RESEARCH ARTICLE

Histological and histochemical study of effect of dietary fibre in motility of stomach in male mice

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Abstract

Objective: To investigate the relationship between dietary fibres and muscularis externa layer.

Method: The experimental study was conducted at the Anatomy Department of the College of Medicine, Al-Nahrain University, Iraq, from November 2020 to April 2021, and comprised adult healthy males. They were divided randomly into experimental group A and control group B. Group A was fed high-fibre diet, and group B was fed standard pellet diet. The tissue samples were harvested at day 30 post-surgery. The stomach samplings were placed in 10% neutral formalin for 24 hours to obtain paraffin sections for routine histological and immunohistochemical staining. The protein expression in stomach's smooth muscle of each group was detected by immunohistochemical staining. Data was analysed using SPSS 24.

Result: Of the 20 mice, 10(50%) were in each of the two groups. Group A exhibited significant weight-gain compared to group B ($p \le 0.01$). There was significant reduction in the thickness of the muscularis layer in group A compared to group B ($p \le 0.01$). Anoctamin 1 expression in group A was significantly lower than that in group B ($p \le 0.01$).

Conclusion: The stress induced by an unstable fibre diet significantly affected the stomach by decreasing the muscularis layer thickness and Anoctamin 1 expression in interstitial cells of Cajal.

Key Words: Anoctamin, Paraffin, Cajal, Formaldehyde

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Introduction

Various nutrients and substances influence motor and sensory functions of the gastrointestinal tract (GIT), resulting in gastrointestinal (GI) symptoms. Factors such as caloric content, physical structure and amount of food play a role in influencing sensations of satiety and fullness.[1] It is now widely acknowledged that a fibre-rich diet is an integral component of a healthy nutrition regimen, and is necessary for regular bowel movements.[2,3]. Muscularis externa orchestrates peristaltic contractions, and comprises three layers of smooth muscle: the inner oblique, middle circular, and outer longitudinal layers[4]. Within the muscularis externa lies Auerbach's nerve plexus (myenteric) and parasympathetic ganglia, positioned between the two muscle layers[5].

The myenteric plexus, a ganglion assembly, extends along the middle of the GIT, providing innervation to the layers of smooth muscle. Positioned between the inner circular and outer longitudinal muscular layers, this nerve network forms the enteric nervous system in conjunction with the submucosal plexus (Meissner). Predominantly governing bowel peristaltic motion, the myenteric plexus operates independently of the central nervous system (CNS).[5]. The potential impacts of a fibre-rich diet on the anoctamin 1 (Ano1) expression in interstitial cells of Cajal (ICCs).

The current study was planned to investigate the relationship between dietary fibres and muscularis externa layer.

Materials and Methods

The experimental study was conducted at the Anatomy Department of the College of Medicine, Al-Nahrain University, Iraq, from November 2020 to April 2021. Approval was obtained from the institutional animal ethics committee, and the study was conducted as per the Guidelines on Ethical Treatment of Experimental Animals issued by the Iraq Provincial People's Government according to the guidelines of the National Institute of Health[6].

Adult healthy male albino mice aged about eight weeks and weighting 20-30g were obtained from the College of Veterinary Medicine, Tikrit University, Iraq, and were divided into experiential group A, which was fed high-fibre (95.5%) diet, and control group B, which was fed a standard pellet diet (70% fibre). The percentage of fibre

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(barley husks) was maintained in line with literature[7]. The intervention lasted 30 days During which all the animals were treated according to the guidelines of the National Institute of Health.

The stomach samplings were put in 10% neutral buffered formalin (NBF) for 24 hours before being histologically processed for paraffin section, de-waxing, staining, and mounting, fixation, dehydration, clearing, impregnation, embedding and sectioning. Tissue preparation for paraffin blocking was in line with an earlier study[8]. The thickness of muscularis externa was measured using Image J. Immunohistochemical process and monoclonal immunoglobulin M (IgM) antibody were used (Sigma Aldrich, Germany; Abcam, United Kingdom). The procedures were carried out in accordance with the instructions provided by the manufacturers.

Data was analysed using SPSS 24. T-test was performed. $P<0.05$ was considered significant.

