Effects of Chia-Enriched Bread on Locomotor Activity and Exploratory Behavior in Alloxan-Induced Diabetic mice

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Abstract:

Diabetes mellitus is associated with behavioral disturbances and neurodegenerative changes that are largely mediated by oxidative stress and inflammation. Chia seeds (Salvia hispanica L.) are rich in omega-3 fatty acids, dietary fiber, and antioxidant compounds that may exert neuroprotective effects. This study investigated the effects of chia-enriched bread on locomotor activity, exploratory behavior, and brain histopathological alterations in alloxan-induced diabetic mice. Eighteen male Albino Swiss mice were allocated into three groups (n = 6/group): a healthy control group (G1), a diabetic group fed bread containing 5% chia seeds (G2), and a diabetic group fed bread containing 15% chia seeds (G3). Diabetes was induced using alloxan administered intraperitoneally. Behavioral assessments included the Open Field Test, Head-Poking Test, and Negative Geotaxis Test. Brain tissues were collected for histopathological examination using hematoxylin and eosin staining. Diabetes induction was associated with reduced locomotor and exploratory activities and impaired neuromotor performance. Chia supplementation improved behavioral parameters in diabetic mice, with the 15% chia group showing greater improvement than the 5% chia group. Histopathological examination revealed marked neurodegenerative changes, inflammatory cell infiltration, and neuronal loss in diabetic animals. These alterations were reduced following chia supplementation, particularly in mice receiving the higher level of chia enrichment.The findings suggest that chia-enriched bread may contribute to improving behavioral performance and reducing diabetes-associated neurodegenerative changes in mice. These effects may be related to the antioxidant and anti-inflammatory properties of chia seeds. Further studies using larger sample sizes and additional diabetic control groups are required to confirm these observations.

Keywords: Chia seeds, Diabetes mellitus, Behavioral tests, Neuroprotection, Histopathology, Alloxan.

Introduction

Diabetes is a complex disorder characterized by chronic metabolic disorder and chronic high blood sugar, caused by an imbalance in the regulation of the metabolism of carbohydrates, fats and proteins. The disease is divided into two main types; the first type occurs as a result of the rapid deterioration of the function of the pancreatic beta-cells, and is sometimes common in children and adults. Type II often arises from insulin resistance by cells with a relative lack of insulin secretion, leading to high levels of

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fatty acids and inflammatory cytokines, decreased muscle glucose transport, increased liver production, and accelerated fat breakdown[5].

Diabetic peripheral neuropathy (DPN) is the most common and difficult to treat complication of diabetes. Studies have shown that nearly half of diabetic patients develop peripheral neuropathy, and this process often begins in the early stages of the disease, and the extent and speed of its development depends on several factors, including the patient's age, the number of years of diabetes, and the level of control of blood sugar level[38].

Synaptic plasticity dysfunction and signal transmission in the hippocampus, which leads to impaired learning and memory, is a complication of diabetes on the central nervous system such as neuropathy. The brain is particularly susceptible to oxidative damage due to its high rate of oxygen consumption, high production of free radicals, and high levels of transition metals, such as iron and copper, which stimulate the production of free radicals. In addition, the membranes of neurons are rich in polyunsaturated fatty acids, which are a source of fat oxidation. Abnormally high levels of free radicals, and the simultaneous decline of antioxidant defense mechanisms, can lead to damage to cellular organelles and enzymes, increased lipid oxidation, and the development of insulin resistance[1] and[21]

Several studies have indicated that mice with diabetes are exposed to oxidative stress, with an increase in biomarkers of oxidative stress in the liver, muscles, pancreatic cells, and brain. Oxidative stress contributes to neuroinflammation and excitatory toxicity, two of the main causes of neuronal death. Studies in mice have shown that feeding them a diet high in sucrose or fat increases anxiety-like behavior[13]

Alloxane (Pyrimidine-2,4,5,6(1H,3H)-tetraone) is a diabetic agent widely used in animal experimental studies, as it stimulates diabetes through selective targeting and destruction of pancreatic beta cells, acts as a toxic analogue to glucose, as it is very similar in structure to glucose, and shows toxicity towards pancreatic beta cells. In addition, it inhibits insulin secretion and promotes the production of free oxygen species (ROS), which cause cellular[7] and [14]

