# ASSESSMENT OF THE BENEFICIAL EFFECT OF CHIA AND QUINOA-FORTIFIED GLUTEN-FREE BISCUIT PRODUCTS ON CELIAC DISEASE IN RATS

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**ABSTRACT**

Celiac disease (CD) is a chronic inflammatory disease of the upper small intestine triggered by gluten protein intolerance, which is prevalent in genetically predisposed individuals. The present study aimed to evaluate the beneficial effect of Chia and Quinoa-fortified gluten-free biscuit products on CD in Rats. Thirty-five male Sprague Dawley rats (225±5 g b.wt. and 8 weeks age) were used. Rats were distributed into 5 equal groups; Group1: negative control, group 2: positive control with CD and groups 3, 4 and 5: with CD and were fed Chia, Quinoa and their combination, respectively. Feed intake (FI), body weight gain (BWG %), and feed efficiency ratio (FER) were calculated. Rats were then anesthetized and blood samples were collected for determination of activities of serum liver and kidney function, total cholesterol (TC) and triglyceride (TG) and lipoprotein fractions were measured. Reduced glutathione (GSH), malondialdehyde (MDA) and lactate dehydrogenase (LDH) were measured. Histopathology of small intestine was also carried out. Results revealed that feeding Chia, Quinoa and their combination decreased FI, BWG% and FER, TC and TG and decreased serum parameters of elevated hepatorenal function as compared to the positive control (+ve) group.Histopathological examination of small intestine demonstrated that feeding combination of Chia and Quinoa ameliorated lesions of CD and inflammation. It could be concluded that Chia and Quinoa would be an effective curative for celiac disease- induced histopathological changes in the small intestinedue to its improvement of intestinal histopathological lesions.

**Keywords:** Celiac disease- Gluten- Chia - Quinoa- Biochemical analysis,Histopathology.

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## INTRODUCTION:

Celiac disease (CD) is a chronic inflammatory disease of the upper small intestine triggered by gluten protein intolerance, which is prevalent in genetically predisposed individuals. The prevalence of the celiac disease in Arab countries varies with sex and age. However, It was found that CD presented similar clinical characteristics independent of the geographic region. **(Paivi *et al.*, 2020).** The classical manifestation of CD is often present with all related signs and symptoms of malabsorption. Moreover, patients also experience diarrhea, steatorrhea, and loss of weight or growth failure. Meanwhile, in children, the classical characteristics are diarrhea, failure to thrive, muscle wasting, poor appetite, abdominal distension, and sometimes emotional distress and lethargy **(Paivi et al., 2020).** Currently the only effective treatment for CD is the gluten free diet (GFD), which is extremely difficult to adhere to and significantly reduces the patients' quality of life. Therefore, investigations are in progress to find new therapies varying from production of genetically modified wheat to blockade of inflammatory cytokines. Meanwhile, the discovery of new endogenous immunoregulatory substances suggests new therapeutic approaches **(Rashtak and Murray, 2012).**

Gluten is the wheat grain protein richly consumed in Western countries with an average daily intake of 10 to 20 grams/person/day **(Cohen *et al.*, 2019)**. It is comprised of prolamin and glutelin proteins. Both proteins abundantly possess glutamine and proline residues, which defy gastrointestinal digestion and promote the deamination process through the tissue transglutaminase (tTG) enzyme. It may lead to mucosal inflammation and villous atrophy, thus causing malabsorption **(Assa and Frenkel., 2017)**.

Chia is commonly known as *Salvia hispanica L.*, an oilseed plant, and is native from southern Mexico and

northern Guatemala. It is a traditional food in Central and South America. Chia seed contains 25 to 40% oil with high essential fatty acids (omega) ω-3 alpha-linolenic acid (60%) and (omega) ω-6 linoleic acid (20%). It is also high in protein (19-23%), dietary fiber (18-30%), vitamins, minerals and antioxidants **(Norlaily *et al*., 2012).** Due to its unique nutritional composition, especially its high unsaturated fatty acid composition, dietary fiber and antioxidant activities its consumption helps to increase satiety index and decreases the risk of various types of diseases such as cardiovascular diseases, cancer, diabetes, inflammatory and autoimmune diseases **(Munoz *et al.*, 2013).**A number of studies have investigated the inclusion of different seeds into gluten-free breads in order to increase their nutritional value. Because of its nutritional composition, particularly the omega-3 fatty acid, fiber and antioxidant content, Chia is an ideal seed for the enrichment of cereal products, and there has been an increase in the number of commercial products that incorporate chia in their formulation in recent years **(Eugenia *et al*., 2014).**

