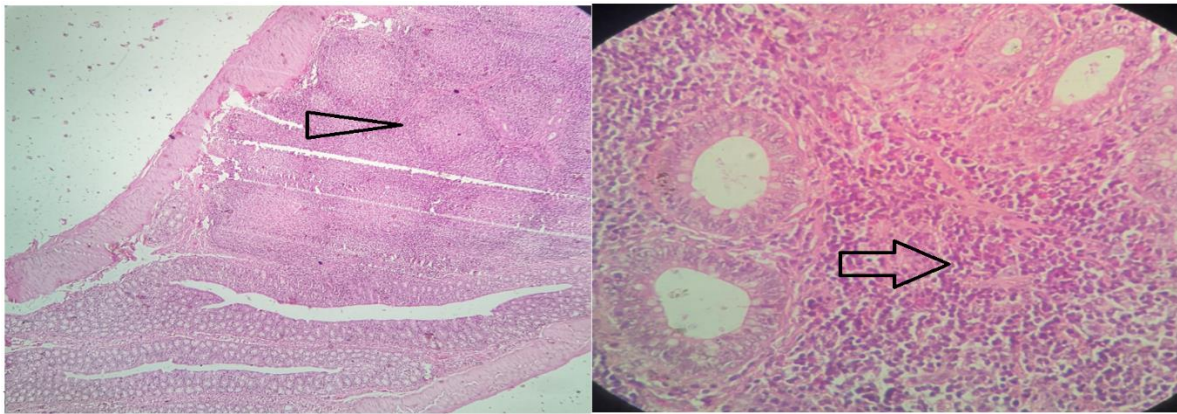


Picture (7) Cross section of rats (colon) of control group (+) at magnification power x 40

control group (-)

The histological part of the gut of rats (colon), diagnosed using the stain (hematoxylin and eosin), showed different pathological changes in a control group (-) that showed Pathological changes represented by infiltration of inflammatory cells (arrow). With an overgrowth of mesenteric lymph tissue and multiple lymph sacs (arrowhead). As a control group (-) was exposed to a lack of nutrition for more than 48 days, which led to a depletion of nutrients and depletion in the various body systems, this made it easy to contract various diseases and infections.

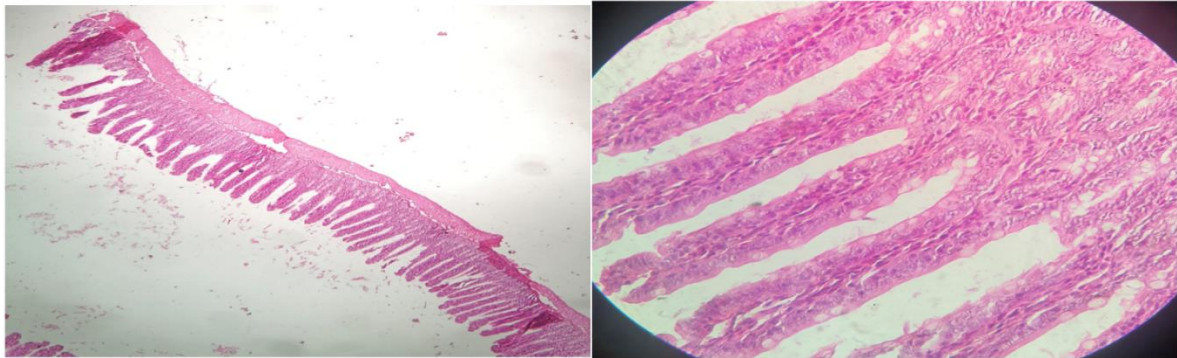
The results are consistent with (Salameh et al., 2019), who stated that the colon increased its permeability to particles outside the body in rats exposed to undernutrition after 35 days, these observations agreed with (Nakajima et al., 2008), who mentioned that the malnutrition in rats led to a loss of mechanical resistance, and the reason for this was a decrease in collagen tissue in the intestine.



Picture (8) Cross section of rats (colon) of a control group (-) at magnification power x 40

First group

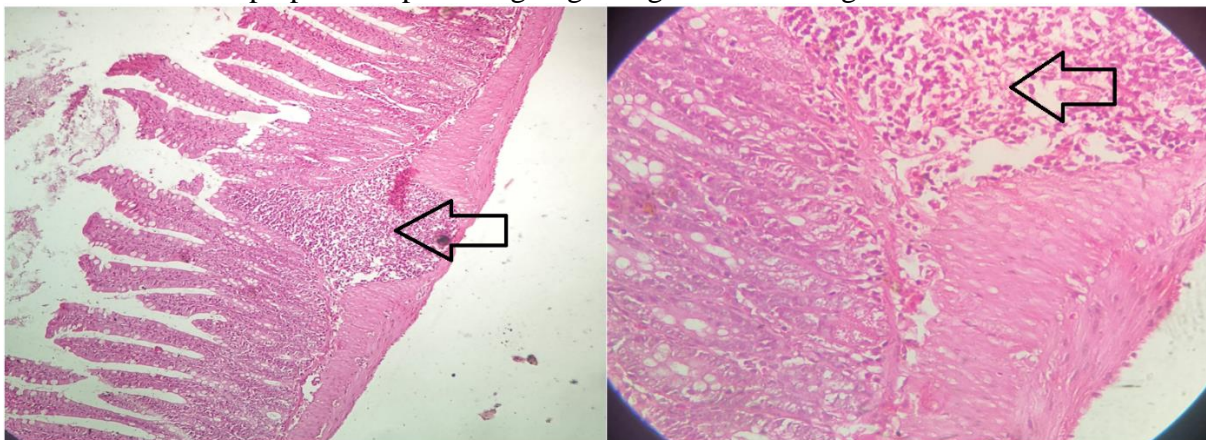
The histological part of the gut of rats (colon) shows normal tissues and without noticing any pathological changes. The diagnosis was made using a stain (hematoxylin and eosin) for the first group. This is due to the efficiency of the first prepared therapeutic nutritional mixture and its richness in essential nutrients.