Chia seeds (Salvia hispanica L.) are rich in unsaturated fatty acids such as omega-3 fatty acids (linolenic acid, 54-67%) and omega-6 (linoleic acid, 12-21%) and are poor in saturated fatty acids. It also contains proteins (15-24%), dietary fiber (18-30%), carbohydrates (26-41%), and minerals (4-6%). The protein content of chia is higher than that of other conventional crops, such as wheat, corn, rice, and oats, and is rich in bioactive compounds with high antioxidant activity, such as sterols, tocopherols, carotenoids, and phenolic compounds, especially flavonol glycosides, chlorogenic acid, and caffeic acid[19].

As[15] pointed out that whole and ground chia seeds have strong antioxidant activity, which enhances antioxidant status and attenuates fat peroxidation, thus preventing diseases associated with oxidative stress. Chia seed powder also boosts antioxidant capacity, growth rate, and immune status, and reduces stress-related markers.

Effect of Diabetes on Behavioral Tests in Mice Diabetes is a chronic metabolic disease whose effects extend to the central nervous system, not just peripheral changes in glucose metabolism. Persistent hyperglycemia leads to a series of

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pathological disorders within the brain, most notably increased oxidative stress, activation of neuroinflammatory processes, and dysregulation of neurotransmitters, which negatively affects the efficiency of the functioning of neurons. Recent evidence suggests that insulin resistance in the brain is a pivotal factor in the deterioration of cognitive and behavioral functions associated with diabetes [24]. The Open Field Test is widely used to measure motor activity, exploratory behavior, and anxiety level, as animals with diabetes show a decrease in general mobility with an increase in anxiety indicators, reflecting the negative impact of diabetes on neurobehavioral function[16] The head pocking test is an important behavioral test for assessing exploratory motivation and anxiety level, as the decrease in the number of head pocking is interpreted as a reflection of increased anxiety and decreased exploratory behavior, as a result of neurological changes associated with metabolic disorders such as diabetes[25] and[27]

In contrast, the Negative Geotaxis Test is used to assess the integrity of neuromotor function and the animal's ability to respond motorically to gravity. Studies suggest that metabolic disorders, including diabetes, may lead to poor performance on this test due to their effect on neuromotor balance and increased oxidative stress that is reflected in nervous system efficiency[36,28] Overall, scientific evidence shows that diabetes induces behavioral and neurological changes that can be accurately measured using multiple behavioral models, such as the open-field test, the head insertion test, and the negative protractor test, making these tests reliable tools for understanding the neural mechanisms underlying the behavioral effects of diabetes in animal models[9] and [35]

Materials and methods

Sample Preparation

In this experiment, 18 male Albino Swiss rats aged 3 weeks old, who were raised in the animal house of the Faculty of Veterinary Medicine at the University of Mosul, were used. The groups were randomly selected and the experiment was conducted under tight laboratory conditions represented by the number of hours of light and the provision of food and water during the 24 hours throughout the experiment period, and the experiment lasted four weeks. The amount of meal consumed by the mice was calculated at the rate of (5-7) g/day, the weights of these mice ranged between 20 to 25 g, and the approximation of the weights of the mice was taken into account in one experiment.

Ethical considerations

All experimental procedures were carried out in accordance with the International Guidelines for the Use of Laboratory Animals, and in accordance with the instructions of the Scientific Research Ethics Committee at the [Faculty of Veterinary Medicine/University of Mosul]. All necessary precautions were taken to reduce pain and stress during the execution of the experiment.

Experience Design

The mice were divided into 3 groups with 6 mice for each group, and the division was as follows:- Group I G1:- Adopted as a control group. Group II: G2: The diabetic group fed on chia seeds 5% Group III: 15% of the diabetic group fed with chia seeds Mellitus type 2 diabetes was introduced in mice for G2 and G3 groups by dosing them with the

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monohydrate alloxan. The final dose was based on the live weight of the animal to ensure biological efficiency and avoid acute toxicity, as the animals were given an injection dose of aloxane by intraperitoneal injection (IP) after an appropriate fasting period. Due to the biphasic effect of alloxane, which in its first phase causes acute breakdown of pancreatic beta cells followed by a sudden release of large amounts of insulin and the accompanying sharp drop in blood glucose level (hypoglycemia), the animals were fed a 5% glucose solution in drinking water for 24 continuous hours immediately after injection, in order to avoid hypoglycemic shock and reduce mortality during the diabetic development phase, depending on previous studies [3]and[32]

