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Research Article

ZOOLOGY

Effect of flaxseed different forms on hematological alteration in Streptozotocin induced diabetic rats

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KEY WORDS

Hematological parameters, Type 2-diabetes, Streptozotocin, Flaxseed

ABSTRACT

Diabetes significantly affected hematological parameters of the experimental rats. Recently, flaxseed is used in different forms such as powder or oil as a natural supplement which may improve diabetic patient health. High-fat diets with specific doses of streptozotocin STZ can induce type 2 diabetes (T2-DM). The aim of this study was to explore the effects of flaxseed forms as a powder and oil on various hematological parameters in STZ induced type 2 diabetes in comparing with those with control group and diabetic group. The study was conducted on 24 albino rats (weight ranging from 150–180 gm) were divided into four groups (N= 6): (Gp1) served as control, Gp2 was diabetic rats, Gp3 served as diabetic rats treated with flaxseed powder 10%, Gp4 was diabetic rats treated with flaxseed oil 4%. After 90 days of treatment, hematological parameters such as red blood cell counts (RBCs), hemoglobin, hematocrit (Hct%), platelet count, and white blood cell counts (WBCs) were examined. Compared to the Gp1, RBC indices were lower in Gp2, but they were in the same range as control in Gp3 and Gp4. Total WBC and neutrophils percentage were increased while lymphocytes were decreased in the diabetic group compared to the control. Also, the platelets counts were increased in diabetic rats compared with control but reduced to normal range in Gp3 and Gp4. In conclusion, flaxseed powder and oil may reverse hematological abnormalities in T2-DM rats and could be effective in preventing diabetes complications such as anemia.

Introduction

Hyperglycemia and abnormalities in insulin secretion are the features of a set of metabolic disorders known as diabetes mellitus (DM). Diabetes risk factors include genetics, unhealthy diets, sedentary lifestyles, obesity, stress, glucose tolerance disorder, and high blood pressure **(Wang et al., 2021)**. In those with diabetes, chronic hyperglycemia is linked to long-term harm and dysfunction of many organs, especially eyes, kidneys, heart, and blood cells which ultimately leads to various diabetic problems **(Al Shehri, 2017; Association, 2013)**. These concerns raise the risk of morbidity and mortality while also affecting patients' quality of life **(Saeedi et al., 2020).** Despite advances in medical technology and extensive studies, diabetes is still a chronic metabolic disorder that is difficult to manage. As a result, preventing problems from occurring, controlling their progression, and improving quality of life are now the main goals of diabetes diagnosis and treatment **(Chatterjee et al., 2017)**.

In diabetic patients, hematological parameters are frequently tested. The majority of blood cells, known as red blood cells (RBCs), are thought to be glucose-consuming. When there is continuous hyperglycemia, the shape, function, and metabolism of erythrocytes are usually at risk for a number of changes, which also impact hemorheology **(Zhou et al., 2018).** RBCs count, Hematocrit, Hemoglobin, platelets, and other related indices such as Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), and Mean Corpuscular Hemoglobin Concentration (MCHC) are used to detect the deformability and hemorrhagic status of red blood cells through quantitative and qualitative investigation of red blood cell characteristics **(Farooqui et al., 2019)**.

Flax is a crop grown for food and fiber. Omega-3 fatty acids, such as alpha-linolenic acid, and dietary fiber are both abundant in flaxseeds. Flaxseeds are significant for human health due to their composition and known health benefits **(Draganescu et al., 2021)**. Alpha-linolenic acid (ALA), lignans, and dietary fiber found in large quantities in flaxseed make it a useful food **(Xi et al., 2022)**. One of the first crops to be cultivated, flaxseed is still prized for its ability to provide food, fiber, and oil as well as for its possible health benefits **(Park, 2014)**. Flaxseed's nutritional profile includes roughly 39% oil, 18% protein, and 30% total dietary fiber **(Carter, 1993)**. The oil comprises 15-18% linoleic, 20% oleic, 6% palmitic, and 4% stearic acids in addition to 51-55% of one of omega 3 fatty acid form which is ALA **(Shim et al., 2014)**. These flaxseed constituents have a variety of health-promoting effects, such as antidiabetic, anti-inflammatory, antiviral, antioxidant, and anti-obesity properties, as well as a reduced risk of some tumors **(Goyal et al., 2014)**.

