Efficacy of Avena Sativa on Regulating Some Biochemical Parameters in Type 2 Diabetic Male Albino Rats

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Abstract

Background: Avena sativa has been recognized as a healthy and nutritious cereal, containing a high concentration of dietary fiber and dense nutrients. Many scientific research studies linked oats as a healthy diet in the fight against Type 2 Diabetes Mellitus (T2DM).
Objective(s): This study aims to assess the proximate analysis of *Avena Sativa* (oats) cultivated in Egypt to evaluate its main nutrient content, and study its effect on blood glucose homeostasis, lipid profile and antioxidant enzymes in type 2 diabetic rats.
Methods: Proximate analysis was measured in oats using standard methods. A total of fifty rats grouped into 10 control rats and four groups of 40 diabetic rats were included in the study. The four diabetic groups were classified into the diabetic control group, and the three experimental groups one of them treated with 200 mg/kg Metformin, the other fed on oat grains only as the main diet and the last one fed on oat grains and treated with metformin.
Results: Oats are rich in dietary fibers, fat, and protein (9.3, 8.75, and 14.12% respectively). Treatment with metformin and oats showed an improvement in blood glucose homeostasis including FBG, insulin, and HOMA-IR (127, 12.44, and 3.92 respectively). Lipid profile showed a statistical change among all studied groups. The lowest decrease in lipid profile (LDL and TC) was in the combined group (79.56 and 151.4 respectively).
Conclusion: Chemical analysis of oats revealed that it's a good source of the main nutrients, containing protein and fibers compared to standard pellets. In addition, it has a promoting healthy effect on blood levels of fast glucose, insulin, Homeostasis Model Assessment for Insulin Resistance (HOMA- IR), low-density lipoprotein (LDL), high-density lipoprotein (HDL), total cholesterol (TC), and malondialdehyde (MDA). Oats revealed a modulatory curative effect on the diseased liver and pancreatic tissues.
Keywords: Oat grains, diabetic rats, glucose homeostasis, lipid profile, liver, pancreas

INTRODUCTION

Type 2 diabetes mellitus T2DM is defined as a complex heterogeneous group of metabolic disorders described by increased levels of blood glucose level owing to deficiency in insulin action and/or insulin secretion.(1) About 90–95% of all types of diabetes are type 2 diabetes mellitus (T2DM), and about 20% of the population over the age of 65 has T2DM. About 5–10% of the total health care budget has been used for T2DM in many countries. T2DM may lead to severe consequences, including kidney failure, blindness, slow wound healing, and arterial diseases.(2) Diabetics usually suffer from high oxidative stress due to persistent and chronic hyperglycemia, which there by reduces the activity of anti-oxidative defense system and thus promotes free radicals' generation. Free radicals are formed excessively in diabetes by oxidation of glucose, oxidative degradation of glycated proteins, and non-enzymatic glycation of protein. These free radicals damage the pancreatic β-cells and induce insulin resistance.(3)

Accumulation of reactive oxygen species (ROS) leads to oxidative stress, which is associated with damage to β-cells and biomolecules. Potential damage to biological molecules, cell membranes, cellular lipids, inflammation, β-cells destruction, and cell

death are mediated by reactive species through direct reaction. Because of the undesirable side effect from medications in type2 DM patients, much interest is focused on the use of natural compounds as a supplement in our dietary life style, which may provide benefits in terms of minimizing the adverse side effects.[4]

High dietary fibers diet increases the sensitivity of peripheral tissues to both endogenous and exogenous insulin. Such diet improves glucose tolerance and lowers the level of serum insulin. In addition, the regulation of carbohydrate releasing could assist in the reduction of cholesterol and saturated fatty acids in the diabetic diet.[5] Dietary fibers help to control the blood glucose level; the intake of dietary fiber is inversely associated with insulin resistance, which supports evidence that say "a high intake of dietary fiber is associated with enhanced insulin sensitivity. Cereals are recognizable as a major source of healthy dietary fibers (soluble and insoluble fibers), the grown area are estimated to be over 73% of the total global harvested zone. Insoluble fibers helps in the prevention and management of type 2 diabetes, which challenge the traditional theory, that solubility and viscosity are the main powerful factors for these beneficial effects of dietary fibers.[6]

Avena sativa (Oats) are unique as one of the richest sources of dietary fibers among cereals; Oats provide more protein, dietary fibers, fat, minerals, B-complex, vitamins, iron and zinc than other whole grains. Oats are said to be unique among cereals as they are therapeutically active against diabetes than other grains such as wheat and rice. Oats considered a very balanced nutritional food item, rich with both soluble and insoluble dietary fibers. The most famous oats dietary soluble fibers are the β -glucan, soluble β-glucans make viscous, shear thinning solutions even at low concentrations. This viscosity is strongly dependent on its concentration. β-glucan usually comprises 3.6–5.1 % of dry weight of the oat grains.[7]