Diet Preparation (Chia Bread) The chia seeds were ground by an electric grinder, and the bread was prepared according to the method mentioned by[2] with some changes, with a weight of 150 grams per loaf, where the mixture consisted of (ground chia seeds, dry bread yeast, salt, and kneading the mixture by hand by adding water until reaching a good dough consistency, and the sample was left to ferment for 30 minutes at room temperature (2+25 °C) and baked on a sheet sheet at a temperature of (400 ± 10) °C for 3-5 minutes, and as shown in the picture (1), the bread was left at room temperature and exposed to air for 2-3 days until the bread dries for the purpose of grinding it for later use in feeding mice by mixing it with laboratory feed at a rate of 5% for group G2 and 15% for group G3.

Image(1) Chia seed bread

Behavioral Tests for Mice

Measurement of motor activity in the open field In this test,

squares crossed test:

and the number of times it stood on the rear legs were calculated in 3 minutes, the mouse to be measured was placed in the center of the test box, and the number of squares it crossed with its four legs and the number of times it stood on its hind legs was calculated according to the method mentioned by[22]

Figure 2 shows the number of squares crossed by a mouse in 3 minutes.

head pocking holes test:

This test shows as shown in Figure (3) the curiosity and movement of the lab-grown mouse, this test was performed using a circular wooden surface, the measurement started by placing the mouse in the center of the perforated circular surface and then according to the number of times the mouse inserted its head into the holes during 3 minutes[27]

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Figure 3: The entry of the mouse's head into the holes

Negative geotaxis

This test shows neuromuscular balance and vestibular function in mice, and the test is performed using a rough wooden surface with an angle of 45 degrees, where the mouse's head is placed under the wooden board longitudinally, and then the time required for the mouse to rotate its entire body by 180 degrees towards the top of the inclined surface, and each mouse is given a maximum of 60 seconds to fully rotate it [37]


Figure 4: Negative Tropism Test

Organ extraction and preservation

Brain extraction

The skull of the mice was opened after they were anesthetized and killed, and surgical instruments were used according to the method developed by them[32] for the purpose of extracting the entire brain, and then the brains were placed in special laboratory boxes with formalin at a concentration of 10% until their measurements were made.


Image (5) Extracting the brain from the head of a mouse

Steps of tissue cutting

Histological Preparations

The tissue sections were prepared according to the method mentioned [34] Sample Fixation: The samples to be studied were stabilized in the brain after being removed with formalin, and after 48 hours, the sample was extracted from and washed several times with water.

Dehydration: Samples were passed through the Ethyl Alcohol concentration series for two hours each concentration for the purpose of gradually drawing out the water inside the tissue.

Clearing: Samples were soaked with Xylene solution twice for an hour at a time to make the samples more transparent and remove the drying solution.

Infiltration: After the completion of infiltration, the samples were immersed in molten paraffin wax and xylene in a ratio of 1:1 to an electric furnace at 50-60 °C∘ and then transferred to other bottles containing molten paraffin wax.

Embedding: The samples were buried in special iron molds with paraffin wax and left

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at laboratory temperature to harden and then separated from the mold and preserved until the time of cutting.

Trimming and Sectioning: The wax molds containing the models are trimmed and then the wax mold is fixed for the purpose of cutting in the rotary microtome and cut to a thickness of 5 micrometers, then the cut strips are transferred to a water bath with a temperature of (45 °C∘) to ensure the texture is well brushed, then the tissue sections are loaded onto clean glass strips and the strips are left to dry on a hot plate at a temperature of 37 °C for an hour and then left at laboratory temperature for the next day.

Staining: Harris and Hematoxylin dye were used according to the following steps adopted[34], and Eosin stain were prepared according to the method adopted by Suvarna et al., 2018. The strips were stained using hematoxylin stain - Eosin based on the method mentioned by[34]

Mounting: The loading material Distrine Plasticizer Xylene D.P.X was used to install the glass slide covers, then the glass slats were left at laboratory temperature for 24 hours to dry after which they were examined with a light microscope.