This study aimed to determine the effects of flaxseed oil and powder on the hematological markers in diabetic rats induced by a high-fat diet (HFD) followed by STZ to trigger their diabetes. As the relationship between consuming flax and hematological indices in diabetes not clearly indicated, the current study was conducted to ascertain whether flaxseed could reverse hematological abnormalities brought on by DM.

Material and Methods

Experimental animals

Male albino rats weighing (150–180 gm) aged between 8-10 weeks old were supplied by animal husbandry, Alexandria University. Animals were kept in cages (6 per cage), with a 12 H/12 H dark/light cycle, in laboratory temperatures, and fed with a high-fat diet for about four weeks after two weeks of acclimatization. It has been proposed that the HFD may be a more effective strategy to start developing the insulin resistance, which is one of the key characteristics of type 2 diabetes. All research on animals was done in Department of Zoology's laboratories, Faculty of Science, Tanta University. Rats were handled in accordance with the ethical standards endorsed by the animal care and use committee.

Diabetic rats

Prior to the streptozotocin (STZ) injection, the rats were starved for 12 hours. The intraperitoneal (i.p.) administration of a single dosage of STZ (35 mg/kg BM) freshly dissolved in 0.1M citrate buffer (pH = 4.5), where 15 minutes resulted in the development of T2DM (**Azad et al., 2020**). The levels of fasting blood glucose (FBG) were measured on days 3 and 7 after injection, which confirmed hyperglycemia. In this investigation, the rats with FBG levels greater than 130 mg/dl were classified as having T2DM.

Experimental design

The experimental animals were divided into four groups (N= 6), and was conducted for 90 days.

Group 1: Control group

Group 2: Diabetic group

Group 3: Diabetic rats treated with flaxseed oil (4%/kg) **(Abduljawad, 2019)**.

Group 4: Diabetic rats treated with flaxseeds powder (10%/kg) **(Abduljawad, 2019).**

Experimental diets and chemicals

Flaxseeds and flaxseed oil were purchased from IMTENAN health shop, Egypt. Streptozotocin was purchased from Sigma-Aldrich. The flaxseeds were weighed and crushed in a blender to obtain the flour and used freshly before feeding time.

Regular diet consisting of 5% fat, 53% carbohydrate, 23% protein, while high-fat diet consisting of 22% fat (saturated) , 48% carbohydrate, and 20% protein.

Blood Sampling

Within 24 hours of the final treatment, blood samples were taken from the rats' abdominal aorta and placed in sterile test tubes with EDTA.

Hematological Evaluation

An automated hematology analyzer was used to calculate the hematological indices, including red blood cells (RBCs), hemoglobin (Hb), hematocrit (Hct), mean corpuscular volume(MCV), mean corpuscular Hb(MCH), mean corpuscular Hb concentration(MCHC), red cell distribution width (RDW), platelet count, , white blood cells (WBCs), lymphocytes, and neutrophils.

For the quantitative measurement of hemoglobin A1c in human blood, the Finecare TM HbA1c Rapid Quantitative Test is a fluorescence immunoassay used in conjunction with the Finecare TM FIA System. The test is used to help individuals with diabetes mellitus track their long-term glycemic state.

Statistical analysis

All statistical calculations were made using SAS 9.4 software. Standard error of the means (SEM) is used to express data. A one-way ANOVA test and Duncan's multiple range test post hoc analysis were used for all statistical comparisons. P value less than 0.05 was regarded as significant.

Ethical approval

The Ethics Committee approved the study protocol from the Faculty of Science, Tanta University, Egypt with No. IACUC-SCI-TU-0314

Results

Effects on hematological parameters (Red Blood Cell Indices)

The hematological parameters of control, diabetic group, and diabetic rats treated with flaxseed oil and powder during a 12-week period are shown in Table (1). The diabetic group showed a significant decrease in Hb concentrations, RBCs, and HCT levels by comparison with the nondiabetic group. MCV, MCH, and MCHC values did not show significance between groups. Diabetic rats treated with flaxseed powder and oil showed a significant increase in RBC count, Hb concentrations, and HCT levels compared to the diabetic group. On the other hand, the RDW exhibited a significant increase ($p<0.05$) in the diabetic group (18.1±0.30), compared to the control group (15.6 ±0.15) while there is a significant decrease resulting from flaxseed powder supplementation (16.9±0.20).