Results

Of the 20 mice, 10(50%) were in each of the two groups. Group A exhibited significant weight-gain compared to group B ($p\leq0.01$) (Table 1).

Table-1: A-Weight of the animal's before and after using high-fibre diet.

Group Name	Mean (gm) ± SD		T-test
	Before	After	
Control	22.56±1.94	22.33±1.22	NS
Fibre	23.33 ±1.87	14.33 ±1.51	1.695 **

SD: Standard deviation, NS: Non-significant.

Table-2: Thickness of muscularis externa layer of stomach in the animals.

Group	Mean (µm) ± SD
Control	122.96 ±23.51
Fibre	105.16 ±16.49
	** (P≤0.01).

SD: Standard deviation.

Table-3: Anti-anoctamin 1 (Ano1) expression.

Group	Mean (µm) ± SD
Control	0.3135 ±0.1363
Fibre	0.5760 ±0.1555
	** (P≤0.01)

SD: Standard deviation.

The Muscularis externa layer of group A contained smooth muscle bundles (Figure 1-A-B).

There was significant reduction in the thickness of the muscularis layer in group A compared to group B ($p\leq0.01$) (Table 2). Ano1 expression was significantly different between the groups (Table 3, Figure 1-C-D; Figure 2).

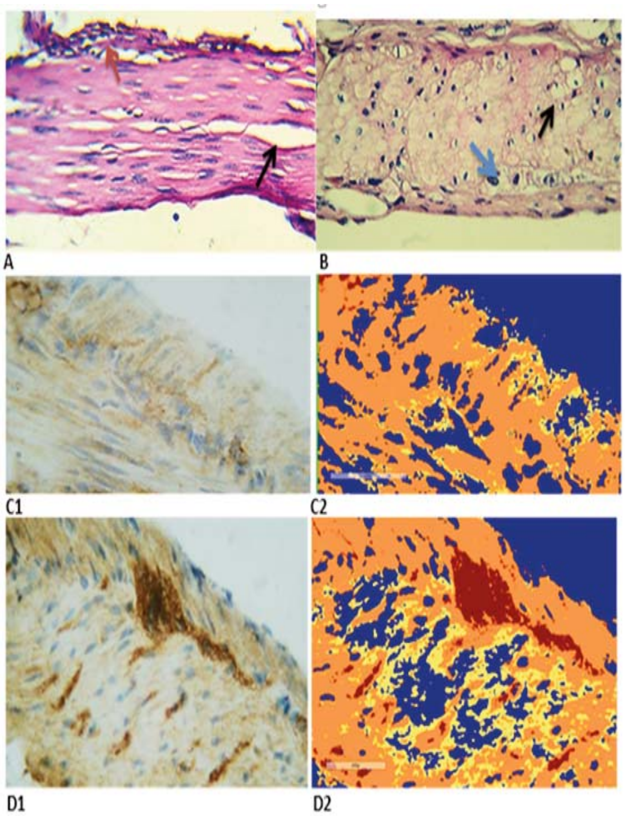

Figure-1: (A,B) Cross-section of the stomach showing muscularis layer of stomach with vacuolation in sarcoplasm (black arrow), hyperplasia of mesothelial cells of tunica serosa (red arrow) and hypertrophy in Auerbach's plexus (blue arrow) (group A, Haemotoxylin and Eosin [H&E] X40); (C1,2) Muscularis externa layer in control group B showing the intensity of the reaction at muscularis externa, and the same tissue analysed by aperio software showing orange discolouration in longitudinal muscular layer and inner circular muscular layer distributed in Muscularis externa layer. The in-between outer longitudinal and inner circular area showing red-brownish discolouration in group A; (D1,2) Muscularis externa layer of group A showing the intensity of the reaction at muscularis externa layer, and the same tissue analysed by aperion software showing orange discolouration in longitudinal muscular layer and the inner circular muscular layer distributed in muscularis externa layer. The small region in-between the outer longitudinal and the inner circular showing red-brownish discolouration in group B.