Microscopic examination and lrh'u photomicrography: Glass slats were examined to identify changes in the studied textilepieces using a light microscope with a digital Canon computer connected to a computer under 40X and 20X 3.

Histological morphomtry: Histological measurements of the slices prepared from brain tissue were recorded by Ocular micrometer (OM) under the 20X power installed in the light microscope. Statistical Analysis The statistical analysis was performed according to the method (Cary, 2012) where the data analysis was performed using the random design CRD by SPSS analysis software, and as the different letters indicate that there are significant differences between the averages according to Duncan's test at the level of ($P \leq 0.05$)

Results and discussion:

Behavioral Tests: Activity Tests Open field

Cross Square Test Table (1) indicates the number of squares cut by the experimental mice within 3 minutes for each control group and after the introduction of the disease and the two feeding groups on the chia seed bakery by 5% and 15%, where it is clear from the table that there is a significant decrease in the number of squares cut for the groups in which diabetes was developed compared to the control group, and this shows the effect of diabetes on neurological indicators in mice with induced diabetes compared to the control group, and these results are consistent with what Sadikan found. Table 1 also shows that there are significant differences after feeding with chia seed baked if the number of squares cut for the baked chia seeds groups is observed compared to the control group, and the baked group with a replacement rate of 15% was significantly superior t
o both the control group and the feeding group on the 5% substitution, which shows the role of chia seeds in treating or stopping the deterioration of neurological markers in mice when fed to them, and this may be due to the richness of chia seeds in antioxidants and omega acids that reduce oxidative stress, and this is consistent with what [15]found.

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Table (1) Cross square test for 5 and 15% diabetes groups before and after feeding with chia seeds in 3 minutes

Treatments	Before Feeding With Chia Bread	After Diabetes Induction	After Feeding With Chia Bread
G1	102.8 ± 1.923 c	-	102.8 ± 1.483 c
G2	103.0 ± 2.345 c	62.2 ± 3.114 d	134.2 ± 4.147 b
G3	104.4 ± 2.302 c	66.4 ± 4.505 d	140.0 ± 6.123 a

*The numbers represent an average of three repeats
*The difference of letters vertically and horizontally indicates the existence of significant differences at the level of significance of 0.05

Test the number of times you stand (Raring in the open field)

Table (2) shows the number of times the mice stood in the open field within 3 minutes for each control group and after the development of diabetes for the 5% and 15% group, where it is clear from the table that there was a significant decrease in the number of standing times for the 5 and 15% diabetic groups before feeding with chia, and this shows the effect of diabetes on neurological indicators in mice and feeling anxious, and these results are consistent with [11] as well as the table shows that there are significant differences for the 5 and 15% groups that were fed on a baked food If it is observed from Table 2 that the number of times standing in the open field increases, this indicates that chia seeds have the ability to improve brain function and cognition, and this may be due to the fact that they contain a high percentage of fatty acids, including omega acids, as pointed out by[23]

Table 2: Rearing test for 5 and 15% diabetes groups before and after feeding with chia seeds in 3 minutes

Treatments	Before Feeding With Chia Bread	After Diabetes Induction	After Feeding With Chia Bread
G1	19.4 ± 3.361 c	-	19.8 ± 1.643 c
G2	18.8 ± 1.303 c	4.4 ± 1.140 d	37.0 ± 2.828 a
G3	19.6 ± 0.547 c	4.4 ± 0.547 d	33.0 ± 2.828 b

*The numbers represent an average of three repeats
*The difference of letters vertically and horizontally indicates the existence of significant differences at the level of significance of 0.05.