Platelet counts were higher in diabetic rats (1124.5±56.50) compared to normal control (447±65.7) but were reduced considerably upon treatment with flaxseed powder (441.7±63.67) and flaxseed oil (650.0±79.58) (Fig. 1).

Effects on White Blood Cell Count and Differentials

Regarding white blood cell count, the present study showed a significant increase ($P < 0.05$) in the diabetic group when compared with the control group and the other treated ones (Table 2). Flaxseed powder and oil addition to the diet in the diabetic rats recover the WBCs values back to the normal range and significantly increased when compared to the diabetic group. The diabetic rats showed non-significant change ($P > 0.05$) in the percentage of eosinophils and monocyte however, there was a significant increase ($P < 0.01$) in neutrophils. On the other hand, lymphocytes showed a significant decrease ($P < 0.01$) when compared with the normal control group throughout the experimental period. Both flaxseed powder and oil supplementation significantly reversed these parameters to be in the normal ranges.

Effects on Plasma Glycated Hemoglobin Concentration (HbA1c)

Figure (2) shows the values of HbA1c in; control, diabetic group, and diabetic rats treated with flaxseed powder and flaxseed oil over the 12 week period. Diabetic controls showed a significant increase in HbA1c concentration by comparison with control ($p < 0:05$). The administration of flaxseed oil and powder significantly decreased HbA1c concentration (4.1 and 4.3%, respectively) compared with the diabetic controls ($p < 0:05$).

Table (1): Effect of flaxseed powder and oil on RBCs and related parameters. P values represent one-way ANOVA of different treatments (Control, Diabetic, Flaxseed powder, and Flaxseed oil), while means with different superscripted letters at the same row are significantly different at $p \leq 0.05$, A > B > C according to Duncan's multiple range test.

Parameters	Control	Diabetic	Flaxseed powder	Flaxseed oil	P value
RBCs (x 10^6/µl)	7.1^{A} ±0.31	6.6^{B}±0.40	7.7^{A}±0.24	7.9^{A}±0.15	0.0314
Hemoglobin (g/dl)	14.2^{A} ±0.72	12.1^{B}±0.50	14.3^{A}±0.46	14.5^{A}±0.26	0.0390
HCT (%)	42.3^{A} ±1.54	36.5^{B}±1.45	42.2^{A}±1.26	42.4^{A}±0.55	0.0310
MCV (fl)	53.1^{A} ±0.73	55.3^{A}±0.90	54.8^{A}±0.54	53.6^{A}±1.59	0.8502
MCH (pg)	17.6^{A} ±0.55	18.3^{A}±0.25	18.5^{A}±0.15	18.4^{A}±0.68	0.9939
MCHC (g/dl)	33.5^{A} ±0.35	33.7^{A}±0.10	33.8^{A}±0.21	34.1^{A}±0.41	0.4621
RDW (%)	15.6^{B} ±0.15	18.1^{A}±0.30	16.9^{B}±0.20	17.3^{BA}±0.30	0.0489

Table (2): Effect of flaxseed powder and oil on WBCs and differentials. P values represent one-way ANOVA of different treatments (Control, Diabetic, Flaxseed powder, and Flaxseed oil), while means with different superscripted letters at the same row are significantly different at $p \leq 0.05$, A > B > C according to Duncan's multiple range test.