Discussion

In the current study, Group B exhibited normal activity, water and food consumption. In contrast, group A animals displayed distinct signs starting on the second day of the experiment. This began with significantly increased activity levels compared to group B, and persisted until the 7th day. However, by the 7th day, these animals showed signs of fatigue, reduced food intake, and

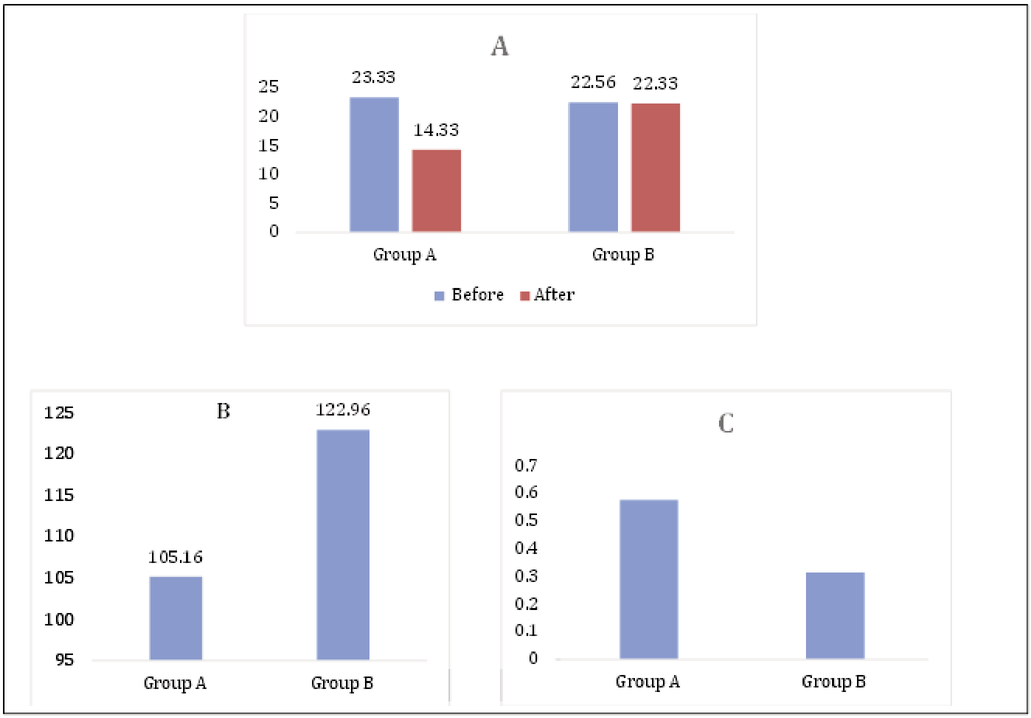

Figure-2: (A) Weight of the animals before and after using the high-fibre diet; (B) Thickness of muscularis externa layer of stomach in control and fibre groups; (C) Comparison between anti- anoctamin 1 (Ano1) expression.

shivering. Also, the faeces of group A animals were noticeably different; being brownish, semi-solid, and larger in volume. Towards the end of the experiment, there was a shift to fewer and more solid faeces.

The current study's finding regarding weight of the mice agreed with earlier studies[9,10] suggesting that dietary fibre helps reduce body weight. Also, the findings agreed with reports that dietary dilution with different levels of wheat reduced body weight gain of turkeys[11]. On the other hand, nutrient release from plants in the oral cavity and following the position of GIT might stimulate the neuronal and hormonal signals that influence gastric processes, such gastric emptying and peristalsis[12] Cell wall properties can vary widely between plant species and even within the same plant[13].

The present study found that group A had a thin muscularis layer. This contradicts the finding that a high fibre diet increased the thickness of muscularis externa. [10]. A strict pure fibre diet led to atrophic changes in the muscularis layer and decrease its thickness. The relevance of nutritional content and meal texture was discovered in a study using magnetic resonance imaging (MRI), reporting a higher sensation of fullness after a high-viscosity meal, and delayed stomach emptying after an energy-rich meal[14]. Hypertrophy of myenteric plexus that works at its maximal limit to keep visceral homeostasis[15] may be marked by a decrease in appearance of ganglionic neuron cell that are closely related together to fibre diet. Some studies found that neurons with lower metabolic activity must become more active to cover the loss of neurons, raising their biochemical processes in an adaptive response, similar to how malnourished neurons increase their biochemical activities[15].