Quinoa, a pseudocereal that belongs to the *Chenopodiaceous* family has a higher nutritive value than traditional cereals. It has high protein content (10-18%) with balanced amino acid composition, supplying high contents of lysine and methionine **(Nowak *et al*., 2016).** The fat content of raw quinoa seeds was 9.7% (dry-weight basis) and it has similar fatty acid composition with soybean oils with high amounts of essential fatty acids linoleic (52.3%) and linolenic acids (3.9%). These essential fatty acids required for good health, cannot be synthesized in human body and must be obtained from the diet **(Costantini *et al*., 2014).**It contains a significant amount of fiber (2.1-4.9%), more calcium, magnesium, potassium, iron, copper, riboflavin (B2), α-Tocopherol (vitamin E), β-Carotene and ascorbic acid (vitamin C) than wheat, barley and rice. It is also a rich source of other bioactive compounds (polyphenols, phytosterols, etc.) **(Aktaş and Levent, 2018).** Quinoa used in the bakery industry because the starch present in the seeds has properties similar to those found in wheat. In addition to augmenting the nutritional value, it is free of gluten, so it can be eaten by people who have CD as well as by those who are allergic to wheat **(Stikic *et al*. 2012)**

## MATERIAL AND METHODS:

### A. Materials

Basal diet was prepared according to the method of **Reeves *et al.* (1993).** It was consisted of 20 % protein (casein), 10 % carbohydrate, 4.7% fat (corn oil), 2% choline chloride, 1% vitamin mixture, 3.5 % salt mixture and 5% fibers. The remainder was corn starch up to 100 %.Raw material Chia seeds, whole quinoa flour, rice flour, fine granulating sucrose, whole egg, oil, cinnamon, salt, baking powder and ethyl vanillin were obtained from Agricultural Research center in Cairo. Kits for blood analysis were purchased from local distributer of (Sigma Chemical), Cairo, Egypt. Gliadin (G-3375; Sigma chemical substance to induction of celiac disease obtained from El-Gomhoryia Company, Cairo, Egypt. Animals: A total number of thirty-five mice (Sprague Dawley strain) weighing (20 to 25 g) body weight, were purchased from the Agriculture Research Center, Giza, Egypt.

### B. Methods:

**Preparation of Flour Formulas:**

The free gluten flour formula consists of (rice flour), chia flour (CF) and quinoa flour (QF) were mixed together for preparation the biscuits by replacement.

**Table (1):** Flour formulas (as weight) for preparation of free gluten bakery products (biscuits).

| Formula | Rice flour (g) | Chia flour(g) | Quinoa flour(g) |
|---|---|---|---|
| Control | 200 | - | - |
| Sample 1 | 190 | 10 | - |
| Sample 2 | 180 | 20 | - |
| Sample 3 | 160 | 40 | - |
| Sample 4 | 190 | - | 10 |
| Sample 5 | 180 | - | 20 |
| Sample 6 | 160 | - | 40 |
| Sample 7 | 190 | 5 | 5 |
| Sample 8 | 180 | 10 | 10 |
| Sample 9 | 160 | 20 | 20 |

**Preparation of Bakery Products (Biscuits):**

Preparation of the different types of free gluten (biscuits). Control gluten-free biscuits sample was prepared using the following recipe: Gluten free flour (rice flour) 200g, fine granulating sucrose (40 g), whole egg (50 g), salt (0.5 g), baking powder (5 g), ethyl vanillin (0.1 g) and sunflower oil (40)g (**A.O.A.C.1995).** The replacement by chia and quinoa flour with the percentage of chia flour (5, 10, 20 %), Quinoa flour (5, 10, 20 %) and mixture of chia: quinoa flour (1:1) as (5, 10, 20%)

**Induction of Celiac Disease (CD):**

To induce celiac disease, Gliadin (G-3375; Sigma) was dilute in 0.02 M acetic acid to form 10% solution. The prepared solution was administered intragastrically by gavage at a dose of 1.5 mg/g body weight for five days as reported by **Nikoukar*et al*., (2014).**

**Experimental Animal Design:**

Thirty-five rats male Sprague Dawley strain rats was housed in well-aerated cages under hygienic conditions, and fed on basal diet for one week for adaptation.