Picture (9) cross section of rats (colon) of the first group at magnification power x 40

Second group

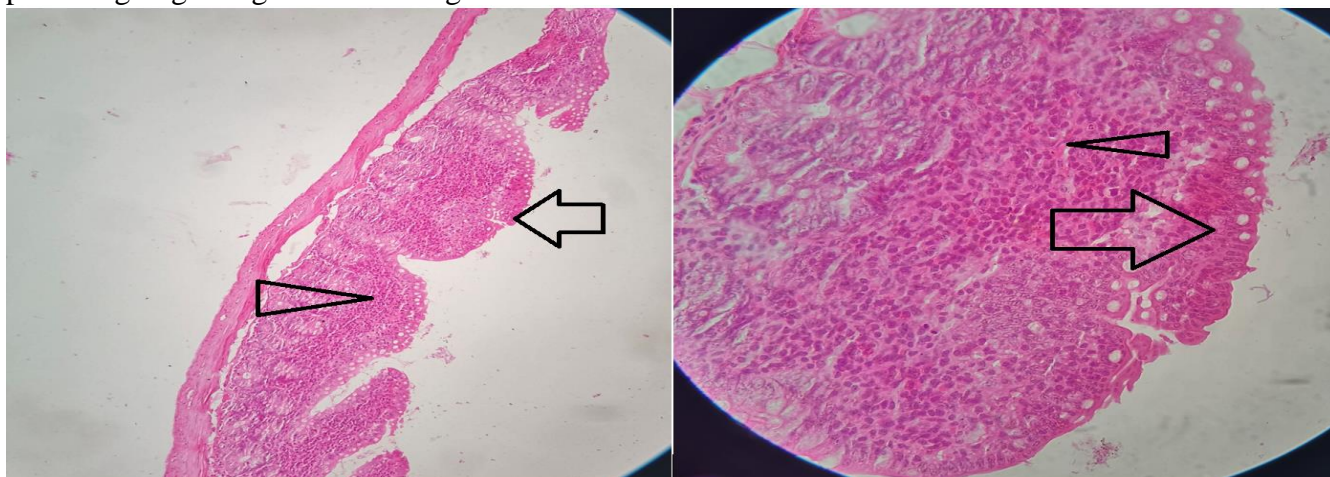
The histological part of the gut of rats (colon) shows diagnosed with (hematoxylin and eosin) stain Infiltration combines and collection of inflammatory cells in the submucosal layer of the colon (arrow). And it got Partial healing compared to a control group (-) due to the effectiveness of the second mixture prepared in providing in gaining Partial healing.



Picture (10) cross section of rats (colon) for the second group at magnification power x 40

Third group

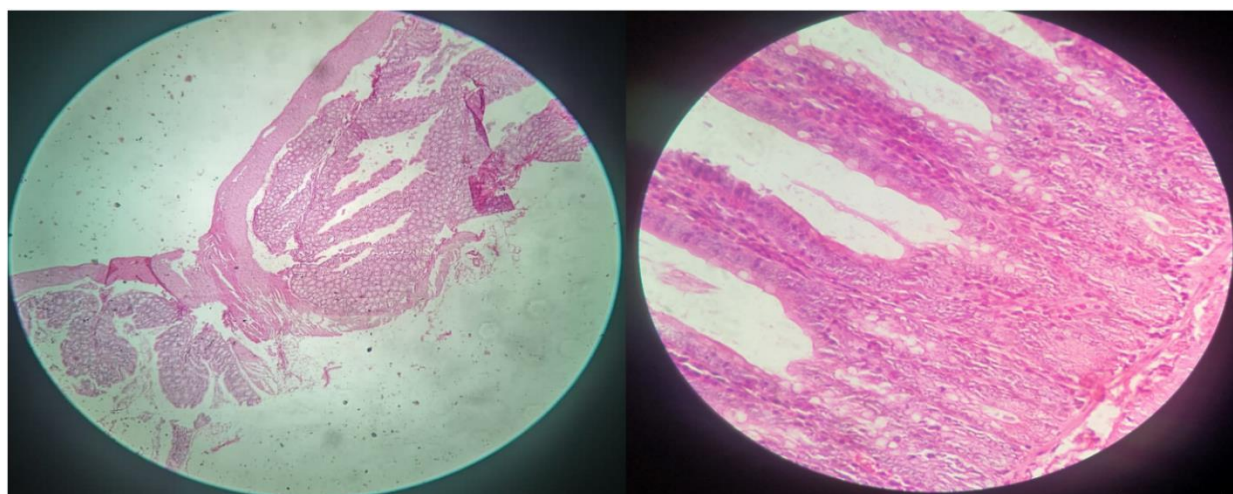
The histological part of the gut of rats (colon), diagnosed with (hematoxylin and eosin) stain, is Shows flatness and union of villi (arrow) with acute inflammation (arrowhead). And it got Partial healing compared to a control group (-) due to the effectiveness of the third mixture prepared in providing in gaining Partial healing.