Parameters	Control	Diabetic	Flaxseed powder	Flaxseed oil	P value
WBCs (x 10^3/µl)	7.3^{B} ±1.86	8.1^{A}±1.25	7.5^{B}±1.54	7.2^{B}±1.56	0.0497
Neutrophil (%)	25.1^{B} ±3.72	52.5^{A}±7.50	20.5^{B}±3.27	15.3^{B}±4.33	0.0024
Lymphocytes (%)	76.27^{A} 5.37	37.0^{B}±7.00	70.0^{A}±3.75	74.7^{A}±4.10	0.0032
Monocytes (%)	6.87^{A} ±0.45	9.5^{A}±0.50	7.7^{A}±0.71	8.0^{A}±0.58	0.3672
Eosinophils (%)	2.1^{A} ±0.51	1.0^{A}±0.00	1.8^{A}±0.40	2.0^{A}±0.00	0.3737

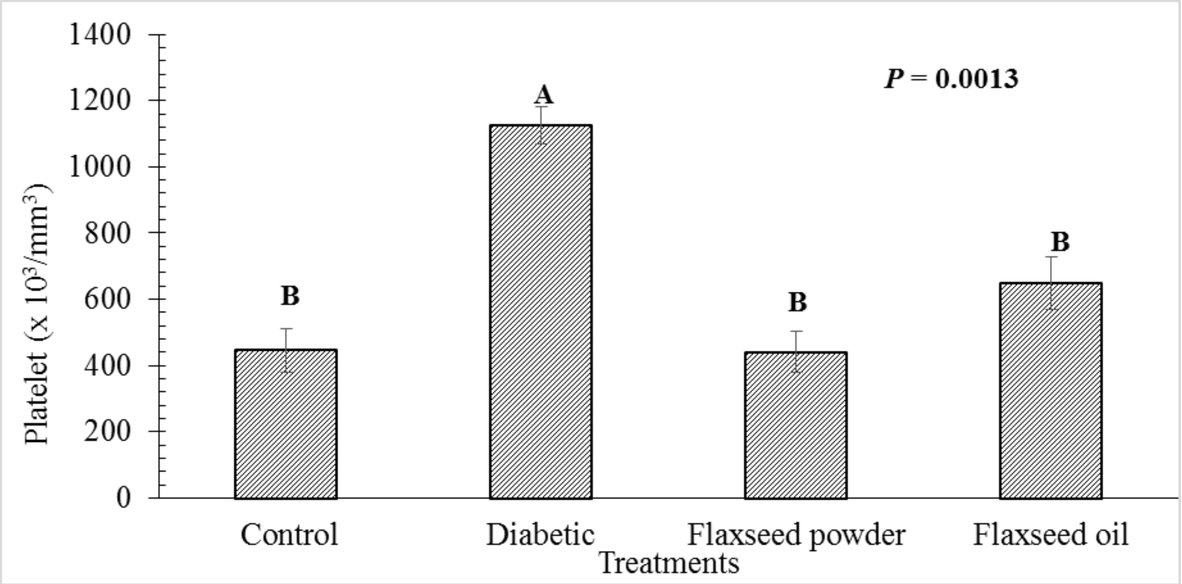

Fig. (1): Effect of flaxseed powder and oil on platelets counts (x $10^3/mm^3$). P values represent one-way ANOVA of different treatments (Control, Diabetic, Flaxseed powder, and Flaxseed oil), while means with different letters are significantly different at $p \leq 0.05$, a > b > c according to Duncan's multiple range test