After the adaptation period, rats will be divided into two main groups, as follows:

**First group:** Negative control group, rats (n=7) was fed on basal diet only during the experimental period.

**Second group:** all remaining rats (n=28), were intragastrically given (1.5 mg/g body weight) from Gliadin diluted in 0.02 M acetic acid to form 10% solution for 5 days to induced celiac disease, and they were divided as follow:

**Subgroup (1):** rats serve as positive control group basal diet only.

**Subgroup (2):** rats were feed Basel diet (with bakery product with chia seed).

**Subgroup (3):** rats were feed Basel diet (with bakery product with quinoa seed).

**Subgroup (4):** rats were feed Basel diet (with bakery product with chia seed and quinoa).

**Biological Evaluation:**

Daily feed intake (FI) was recorded day after day throughout the experimental period (3 weeks). Body weight gain (BWG) was calculated. Determination of body weight gain percent and feed efficiency ratio (FER) were assessed according to the method described by **Chapman *et al.* (1959)** using the following equations:

**BWG % =** final weight (g) – Initial weight (g) / Initial weight (g) ×100

**Feed efficiency ratio (FER)** = Weight gain (g) / feed consumed (g)

**Blood Collection and Serum Separation:**

At the end of the experimental period (2 weeks), rats were fasted overnight and authorized by ether, intestinal was dissected out and kept in 10% neutral buffered formalin for histopathological examination and blood samples were collected from each rat and centrifuged at 3000 rpm for 15 min to obtain the serum for biochemical analysis.

**Biochemical Analysis:**

Total cholesterol (TC) was determined according to **Richmond, (1973)**. Triglyceride (TG) was determined according to **Wahlefeld, (1974)**. High density Lipoprotein cholesterol (HDL-C) was determined according to **Albers *et al*., (1983)**.Low density Lipoprotein cholesterol (LDL-C) and very Low density Lipoprotein cholesterol (VLDL-C) were estimated according to the equation described by **Friedewald *et al*., (1972)**.Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) concentration were determined according to **Young, (2001)**. Alkaline phosphatase (ALP) concentration was determined according to **Roy, (1970)**.Serum uric acid and creatinine levels were estimated as described by **Lorentz and Brendt (1967)** andGlutathione peroxidase (GPx) was determined according to **Hissin and Hilf, (1976)**.Serum lactate dehydrogenase was determined according to**Vassault (1986)**. Serum malondialdehyde (MDA) was determined by the method of**Ohkawa *et al*., (1979).**

**Histopathological Examination:**

Immediately after scarifying of rats, intestinal dissected out and kept in 10% neutral buffered formalin for histopathological examination according to **Bancroft and Stevens (1996)**.Histopathological examination was carried out at the Department of Pathology, Faculty ofVeterinary medicine, Cairo University, Egypt.

## STATISTICAL ANALYSIS:

All data obtained results were analyzed using Statistical Package for the Social Sciences (SPSS) for Windows, version 20 (*SPSS Inc., Chicago, IL, USA*). Collected data was presented as mean± standard deviation (SD). Analysis of Variance (ANOVA) test was used for determining the significances among different groups according to **Armitage and Berry (1987)**. All differences were considered significant if P-values were ($P< 0.05$).

## RESULTS AND DISCUSSION:

Data in table (2) show that feeding of rats with Chia and Quinoa-fortified gluten free biscuit products decreased FI, BWG% and FER in the positive group (rats with celiac disease) as compared to the normal control rats. Feeding Chia, Quinoaand their combination to rats significantly increase. FI, BWT% and FER.

These findings were similar to those reported by**Sadeek*etal*., (2021**) who concluded that the

incorporation of chia and quinoa flour with wheat flour have beneficial effects for improving the chemical, nutritional, rheological, and sensory properties of food product pasta.