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Head poking test

Table 3 shows the number of times the mouse head was inserted into the holes for both the control group and the groups of 5 and 15% chia seeds, and it is observed from the table that the number of times the mouse head was inserted into the holes after the development of diabetes, and this shows the effect of diabetes that leads to neurological and behavioral disorders that include decreased exploratory activity and an increase in anxiety-like behavior as a result of oxidative stress, neuroinflammation and central neurotransmission disorder, which is reflected in a clear decrease in the performance of the animals within the exploratory behavior tests This is consistent with what [31]found, and the table shows that there was a significant improvement in the group fed chia baked chia by 15%, and this may be due to the fact that chia seeds contain active bioactive compounds such as fatty acids, omega-3 acids, polyphenols, dietary fiber, and antioxidants, which have shown the ability to improve glucose metabolism and reduce oxidative stress and inflammation in diabetic animal models. Reducing neuroinflammation and improving behavioral and cognitive function [18]. Accordingly, it can be hypothesized that giving chia seeds may contribute to improving exploratory behavior and increasing the frequency of head insertions into holes in diabetic animals by reducing the negative neurological effects associated with the disease[4].

Table (3) Head poking test of control mice and groups 5 and 15% in 3 minutes

Treatments	Before Feeding With Chia Bread	After Diabetes Induction	After Feeding With Chia Bread
G1	33.2 ± 1.303 c	-	32.4 ± 1.341 c
G2	33.4 ± 1.140 c	11.4 ± 2.302 d	31.4 ± 2.073 c
G3	42.2 ± 1.483 b	10.6 ± 1.341 d	48.4 ± 8.018 a

*The numbers represent an average of three repeats

*The difference of letters vertically and horizontally indicates the existence of significant differences at the level of significance of 0.05.

Negative geotaxis test

Table (4) shows the negative geotaxis test for both the control group and the 5 and 15% groups of chia seeds, which is performed within 60 seconds to calculate the time required for the mouse to rotate on the oblique surface, where it is clear from the table that there are significant differences between the control group and the 5 and 15% chia groups, as the time required for the mouse head rotation increased after the development of diabetes for both groups 5 and 15%, due to the effect of diabetes on neurological markers and the brain, and this is consistent with what[31] said. The table also shows that there was a significant improvement after feeding on the chia baked goods for both groups compared to the control group if the time required for the mice to turn around was reduced, and this improvement was also evident compared to the groups before feeding with chia.

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Table (4) Negative geotaxis test for control and groups 5 and 15% within 60 seconds

Treatments	Before Feeding With Chia Bread	After Diabetes Induction	After Feeding With Chia Bread
G1	5.8 ± 1.303 b	-	5.6 ± 1.140 b
G2	5.6 ± 1.140 b	10.0 ± 1.309 a	3.1 ± 0.578 c
G3	6.4 ± 1.140 b	9.7 ± 1.439 a	3.1 ± 0.228 c

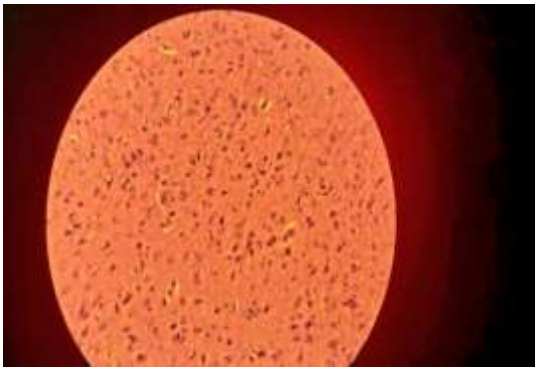
*The numbers represent an average of three repeats
*The difference of letters vertically and horizontally indicates the existence of significant differences at the level of significance of 0.05.

Histopathological examination

After the histological cutting of brain tissue for both the control group and the 5 and 15% groups fed chia seed bakery.

G1 Group Histopathological examination

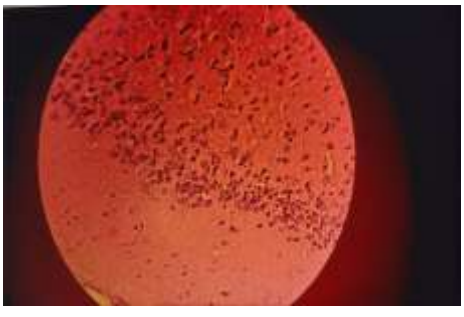
Figure 1 shows the brain tissue of the control group that did not have diabetes and did not feed on chia seed bakery. This group was used for the purpose of comparison with the 5 and 15% groups that were fed chia seed bakery, where the image showed that there was no loss of nerve cells, no necrosis in the nerve cells, no nerve gaps, no inflammatory cell infiltration, and no glial fibrosis, which indicates the safety of the mice in the experiment.