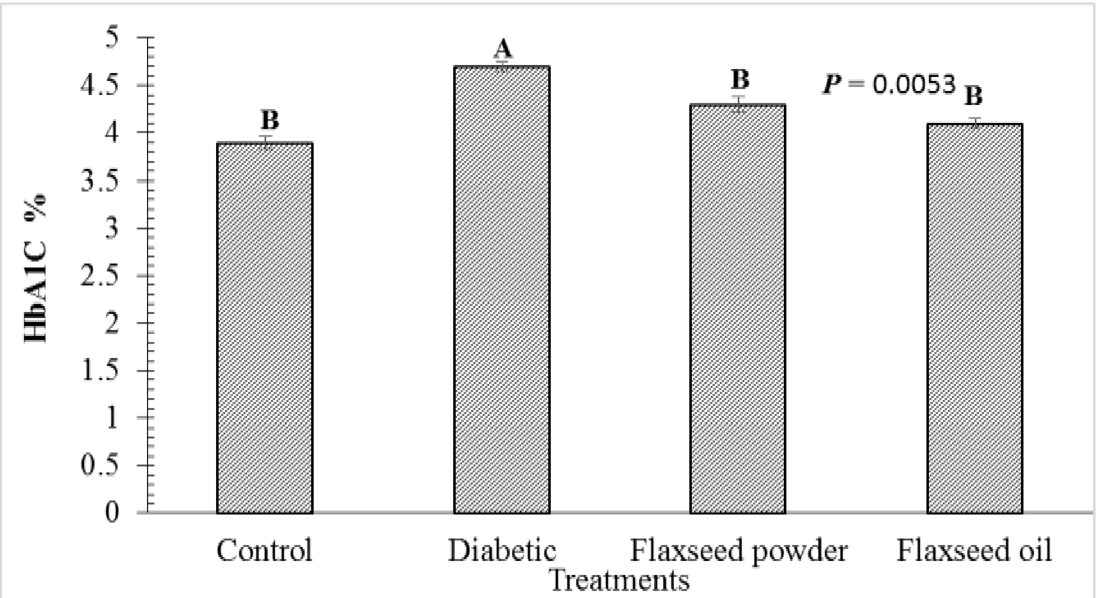

Fig. (2): Effect of flaxseed powder and oil on HbA1C %. P values represent one-way ANOVA of different treatments (Control, Diabetic, Flaxseed powder, and Flaxseed oil), while means with different letters are significantly different at $p \leq 0.05$, a > b > c according to Duncan's multiple range test

Discussion

The results of this study revealed that there is a considered relationship between diabetes and hematological indices that need to be clarified. Hematological measurements provide information on the physiological states of blood cells and type 2 diabetes mellitus may cause abnormalities in these parameters. The structure and function of erythrocytes are disturbed by persistent hyperglycemia, and several hematological measures can be used to diagnose and prevent diabetes and its consequences **(Wang et al., 2021)**. This study found that the indices (RBC, WBC, and platelet indices) were changed in STZ diabetic control rats compared to normal rats, which is consistent with other study findings **(Essiet et al., 2020)**. STZ is commonly used to create diabetic animal models that are imitators of human diabetes **(Fırat et al., 2012)**.

The current study is a step to assess and update the impact of flaxseed in different forms (powder and oil) on some hematological indices and the related parameters in relation to diabetes. Flaxseeds have a significant impact on human health due to their composition and well-known beneficial effects **(Draganescu et al., 2021)**. According to the results of the current study, when using flaxseed powder or oil, most of the hematological indices return to normal. In other words, treatment with flaxseed oil and powder helped to reverse a general decline in all hematological parameters in diabetic rats. Flaxseed powder values in most parameters seem to be closer to nondiabetic control values which may give it more advantage than the oil. This may be due to the high content of soluble fiber and lignans, which contain phytoestrogens that are present in the flaxseed powder and absent in oil. Moreover, Flaxseeds are considered a whole food while flaxseed oil is not. Hence, flaxseed powder has a wider range of vitamins and natural nutrients than flaxseed oil, it has far greater health advantages.

The current research showed that while the RBC count, Hb concentration, and Hct values were lower in the diabetic group than in the control, they were higher in the flaxseed-treated diabetic rats than in the untreated diabetic ones. The declines were suggested to happen most likely due to RBC membrane structural changes brought on by an excess of ROS produced in diabetes **(Tanko et al., 2011)**. These results are suggested to caused due to anemia which may possibly be explained by RBC destruction and a slower rate of release from the bone marrow into the blood. A similar study showed a significant decrease in the mean of RBC count, Hb, HCT, and MCV of diabetics when compared to non-diabetic individuals, they suggested that diabetic patients with poor control are more likely to have anemia **(Farooqui et al., 2019)**. The number of erythrocytes can be used to track the effects of drugs or treatments for blood problems. Anemia, also known as a low erythrocyte count, is a condition in which there are fewer oxygen-carrying cells in the blood **(Wang et al., 2021).** Patients with T2DM had decreased hemoglobin values, according to **(Al Salhen & Mahmoud, 2017)** as they suggested that low hemoglobin concentration may contribute to numerous clinical features of diabetes or its progression including anemia. Another study proposed that hemoglobin glycosylation which commonly occurs in diabetics may result in low Hb concentration **(Gerner et al., 2013)**. Related outcomes displayed that the mean values of hemoglobin, and RBC were significantly decreased in the diabetic group compared to the control group. As opposed to the nondiabetic group, the red cell distribution width dramatically increased in the diabetic group versus the control group **(Al Shehri, 2017)**.