**Table (2):** Effect of Chia and Quinoa-fortified gluten-free biscuit products on feed intake (FI), body weight gain (BWG %) and feed efficiency ratio (FER) in rats

| Parameters<br>Groups | FI<br>(g/day/rat) | BWG<br>(%) | FER |
|---|---|---|---|
| **Control (-ve)** | 10.0±0.71a | 48.56±2.71a | 0.230±0.009a |
| **Control (+ve)** | 9.0±0.67c | 24.07±2.67c | 0.120±0.003b |
| **Chia** | 12.0±0.34bc | 30.35±2.34bc | 0.121±0.007b |
| **Quinoa** | 12.5±0.10bc | 32.88±2.10bc | 0.128±0.006b |
| **Chi and Quinoa** | 13.0±0.71b | 36.28±2.71b | 0.132±0.008b |

Means in the same column with different letters are significantlydifferent at P≤0.05

**Table 3.** Effect of Chia and Quinoa-fortified gluten-free biscuit products on total(TC) and triglycerides (TG) in rats. (n=7 rats)

| Parameters<br>Groups | Total Cholesterol<br>(mg/dL) | Triglycerides<br>mg/dL |
|---|---|---|
| **Control (-ve)** | 96.14±1.07b | 98.42±2.12b |
| **Control (+ve)** | 122.42±1.43a | 122.71±2.47a |
| **Chia** | 92.28±1.68bc | 83.71±2.14cd |
| **Quinoa** | 91.42±1.35bc | 86.57±1.60c |
| **Chi and Quinoa** | 88.71±1.83c | 74.28±1.83d |

Means in the same column with different letters are significantly different at P≤0.05

Results in table (3) revealed that feeding of rats with celiacdisease significantly increased TC and TG in the positive group as compared to the normal control group. Rats fed Chia, Quinoa and their combination had a significant decrease in TC and TG.

These results agreed to those reported by**Eugenia *et al.* (2014), Nowak *et al.* (2016) and Aktaş et.al. (2018).**The previous authors reported thatChia, Quinoa lowered TC and TG in rats with celiac disease.

**Table (4).** Effect of Chia and Quinoa-fortified gluten-free biscuit products on lipoprotein fraction (high density lipoprotein (HDL-c), low density lipoprotein (LDL-c) and very low density lipoprotein (VLDL-c). (n=7 rats)

| Parameters<br>Groups | HDL-c<br>(mg/dL) | LDL-c<br>(mg/dL) | VLDL-c<br>(mg/dL) |
|---|---|---|---|
| **Control (-ve)** | 50.57±1.23a | 25.88±1.65b | 19.68±0.42b |
| **Control (+ve)** | 42.14±0.50c | 55.74±1.77a | 24.54±0.69a |
| **Chia** | 46.57±1.49b | 28.79±2.07b | 16.47±0.62cd |
| **Quinoa** | 46.58±0.93b | 27.25±1.99b | 17.31±0.32c |
| **Chi and Quinoa** | 49.57±0.57ab | 24.28±1.79b | 14.85±0.16d |

Means in the same column with different letters are significantly different at P≤0.05.

As shown in table (4), Celiac diseases in rats significantly decreased HDL-c, and increased LDL-c and VLDL-c. Rats fed Chia, Quinoa and their combination had a significant increase in HDL-c and a decrease in LDL-c and VLDL-c.
These findings were similar to those of**Norlaily *et al.* (2013); Nowak *et al.* (2016) and Khatri*et al.*(2023).**

Moreover, many studies have described chia and quinoa as protecting against a variety of diseases, particularly cancer, allergies and, inflammatory diseases, and they may reduce the risk of cardiovascular diseases (**Sadeek et al., (2021).**

**Table (5).** Effect of Chia and Quinoa-fortified gluten-free biscuit products on liver enzymes aspartateaminotransferase (AST); alanine aminotransferase (ALT) and alkaline phosphates (ALP) in rats.(n=7 rats)

| Parameters<br>Groups | AST<br>(U/L) | ALT<br>(U/L) | ALP<br>(U/L) |
|---|---|---|---|
| **Control (-ve)** | 14.71±0.42c | 28.28±0.86d | 115.00±1.38d |
| **Control (+ve)** | 39.57±0.57a | 32.57±0.64a | 126.71±1.20a |
| **Chia** | 19.71±0.42b | 29.28±0.80bc | 118.42±1.48b |
| **Quinoa** | 19.57±0.42b | 26.57±0.97b | 117.58±1.12c |
| **Chi and Quinoa** | 16.14±0.63c | 25.00±0.81cd | 116.57±1.84c |

Means in the same column with different letters are significantly different at P≤0.05.