Image(1) Brain tissue of the control group

Histological anatomy of groups G2 and G3 after diabetic events and before chia feeding

Figure 2 shows the brain segment of a diabetic mouse where the result of alloxane is exposed to severe neurodegenerative changes characterized by noticeable deterioration and loss of neurons, the formation of extensive gaps in the neural tissue and around neurons, vascular congestion, and reactive glial cell proliferation, with multifocal neuronecrosis, indicating advanced brain damage caused by toxicity or oxidative stress



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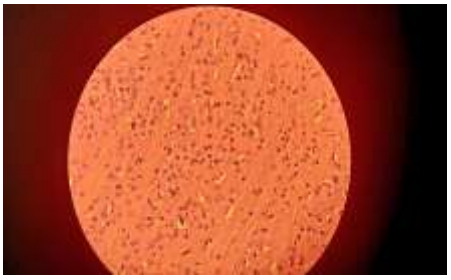
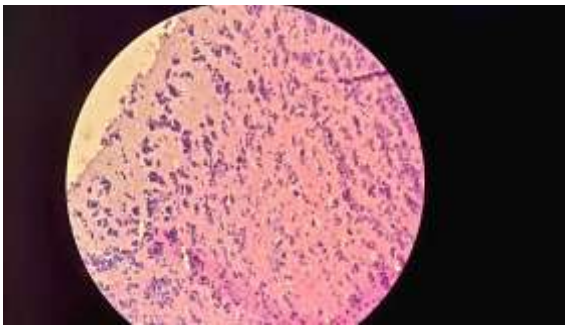
Image(2) Brain tissue of a diabetic mouse before feeding on 5% chia bread

Figure 3 shows a slight improvement in brain tissue after feeding on 5% peptides chia seed bread, and the study showed that chia seeds are a rich source of high-value protein and essential amino acids, in addition to containing bioactive properties that have antioxidant, anti-inflammatory, hypertension and diabetes properties. The review also noted that the degradation of chia proteins produces peptide compounds capable of inhibiting oxidative stress, reducing cellular damage, and improving metabolic response. The study also showed that regular consumption of chia seeds may contribute to the prevention of chronic diseases, including diabetes, cardiovascular disorders, and neurological disorders associated with oxidative stress, as a result of their omega-3 fatty acids, dietary fiber, and bioactive phenolic compounds, which is consistent with[12]



Image(3) Brain tissue of a diabetic mouse after feeding on 5% chia bread

Histological anatomy of groups G2 and G3 after diabetic events and after chia feeding Brain segments showed marked inflammatory cell infiltration accompanied by neurodegeneration and localized loss of neurons (H&DX40 dye) as shown from Figure 4. Whereas, the observed histological changes are dense infiltration of inflammatory cells in the brain tissue, the majority of which are mononuclear cells and noticeable neurodegeneration, where many neurons exhibit shrunken cell bodies and condensed nuclei with localized loss of neurons, leading to irregular cellular distribution. Disturbance of normal tissue structure as well as mild to moderate glial fibrosis is observed in adjacent areas with the presence of minor to moderate nerve gaps.



Image(4) Brain tissue with auxan-induced diabetes before feeding on 15% sheba bread

Figure 5 shows brain segments of diabetic mice after being fed 15% chia seeds, where mild to moderate neurodegeneration is observed, with scattered neurons showing shrinking cell bodies and slightly dense nuclei and a slight decrease in the density of neurons compared to normal tissues with small gaps in the nerve tissue, indicating early or healing degenerative changes, as well as slight infiltration of inflammatory cells. From these results, we conclude the protective effect to the high content of chia seeds of unsaturated fatty acids, especially omega-3 acids – omega-3 acids and antioxidants play an important role in promoting tissue repair processes by reducing cell membrane damage, stimulating cell turnover, improving blood perfusion and reducing inflammatory infiltration. Therefore, histological studies in chia-treated animals have shown a reduction in the severity of cytodegeneration and the preservation of the overall structure of the