Avena sativa (Oats) is a rich source with various oxidative components such as phenolic compounds (avenanthramides), vitamin E (tocopherols and tocotrienols), flavonoids, phytic acids, and sterols that protect its lipids from oxidation. The potential antioxidants activity of oats to scavenge free radicals and protect its source plant from oxidation is transferred to the human body when oats or other antioxidant-rich foods are consumed.[8] Therefore, our study was conducted to investigate the effect of oats as a main meal that is rich in dietary fibers in controlling blood glucose homeostasis in diabetic rats and improving other biological parameters in diabetic rats. In addition, studying its role in modulating the deformations occurred in liver and pancreas tissues because of induction of diabetes.

METHODS

The study was done in the central laboratories, High Institute of Public Health and animal facility of Faculty of Agriculture, Alexandria University. An experimental design was conducted.

Chemicals and drugs:
Streptozotocin (STZ) was purchased from Sigma Chemical Co. (USA). Metformin was obtained in the form of tablets manufactured by the Pharmaceutical Company Merck limited-Egypt, Egypt. Each tablet contains 500mg Metformin hydrochloride. Tablets were dissolved in distilled water. All other chemicals used in the present study were of analytical grade available from commercial sources.

High fat diet:
The animal fat used in this study was purchased from the local market. A standard compressed pellet (11.43% protein, 0.67% fiber, and 8.95% fat) was mixed with 40% melted lard to prepare a high fat diet.

Oat grains:
A total of ten kilograms of oat grains (*Avena Sativa species*) were used in this study. It was purchased from the National Research Centre of Dokki, Giza Governorate, Egypt, where it was cultivated. Oat grains were manually hulled then the crushed grains were passed into a sieve of 0.5 mm to exclude the outer husks, and then the hull-less oat was transferred into a dried clean container.

Chemical composition of oat grains
Proximate analysis of hulled oat grains including; carbohydrate, fat, protein, crude fibers, and moisture were conducted in the Central Laboratory of High Institute of Public Health, Alexandria University. All tests were done in triplicates.

Moisture percentage
Moisture percentage is the measurement of water evaporated at or near the boiling point. Moisture was measured according to the Association of Official Analysis Chemists (AOAC) method 990.19.[9]

Moisture (%) = (W1-W2) x 100 ÷ wt of sample in grams
Where
W1 = weight of crucible + sample prior to drying
W2 = weight of crucible + sample after drying

Ash percentage:
Ashing refers to the inorganic deposits lasting after complete ignition or oxidation at temperature of 500°C using muffle furnace. Organic substances are burned in the presence of oxygen to CO_2 and oxides of N_2 according to AOAC 900.02 A.[10]

Ash wt. % =

$$\frac{(wt.\ crucible\ and\ ash\ -\ wt.\ crucible)}{(wt.\ crucible\ and\ sample\ -\ wt.\ crucible)}\ X\ 100$$

Fat percentage
Soxhlet method was used for crude fat determination established by AOAC in method of 945.16 (2012).[11]
% Crude fat = weight of fat / weight of sample x 100

Crude protein percentage:
Oat grain was digested with sulfuric acid in the presence of catalysts. Total nitrogen was converted to ammonium sulfate. The digested solution was neutralized with alkali and distilled into boric acid solution. The borate anions formed were titrated with hydrochloric acid. The results of the analysis represent the nitrogen content of the food; since nitrogen also comes from non-protein components hence it should be multiplied by factor (5.83) to convert nitrogen content to crude protein. The used method was the semi-micro Kjeldahl method, which has been established by AOAC in methods 976.06, 976.05, and 960.52 (2012). [9]

Crude fiber percentage:
Crude fiber is the insoluble and oxidized organic residue remaining after sample treatment according to prescribed conditions. The most common conditions are consecutive treatments with light petroleum, boiling diluted sulfuric acid, boiling diluted sodium hydroxide, diluted hydrochloric acid, alcohol and ether. This empirical treatment provides a crude fiber consisting largely of the β-glucan, cellulose content together with a proportion of lignin and hemicellulose content of the sample according to AOAC by method number 991.43. [9]

Carbohydrate percentage:
Carbohydrate content was calculated by difference method according to AOAC using the following equation, [9] Carbohydrates (%) = 100 – (Protein % + Fat % + Ash% + Moisture % + Fiber %).