Data in table (5) show that Celiac diseases in rats significantly increased liver enzymes AST, ALT and ALP. Rats fed Chia, Quinoa and their combination had a significant decrease in the levels of elevated liver enzymes.

The present results agreed with those of **Rashtak *et al*. (2012) Muñoz*et al*. (2013); Khatri, *et al*., (2023).** The previous author concluded that feeding Chia and Quinoa to rats lowered the level of liver enzymes.

**Table (6).** Effect of Chia and Quinoa-fortified gluten-free biscuit products on kidney function parameters uric acid (UA) and creatinine (Creat.) in rats. (n=7 rats)

| Parameters<br>Groups | UA<br>(mg/dL) | Creat.<br>(mg/Dl) |
|---|---|---|
| **Control (-ve)** | 16.71±0.56c | 0.78±0.036c |
| **Control (+ve)** | 24.71±0.68a | 1.79±0.058a |
| **Chia** | 19.14±0.50bc | 1.13±0.087b |
| **Quinoa** | 19.42±0.64b | 1.05±0.46b |
| **Chi and Quinoa** | 17.14±0.63bc | 0.82±0.043c |

Means in the same column with different letters are significantly different at P≤0.05.

As shown in table (6), Celiac diseases in rats significantly increased kidney function parameters uric acid (UA) and creatinine (Creat.) in control positive groups. Rats fed Chia, Quinoa and their combination had a significant decrease in the levels of elevated kidney parameters (uric acid and creatinine).

These results were in agreement with those of **Mohd*et al.* (2012) Muñoz *et al.*(2013) and Costantini*et al.*, (2014).**

**Table (7).** Effect of Chia and Quinoa-fortified gluten-free biscuit products on glutathione (GSH); malondialhyde (MDA) and lactate dehydrogenase (LDH) in rats. (n=7 rats)

| Parameters<br>Groups | GSH<br>(mg/dL) | MDA<br>(mg/dL) | LDH<br>(U/L) |
|---|---|---|---|
| **Control (-ve)** | 5.09±0.023a | 2.60±0.018c | 118.28±3.44c |
| **Control (+ve)** | 3.43±0.119d | 3.67±0.103a | 316.14±4.33a |
| **Chia** | 4.80±0.020b | 2.83±0.064b | 144.57±2.71b |
| **Quinoa** | 4.50±0.038c | 2.73±0.073bc | 139.00±2.41bc |
| **Chi and Quinoa** | 4.74±0.034bc | 2.28±0.040d | 129.00±2.60bc |

Means in the same column with different letters are significantly different at P≤0.05.

The current results revealed that Celiac diseases in rats significantly increased LDH and MDA but decreased GTH in the positive control group as compared to the negative control group. Rats fed Chia, Quinoa and their combination had a significant decrease in LDH and MDA and a significant increase in GTH.

These findings were nearly similar to those of **Muñoz et al., (2013) and Aktaş and Levent(2018).** The previous authors reported thatfeeding of rats with Chia, Quinoa and their combination had a significant decrease in LDH and MDA and a significant increase in GTH.

**Histopathology Examination**

Evaluation of microscopic lesion score of H&E stained sections of small intestine segments were demonstrated in