More fibre in the diet leads to constipation and a colon full of faeces, and decreasing fibre diet would lower the bulk and volume of faeces, making evacuation of smaller, thinner faeces easy. The addition of fibre to the mix would merely add bulk and volume, making evacuation even more difficult. Consumption of small amounts of fibre does not lead to constipation.[16], and patients with chronic constipation have the same amount of fibre as healthy people.[17,18] When fibre diet intake is increased, patients with constipation could have worse symptoms. Compared to fibre diet, lactulose was found to be more helpful in alleviating constipation[19].

In the current study, the expression of Ano1 in the muscularis layer in group A revealed a low expression of Ano1 compared to group B. This agreed with a study[20]. The decrease in the expression of Ano1 is due to inhibition of kinase enzyme activity of Ano1 protein in the induction of cell motility as well as cell division and proliferation.

The current findings were in contrast to a study[21] which revealed that 4% protein malnutrition diet led to a reduction in both weight and organ size.

The observed innervation impairment in the current study could be attributed to irregularities in Ano1 expression within ICCs. It is well established that ICCs play a crucial role in generating electrical slow waves required for smooth muscle activity[22]. In the absence of proper electrical slow waves, contractile function becomes compromised and imbalanced, ultimately resulting in decreased gut motility, often manifesting as constipation [23]. An abnormal decrease in ICCs might lead to irregular and decreased activity of slow waves, therefore leading to reduced smooth muscle contractile movement, resulting in slow peristalsis, and consequently causing constipation. The ICCs have been proposed to act as neural signal transducers and amplifiers in smooth muscle cells[24].

Conclusion

An excessive intake of dietary fibre has a notable impact on the Muscularis Externa of the stomach, leading to alterations in the thickness of the Muscularis layer and a decrease in Anoctamin 1 (Ano1) expression within Cajal cells. Consequently, this disruption adversely affects gut motility, resulting in the emergence of motility irregularities. These irregularities serve as a significant contributing factor to the development of various gastrointestinal disorders.

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Conflict of Interest: None.