Experimental animal model
Animals:
A total of fifty healthy (which didn't show any signs of illness, appeared to have normal muscular activity and behavior together with normal adequate food and water intake) adult male albino rats of Wistar strain (*Rattus norvegicus*) weighing around 140 g (aged 6-8 weeks) were procured from the animal house in Faculty of Agriculture, Alexandria University, Egypt. Animals were housed in clean polyethylene cages (5 rats/ cage). Rats were housed in a well-ventilated animal facility and maintained in natural environment with a 12 h/12 h light and dark cycle at room temperature (22 – 27 °C). Animals were given a standard rodent diet and given distilled water *ad libitum*. Rates were acclimatized to the laboratory conditions for two weeks before commencement of the experiment. Male rats were selected because their growth potential is much higher than female rats and thus the biological impact of the high dietary fiber diet (oat grains) as manifested by the difference in the body weight could be identified.

Induction of Hyperglycemia:
To induce experimental diabetes, only 40 normal rats were fed on a high fat diet (60% standard rodent diet + 40% animal fat) for 4 weeks, then injected intra-peritoneal with low dose of Streptozotocin (STZ) (50 mg/kg) dissolved in citrate buffer (pH 4.4) for 3 consecutive days. During the first 73 hours of diabetes induction, STZ treated animals could drink 5% glucose solution to overcome drug induced hypoglycemia. Three days after STZ administration, diabetes was confirmed by the presence of hyperglycemia. Rats with fasting blood glucose level > 200 mg/dl were designated diabetic and were therefore included in the present study. Also, they were considered to be diabetic when glycosuria was present for 3 consecutive days.[12]

Experimental design:
The rats were randomly divided into five equal groups, ten animals each, and were divided into the following groups.
Group I: (Control group) rats were fed on standard rodent diet.
Group II: 10 diabetic rats were left without treatment fed on standard rodent diet.
Group III: 10 diabetic rats were fed on oat grains.
Group IV: 10 diabetic rats were fed on standard rodent diet + orally administered with Metformin (200 mg/ kg rats daily).[31]
Group V: 10 diabetic rats were fed on oat grains + Metformin drug (200 mg/ kg orally administered daily).[31]

Sample collection and processing:
Rats were fasted overnight and blood samples were collected at the end of the experimental period from aorta of euthanized rats by isoflurane inhalation > 5%. The separated serum was used for the biochemical analysis: fasting blood glucose, insulin levels and insulin resistance by homeostasis model assessment (HOMA – IR), serum lipid profile [total triglycerides, total cholesterol, low density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) levels], and serum malondialdehyde (MDA) concentration.

Measurement of body and organs weight of rats:
The body, liver and pancreas of all rats were measured to make sure that oat grains are nearly enough to meet

rats' macro-nutrient requirements. They were measured by an automatic balance (AND GX-600, Japan). Body weight was measured every week to see the effect of treatment on the animals. Adipose tissue was removed from the liver before weighing.

Biochemical Estimations:
Estimation of serum glucose concentration:
Glucose in serum was estimated by using enzymatic method according to Keilin and Hartree.[13]

Estimation of serum insulin levels:
The serum insulin was measured by RayBio Rat Insulin ELISA Kits, by using a standard curve method in detecting the actual insulin concentration in the serum samples.[13]

Estimation of HOMA-IR:
Insulin resistance index (IRI) was derived using the homeostasis model assessment (HOMA- IR) as follows:
IRI= fasting insulin (µIU/ml) × fasting glucose (mmol/L) / 22.5. [13]

Estimation of serum lipid profiles:
Moreover, serum triglycerides (TG), total cholesterol, high-density lipoproteins (HDL) and low-density lipoproteins (LDL) were assayed using the kit following the manufacturer's instructions of Bio-Systems.

Estimation of MDA levels:
Liver tissue homogenate was prepared using the spectrophotometric method according to the manufacturer's protocol of Biodiagnostic, Egypt. The malondialdehyde (MDA) content was assayed in the form of thiobarbituric acid-reactive substances (TBARS) in the liver according to the method described previously.[14]

Histopathological study:
To assess histological changes, the chosen organs (pancreas and liver) from the rats of all experimental groups were excised, saline-washed and fixed in 10% buffered-formalin at room temperature for 24 hours. Specimens were dehydrated using ascending ethanol concentrations, double cleared in xylene for one hour each. Tissue sections were cut, cleared, hydrated, and double stained with hematoxylin and eosin (H&E)[15] and then observed by light microscope.