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tissues compared to the untreated infected groups.[6] It is known that diabetes leads to increased production of reactive oxygen species (ROS) and the consequent cellular damage and disruption of the integrity of neurons, which is reflected in the form of neurodegeneration, loss of nerve cells, and the appearance of tissue gaps[30] as it shows a slight neurodegeneration with small gaps in the nerve tissue, while maintaining the overall structure of the brain. The beneficial effects observed following chia supplementation may be attributed to its high content of omega-3 fatty acids, polyphenols, dietary fiber, and antioxidant compounds. Previous studies have demonstrated that chia seeds can reduce oxidative stress, attenuate inflammatory responses, and improve metabolic regulation, all of which may contribute to the preservation of neuronal integrity and behavioral function in diabetic conditions. Furthermore, the greater improvement observed in the 15% chia group suggests a possible dose-dependent response, although further investigations are needed to confirm this relationship.

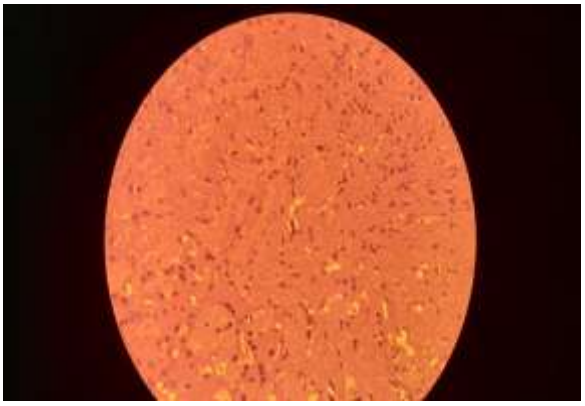

Figure 5: Brain tissue of a diabetic mouse after feeding with chia bread 15%

Study Limitations

This study has several limitations that should be considered when interpreting the findings. First, the sample size was relatively small (n = 18), which may limit the statistical power of the analysis. Second, a diabetic untreated control group was not included, making it difficult to distinguish the specific contribution of chia supplementation from potential spontaneous recovery effects. Third, biochemical markers of oxidative stress, inflammation, and glycemic control were not evaluated. Therefore, future studies incorporating larger sample sizes, diabetic control groups, and mechanistic biomarkers are recommended to further validate the observed neurobehavioral and histopathological effects of chia supplementation. The observed improvement in behavioral performance and brain histopathological features following chia supplementation may be explained by the unique nutritional composition of chia seeds. Chia seeds are rich in omega-3 fatty acids, particularly α-linolenic acid, as well as polyphenols, dietary fiber, and antioxidant compounds. These bioactive constituents have been reported to reduce oxidative stress and inflammatory responses, which are considered major contributors to diabetes-induced neuronal damage. Previous studies have demonstrated that oxidative stress plays a central role in the development of neurodegeneration and behavioral impairments associated with diabetes. Therefore, the reduction in neuronal degeneration and inflammatory cell infiltration observed in the present study may be attributed to the antioxidant and anti-inflammatory properties of chia seeds. Furthermore, the superior performance of the 15% chia group compared with the 5% group suggests a possible dose-dependent protective effect. These findings are in agreement with previous reports that highlighted the beneficial role of chia seeds in improving metabolic status, reducing

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oxidative damage, and preserving neural tissue integrity.

Proposed Mechanisms of Chia Neuroprotection

The present findings are consistent with those reported by [15], who demonstrated that chia seed oil alleviated neurobehavioral disturbances through antioxidant and anti-inflammatory mechanisms. Similarly, [12]reported that chia-derived bioactive peptides possess protective biological activities that may contribute to the prevention of chronic metabolic and neurological disorders. The histopathological improvements observed in the current study further support the hypothesis that chia supplementation may help attenuate diabetes-associated neural damage and improve behavioral outcomes

Conclusion

The present study demonstrated that alloxan-induced diabetes was associated with impaired locomotor activity, reduced exploratory behavior, and histopathological alterations in brain tissue. Dietary supplementation with chia-enriched bread was associated with improvements in behavioral performance and attenuation of diabetes-related neurodegenerative changes. The beneficial effects observed in the 15% chia group were generally greater than those observed in the 5% chia group. These findings suggest that chia seeds may provide neuroprotective benefits through mechanisms related to their antioxidant and anti-inflammatory properties. However, further investigations involving larger experimental populations and appropriate diabetic control groups are necessary to confirm these findings and clarify the underlying mechanisms.

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