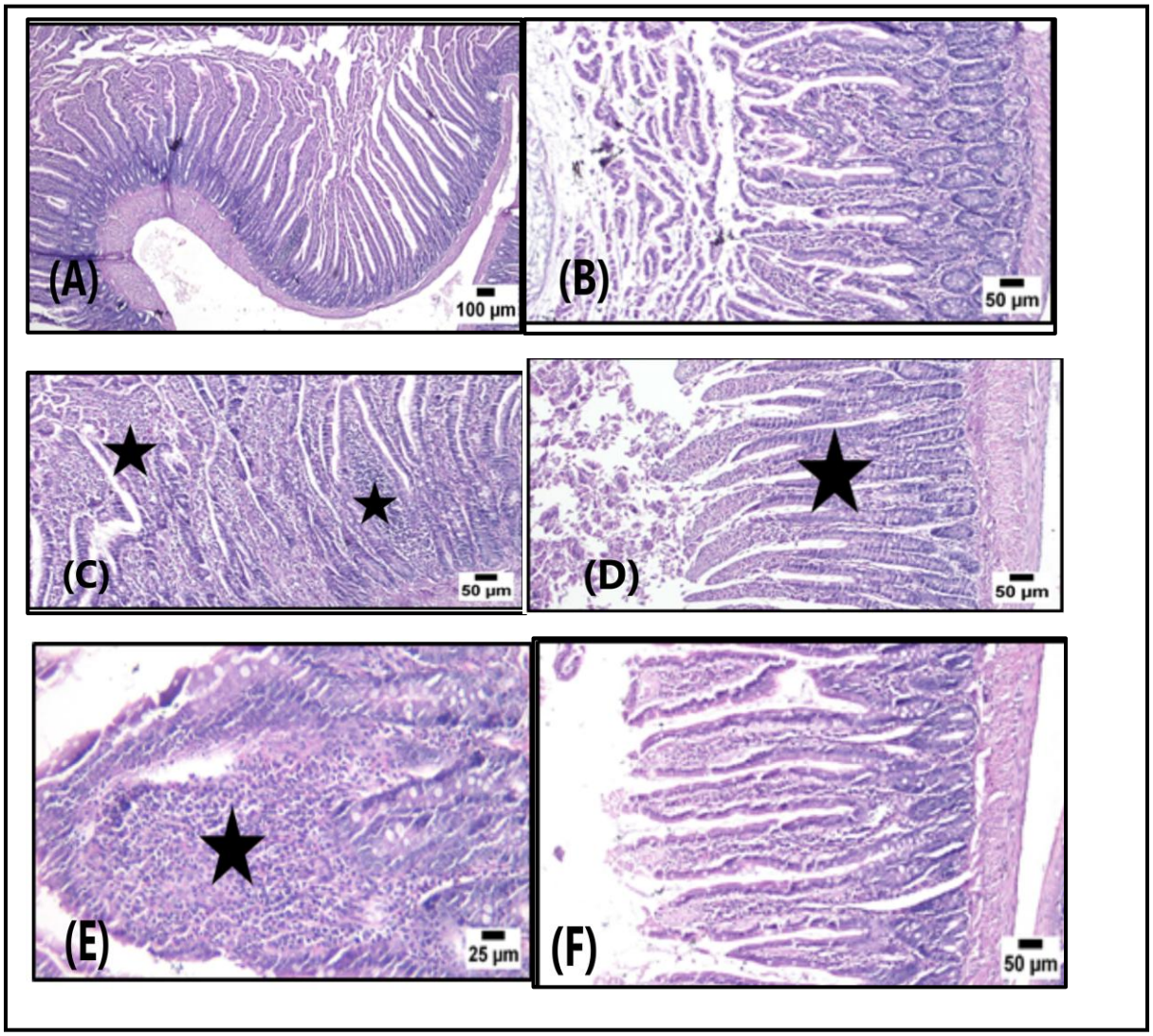


Fig (1).

**Fig (1):** Cross sections of small intestine of rats from:

**(A)** Normal control group showing normal histological structure all layers (H&E), **(B)** Positive control group showing marked atrophied intestinal villi with hyperplasia of crypts (H&E), **(C)** Positive control group showing expansion of the lamina propria with numerous infiltrated inflammatory cells (stars) (H&E), **(D)** Group fed Chia-fortified gluten free biscuit product showing moderate atrophy of the intestinal villi associated with hyperplasia of the crypts (Star) (H&E), **(E)** Group fed Quinoa -fortified gluten free biscuit product showing variable number of inflammatory cells infiltration in the intestinal mucosa (Star) (H&E) and **(F)** Group fed Chia and Quinoa -fortified gluten free biscuit product showing apparently normal histological structure.

The histopathological alterations seen in small intestine in rats with CD rats, in this study, were parallel with the reported biochemical parameters. Theses histopathological alterations in the present study were similar to those demonstrated by **Muñoz et al. (2013)**,**Khatri et.al. (2023)**who reported that Chia and Quinoa would be an effective curative for celiac disease- induced histopathological changes in the small intestine.

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