Statistical analysis:
Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp). Qualitative data were described using numbers and percent. The Kolmogorov-Smirnov test was used to verify the normality of distribution. Quantitative data were described using range (minimum and maximum), mean, standard deviation (SD) and median. Significance of the obtained results was judged at the 5% level. ANOVA test was used for normally distributed quantitative variables, to compare between more than two groups, and Post Hoc test (Tukey) for pairwise comparisons.[16]

RESULTS

Chemical analysis of oat grains and rodent pellets:
Table 1 showed the chemical analysis of oat grains in comparison with the rodent pellets to assure that oats diet is nearly enough to meet rats' main nutrients requirements. The results revealed that oat is a rich source of protein, fat and carbohydrates (14.12, 8.75 and 53.22%) compared to rodent pellets (11.43, 1.95 and 73.36%) respectively. On other hand the analysis showed the high content of dietary fibers of used oats diet in comparison with the used rodent pellets; that could help in the present study to assess its different effect on the diabetic type 2 rats.

Table (1): Comparison between the chemical composition of oat grains and the standard rodent pellets

	Moisture %	Protein %	Fat %	Crude fiber %	Ash %	Carbohydrate %
Oat grains	10.20	14.12	8.75	9.33	4.38	53.22
Rodent pellets	11.94	11.43	8.95	0.67	0.65	66.36

Evaluation of body and organs weights:
Death was not observed in any of the experimental groups during the experimental period. Data presented in figure (1) reveal that the mean body weight of the experimental groups that fed on oat grains wasn't significantly changed after 4 weeks of treatment, while there was obvious decline in body weight in positive control group (II). Table (2) reveal that nearly no significant difference in the body organs weight between the different groups of the study, except a slightly significant difference in the mean liver weight in the groups treated with metformin, that was in comparison with the experimental untreated group.

Evaluation of serum Glucose, Insulin and HOMA-IR Levels:
Figures 2 (A, B, & C) illustrate that the lowest serum glucose level after fasting for 12 hours among the experimental groups, was in the group (V) fed on oats and treated with metformin (127.5 mg/dL ± 15.40), while the untreated experimental group (II) had the highest fasting blood glucose level (386.1 mg/dL ± 29.49). The lowest serum insulin level and HOMA-IR were found in treated experimental group with metformin and fed on oats (group V) (12.44 µIU/ml ± 0.33 and 3.92 ± 0.95) respectively, followed by clinical groups (13.52 µIU/ml± 0.26 and 5.24

± 0.22) respectively, compared to the control group (8.52 µIU/ml± 0.23 and 1.69 ± 0.04) respectively.

Evaluation of lipid profiles:

Table 3 revealed that there is a statistically significant (*p*<0.001) increase in LDL, and total cholesterol in all studied groups compared to control group (39.60, and 96.20 mg/dl) respectively. The highest percent decrease in LDL, and TC was observed in combined group in which rats treated with Metformin and fed on oats (35.79 and 19.55%) respectively, followed by rats fed on oats only (14.12, and 8.87%) respectively. HDL had the highest level in control group 40.3± 2.27 mg/dl followed by combined group 35± 2.11 mg/dl, while untreated experimental group (group II) had the lowest content 23.0 ± 2.83 mg/dl. It shown that some increasing in the level of triglycerides was remarked in the group fed on oats in comparison with other experimental groups.
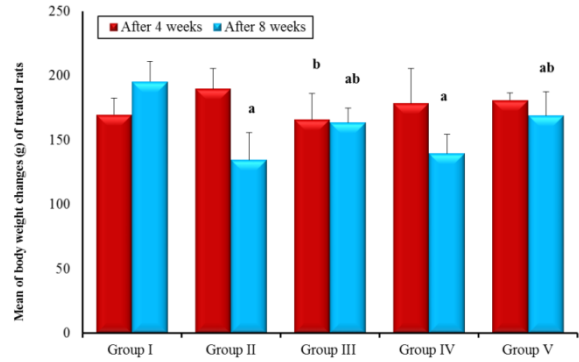

Figure (1):   **Body weight of different studied groups after 4 and 8 weeks**
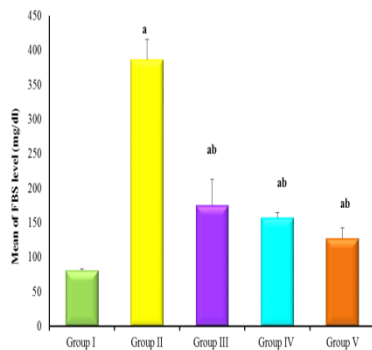
a: Significant with group I
b: Significant with group II