Picture (11) cross section of rats (colon) for the third group at magnification power x 40

Fourth Group

The histological part of the gut of rats (colon) shows normal tissues and without noticing any pathological changes. The diagnosis was made using a stain (hematoxylin and eosin) of the fourth group, which was fed the standard food mixture F-100.



Picture (12) cross section of rats (colon) for the Fourth group at magnification power x 40

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تأثير خلطات الغذاء العلاجية المحضرة على انسجة امعاء الجرذان المصابة بسوء التغذية

علي صباح محمد صالح التكريتي فريال فاروق حسين بيان ياسين العبدالله

قسم علوم الأغذية – كلية الزراعة – جامعة تكريت – العراق.

الخلاصة

اجريت الدراسة الحالية في مختبرات كلية الزراعة والبيت الحيواني التابع لكلية الطب البيطري في جامعة تكريت للفترة من 2020/5/5 لغاية 2020/10/29. وهدفت الى إنتاج خلطات غذائية علاجية لعلاج المصابين بسوء التغذية ومعرفة تأثيرها على صحة الأمعاء الدقيقة والأمعاء الغليظة. حيث تم تحضير ثلاثة خلطات غذائية علاجية جاهزة الاستعمال (لا تحتاج الى تحضير او طهي قبل تناولها) مكونة من الحليب والشوفان المنبت والشوفان غير المنبت والحمص والسهم والرز والتمر والموز والتفاح والبطاطا والزيت، على ان لا تزيد نسبة الرطوبة والدهن عن 2.5% و 32%، ولا تقل نسبة البروتين والكاربوهيدرات عن 14% و 40% على التوالي، وتم إعطاؤها لثلاثين من الجرذان السليمة وقسمت الى ست مجاميع بالتساوي، مجموعة سيطرة (+) الطبيعية، مجموعة سيطرة (-) (مصابة بسوء التغذية مدة 48 يوم)، المجموعة الأولى، المجموعة الثانية، المجموعة الثالثة، المجموعة الرابعة. تم إصابة المجاميع المدروسة بسوء التغذية مدة 24 يوم ثم اعطيت الخلطة الأولى للمجموعة الأولى، الخلطة الثانية للمجموعة الثانية، الخلطة الثالثة للمجموعة الثالثة، خلطة الغذاء القياسي F-100 للمجموعة الرابعة. وبعد 24 يوم من تناول الخلطات الغذائية المحضرة فحصت انسجة الأمعاء الدقيقة والغليظة مجهرياً. وظهر التحليل النسيجي للأمعاء الدقيقة ان المجموعة الأولى والمجموعة الثانية قد أعطت انسجة سليمة كلياً للأمعاء الدقيقة مشابهة لمجموعة سيطرة (+) والمجموعة الرابعة، في حين أظهرت المجموعة الثالثة احتقان في الاوعية الدموية مع فرط في تنسج الخلايا الطلائية المعوية مقارنة بمجموعة سيطرة (-) والتي اظهرت تنكسات خلوية عالية الحدة في خلايا بطانة الأمعاء مع وجود التهابات مزمنة وحادة. اما الامعاء الغليظة فيظهر ان المجموعة الأولى قد اعطت شفاءً كلياً لأنسجة القولون ومشابها لمجموعة سيطرة (+) والمجموعة الرابعة، في حين اظهرت المجموعة الثانية والثالثة تغيرات مرضية شملت ارتشاح وتجمع موضعي للخلايا الالتهابية في الطبقة تحت المخاطية للقولون وتسطح الزغابات واتحادهما مع التهاب حاد مقارنة بمجموعة سيطرة (-) والتي اظهرت تغيرات مرضية متمثلة بارتشاح الخلايا الالتهابية مع فرط في النسيج اللمفاوي المساريقي وتعدد الحويصلات اللمفية. ويتبين ان الخلطة الأولى قد أعطت نتائج مشابهة لنتائج مجموعة سيطرة (+) والمجموعة الرابعة.

الكلمات المفتاحية:

سوء التغذية، اغذية علاجية جاهزة الاستعمال (RUTF)، أغذية الأطفال، الأمعاء الدقيقة، الأمعاء الغليظة.