Erythrocyte size heterogeneity is referred to as RBC volume distribution width (RDW). Greater heterogeneity in MCV is reflected by higher RDW values, which are typically brought on by disturbances in erythrocyte maturation or degradation. Increases in RDW suggest that the erythrocyte size is inconsistent **(Wang et al., 2021)**. Similar results showed that RDW was positively related to HbA1c and the occurrence of diabetic retinopathy **(Blaslov et al., 2019)**. The RBC count report produced by a hematology analyzer contains the test findings for RDW. Therefore, RDW can be widely used for the diagnosis of diabetes because it is an affordable, practical, and minimally intrusive testing approach **(Kor et al., 2018)**.

Platelets count is known to play an important role in wound healing, hemostasis, as well as the pathogenesis of many inflammatory diseases, and it may be considered from the indicators of diabetic diagnosis. The study's results demonstrated that the diabetes group had higher mean platelet values, than the control group. This rise could be a result of platelet activation. The present findings are in agreement with prior results which have revealed that patients with diabetes mellitus show altered platelet function including high platelet counts **(Al Shehri, 2017)**. Another study also yielded higher platelet indices for diabetic controls compared to normal rats **(Essiet et al., 2020)**.

The total WBCs count in the diabetic group revealed a significant increase. A traditional indicator of inflammation is an elevated WBC count which is linked to type 2 diabetes mellitus. This is confirmed by another study which showed that patients with hyperglycemia had dramatically changed total and differential leukocyte counts **(Al Salhen & Mahmoud, 2017)**. Additionally, the finding of a recent study presented that the WBCs count is considerably higher in the diabetic group compared to the control **(Kheradmand et al., 2021)**. Also a higher WBC count indicates an increased risk of T2DM according to **(Park et al., 2021)** who suggested that WBC count may help in identifying people who are likely to develop type 2 diabetes.

In this study, groups treated with flaxseed oil and powder had higher WBC counts, higher neutrophil percentages, and lower lymphocyte counts as these values turned back to normal compared to a nondiabetic group, which may have been caused by changes in immunological parameters brought about by these supplements during and after treatment. This suggests that using flaxseed oil and powder to treat the condition affects the body's defenses and immune system. Accordingly, administration of flaxseed in dietary routine may aid to lessen lifestyle related dysfunctions **(Afzal et al., 2020)**.

In the diabetic group, lymphocytes decreased in number significantly. Since lymphocytes are crucial for maintaining the body's immune system and defense, the drop in their numbers could be a reaction to the stress brought on by the disease. Since lymphocytes are responsible for achieving the body's defense system, this could be a reaction to stressful situations, or it could be caused by the production of specific or nonspecific antibodies against various antigens **(El Feki et al., 1997)**. Regarding increased neutrophils which recorded in this study particularly in diabetic group compared to other groups, it was in accordance with other finding which suggested that as diabetics are more susceptible to infection and that neutrophils participate in phagocytic processes against diverse antigens, it is possible that the increase in neutrophils is related to this **(Hawkins et al., 2007)**.

Glycosylated hemoglobin (HbA1c) is one of the nonenzymatic glycosylation productions of Hb which displays the mean blood glucose level over the prior two to three months. Clinically, HbA1c is frequently employed as a key diabetes diagnostic marker. Glucose binds to hemoglobin (Hb) in erythrocytes as blood glucose concentration rises. Once

HbA1c is created, it is difficult to break down. Higher HbA1c concentrations make it more difficult for the body to release oxygen into cells and impair the ability of erythrocytes to carry it **(Association, 2012)**. Hence, HbA1c is a useful indicator for preventing and managing diabetic complications as a result. When paired with other erythrocyte markers **(Bianchetti et al., 2021)**. In this study, results indicated that the supplementation of flaxseed to diabetic STZ rats reduced the glycosylation of hemoglobin in diabetic rats. The current results are in agreement with another study which indicated that flaxseed administration produced promising modifications in glycosylated hemoglobin in the treated diabetic rats **(Draganescu et al., 2021)**. Additionally, recent findings showed that persons with T2DM who took flaxseed supplements had significantly lower hemoglobin A1c (HbA1c) levels than those in the control group **(Xi et al., 2022)**.