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References

1. Livovsky DM, Pribic T, Azpiroz F. Food, Eating, and the Gastrointestinal Tract. Nutr 2020;12:986. doi: 10.3390/nu12040986.
2. Barber TM, Kabisch S, Pfeiffer AFH, Weickert MO. The Health Benefits of Dietary Fibre. Nutr 2020;12:3209. doi: 10.3390/nu12103209.
3. Majeed K. The Effect of Diet Modality on ano-1 Cajal Cell Marker Expression in Nervous Plexus of Stomach In Male Mice. Baghdad, Iraq: Al-Nahrain University, 2021.
4. Chaudhry SR, Liman MNP, Peterson DC. Anatomy, Abdomen and Pelvis: Stomach. Fl, US; Statpearls publishing, 2022.
5. Shahrestani J, Das JM. Neuroanatomy, Auerbach Plexus. Fl, US; Statpearls publishing, 2023.
6. National Research Council. Guide for the Care and Use of Laboratory Animals. 8th edition. Washington (DC), USA: National Academies Press (US), 2011; pp 1-246. Available from: https://www.ncbi.nlm.nih.gov/books/NBK54050/
7. Bhavan M, Bahadur MBS. Animal Feeding Stuff - Determination of Nitrogen Content and Calculation of Crude Protein Content - Kjeldahl Method [FAD 5: Livestock Feeds, Equipment and Systems]. IS 14825. New Delhi: Bureau of Indian Standards; 2000.
8. Survana KS, Layton C, Bancroft JD. Bancroft's Theory and Practice of Histological Techniques, 8th edition. Elsevier, 2019; pp 1-557. doi: 10.1016/B978-0-7020-6864-5.05002-7.
9. Al Hammadi E. Effects of dietary fiber intake on body weight and waist circumference. Arab J Nutr Exerc 2016;1:77-84. doi: 10.18502/ajne.v1i2.1225.
10. Jin S, Wijerathne CUB, Au-Yeung KKW, Lei H, Yang C, O K. Effects of high- and low-fiber diets on intestinal oxidative stress in growing-finishing pigs. J Anim Sci 2022;100: 306. doi: 10.1093/jas/skac306.
11. Zdunczyk Z, Jankowski J, Mikulski D, Przybylska-Gornowicz B, Sosnowska E, Juskiewicz J. Gastrointestinal morphology and function in turkeys fed diets diluted with whole grain wheat. Poult Sci 2013;92:1799-811. doi: 10.3382/ps.2012-02482.
12. Maljaars PW, Peters HP, Mela DJ, Masclee AA. Ileal brake: a sensible food target for appetite control. A review. Physiol Behav 2008;95:271-81. doi: 10.1016/j.physbeh.2008.07.018.
13. Edwards CH, Ryden P, Mandalari G, Butterworth PJ, Ellis PR. Structure-function studies of chickpea and durum wheat uncover mechanisms by which cell wall properties influence starch bioaccessibility. Nat Food 2021;2:118-126. doi: 10.1038/s43016-021-00230-y.
14. Marciani L, Gowland PA, Spiller RC, Manoj P, Moore RJ, Young P, Fillery-Travis AJ. Effect of meal viscosity and nutrients on satiety, intragastric dilution, and emptying assessed by MRI. Am J Physiol Gastrointest Liver Physiol 2001;280:G1227-33. doi: 10.1152/ajpgi.2001.280.6.G1227.
15. Castelucci P. Morphoquantitative analysis of the myenteric plexus of the large intestine of rats subjected to pre- and post-natal protein malnutrition and post-natal renutrition [thesis]. São Paulo: University of São Paulo; 1999.
16. de Carvalho EB, Vitolo MR, Gama CM, Lopez FA, Taddei JA, de Morais MB. Fiber intake, constipation, and overweight among adolescents living in Sao Paulo City. Nutrition 2006;22:744-9. doi: 10.1016/j.nut.2006.05.001.
17. Bellini M, Tonarelli S, Barracca F, Rettura F, Pancetti A, Ceccarelli L, Ricchiuti A, et al. Chronic Constipation: Is a Nutritional Approach Reasonable? Nutrients 2021;13:3386. doi: 10.3390/nu13103386.
18. Towers AL, Burgio KL, Locher JL, Merkel IS, Safaeian M, Wald A. Constipation in the elderly: influence of dietary, psychological, and physiological factors. J Am Geriatr Soc 1994;42:701-6. doi: 10.1111/j.1532-5415.1994.tb06527.x.
19. Quah HM, Ooi BS, Seow-Choen F, Sng KK, Ho KS. Prospective randomized crossover trial comparing fibre with lactulose in the treatment of idiopathic chronic constipation. Tech Coloproctol 2006;10:111-4. doi: 10.1007/s10151-006-0262-5.
20. Sharm K, Sung J, Kim HJ, Oak MH, Yi E. Rice Bran Extract Inhibits TMEM16A-Involved Activity in the Neonatal Rat Cochlea. J Nanosci Nanotechnol 2017;17:2390-3. doi: 10.1166/jnn.2017.13333.
21. Pinto MC, Schreiber R, Lerias J, Ousingsawat J, Duarte A, Amaral M, et al. Regulation of TMEM16A by CK2 and Its Role in Cellular Proliferation. Cells 2020;9:1138. doi: 10.3390/cells9051138.
22. Huizinga JD, Thuneberg L, Klüppel M, Malysz J, Mikkelsen HB, Bernstein A. W/kit gene required for interstitial cells of Cajal and for intestinal pacemaker activity. Nature 1995;373:347-9. doi: 10.1038/373347a0.
23. Zhao Q, Chen YY, Xu DQ, Yue SJ, Fu RJ, Yang J, et al. Action Mode of Gut Motility, Fluid and Electrolyte Transport in Chronic Constipation. Front Pharmacol 2021;12:e630249. doi: 10.3389/fphar.2021.630249.

24. Tanahashi Y, Komori S, Matsuyama H, Kitazawa T, Unno T. Functions of Muscarinic Receptor Subtypes in Gastrointestinal Smooth Muscle: A Review of Studies with Receptor-Knockout Mice. Int J Mol Sci 2021;22:926. doi: 10.3390/ijms22020926.