Conclusion

Type 2 Diabetes is a severe public health issue that may result in hematological abnormalities and cause long-term problems or poor quality of life. As a result, it's imperative to closely examine and evaluate hematological markers in diabetes individuals.

The current study shows how flaxseed forms affect the hematology of streptozotocin STZ-induced diabetic rats. It is concluded that in a diabetic rat model, hematological parameters including RBC, Hb, and Hct are greatly lowered and exert additional disorders in the related indices. According to these findings, flaxseed oil and powder may be able to treat male albino rats who have developed diabetes after being given the HFD/STZ drug. As RBC, WBC, and platelet indices improved, and serving as indicators of the benefits that happened by flaxseed by reversing verse the values to the normal. Hematological parameters can be used to track the development of diabetes and associated complications as these parameters can sense blood glucose changes early.

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تأثير الصور المختلفه من بذور الكتان على تغيرات الدم في الفئران المصابة بالسكر والمستحثه بماده الستربتوزوتوسين

منة الله منير عبدالله حربي

قسم علم الحيوان – كليه العلوم – جامعه طنطا

تتأثر القياسات الخاصة بالدم بشكل كبير بمرض السكري. في الآونة الأخيرة ، يتم استخدام بذور الكتان في أشكال مختلفة مثل المسحوق أو الزيت كمكمل طبيعي قد يحسن صحة مرضى السكري. يمكن استحداث نموذج مرض السكر من النوع الثانى عن طريق اعطاء الفئران أنظمة الغذائية عالية الدهون مع حقن جرعات محددة من الستربتوزوتوسين(STZ) . الهدف من هذه الدراسة هو استكشاف تأثير أشكال بذور الكتان المختلفة كمسحوق أوزيت على قياسات الدم المختلفة في مرض السكري من النوع ٢ الذي يسببه STZ ومقارنتها مع الأشخاص غير المصابين بالسكري (المجموعة الضابطة) ومجموعة مرضى السكري غير المعالجة. أجريت الدراسة على ٢٤ جرذًا ألبينو (وزن يتراوح بين ١٥٠-١٨٠ جرامًا) تم تقسيمها عشوائيًا إلى أربع مجموعات ، ستة في كل مجموعة: المجموعة الأولى كمجموعة ضابطة غير مصابة بالسكري ، والثانية: الجرذان المصابة بداء السكري ، والثالثة: الفئران المصابة بداء السكري المعالجة ببذور الكتان. المسحوق الرابع: الجرذان المصابة بداء السكري المعالجة بزيت بذور الكتان. تم عمل قياسات الدم بما في ذلك: عدد كرات الدم الحمراء، الهيموجلوبين ، Hct٪ ، عدد الصفائح الدموية ، تعداد كريات الدم البيضاء الكلية والتفاضلية. بعد ٩٠ يومًا من العلاج أظهرت نتائج الدراسة انخفاض مؤشرات RBC في مجموعة مرضى السكري مقارنة بالمجموعة الغير مصابة (C) ولكنها سجلت معدلات طبيعية في الفئران المصابة بداء السكري المعالجة ببذور و زيت الكتان مقارنة بمجموعة المصابة بالسكر كما وجد زيادة فى مجموع WBC بينما انخفضت الخلايا الليمفاوية في المجموعة الثانية المصابة بالسكر مقارنة بـا لمجموعة الضابطة .أيضا ازداد عدد الصفائح الدموية في المجموعة الثانية مقارنة مع المجموعة الاولى ولكن انخفضت فى المجموعات الاخرى التى تلقت العلاج ببذور و زيت الكتان. في الختام ، أظهرت الدراسة أنه يمكن أن يعالج مسحوق بذور الكتان أو زيت بذر الكتان الخلل الذى حدث فى قياسات الدم في الفئران البيضاء المصابة بداء السكري من النوع ٢ وبالتالى قد تكون مفيدة في منع مضاعفات مرض السكري مثل مرض فقر الدم.