Table (2): Comparison between the different studied groups according to body organs weight (liver & pancreas) after 8 weeks

Body organs weight	Group I (n=10) Mean ± SD.	Group II (n=10) Mean ± SD.	Group III (n=10) Mean ± SD.	Group IV (n=10) Mean ± SD.	Group V (n=10) Mean ± SD.
Liver	5.67 ± 0.26	4.85 ± 0.79	5.38 ± 0.54	6.79 ± 1.19	6.53 ± 0.87
% of change 1		↓14.46	↓5.11	↑19.75	↑15.17
% of change 2			↑10.93	↑40.0	↑34.64
p_1		0.164	0.927	0.023*	0.129
p_2			0.574	<0.001*	<0.001*
Sig. between groups.			p_3=0.002*,p_4=0.019*,p_5=0.948		
Pancreas	0.29 ± 0.05	0.28 ± 0.05	0.33 ± 0.03	0.32 ± 0.06	0.28 ± 0.04
% of change 1		↓3.45	↑13.79	↑10.34	↓3.45
% of change 2			↑17.86	↑14.29	0.0
p_1		0.987	0.280	0.597	0.943
p_2			0.107	0.304	0.999
Sig. bet. grps.			p_3=0.981,p_4=0.061,p_5=0.196		

Pairwise comparison bet. each 2 groups was done using Post Hoc Test (Tukey) for ANOVA test
p_1: p value for comparing between each group and Group I
p_2: p value for comparing between each group and Group II
p_3: p value for comparing between Group III and Group IV
p_4: p value for comparing between Group III and Group V
p_5: p value for comparing between Group IV and Group V
*: Statistically significant at p ≤ 0.05

Figure(2A): Comparison between the different studied groups according to fasting blood glucose level (mg/dl) after 8 weeks of baseline
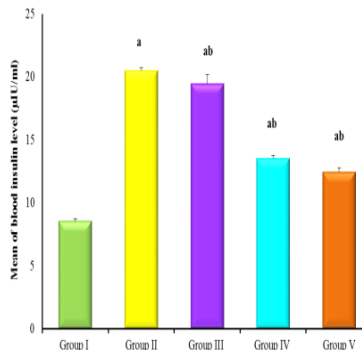a: Significant with group I
b: Significant with group II

Figure (2B): Comparison between the different studied groups in blood insulin level (µIU/ml) after 8 weeks of baseline
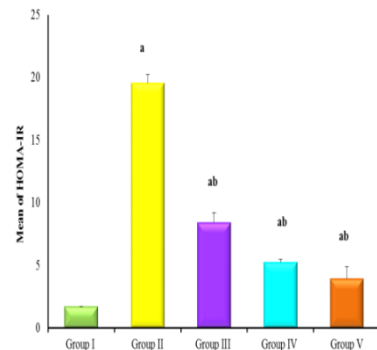a: Significant with group I
b: Significant with group II

Figure (2C): Comparison between the different studied groups according to blood HOMA level after 8 weeks of baseline
a: Significant with group I
b: Significant with group II

Table (3): Comparison between the different studied groups according to triglycerides, total cholesterol, HDL-cholesterol and LDL-cholesterol after 8 weeks

	Group I (n=10) Mean ± SD.	Group II (n=10) Mean ± SD.	Group III (n=10) Mean ± SD.	Group IV (n=10) Mean ± SD.	Group V (n=10) Mean ± SD.
Triglycerides (mg/dL)	81.50 ± 4.50	199.3 ± 7.41	206.7 ± 5.77	174.0 ± 7.51	184.2 ± 3.61
% of change 1		↑144.54	↑153.62	↑113.50	↑126.01
% of change 2			↑3.71	↓12.69	↓7.58
p_1		<0.001*	<0.001*	<0.001*	<0.001*
p_2			0.059	<0.001*	<0.001*
Sig. between groups.				P_3<0.001*,p_4<0.001*,p_5=0.004*	
Total Cholesterol (mg/dL)	96.20 ± 7.80	188.2 ± 6.94	171.0 ± 7.29	171.5 ± 8.06	151.4 ± 6.11
% of change 1		↑95.63	↑77.75	↑78.27	↑57.38
% of change 2			↓9.14	↓8.87	↓19.55
p_1		<0.001*	<0.001*	<0.001*	<0.001*
p_2			<0.001*	<0.001*	<0.001*
Sig. between groups.				P_3=1.000,p_4<0.001*,p_5<0.001*	
HDL-Cholesterol (mg/dL)	40.30 ± 5.27	23.0 ± 2.83	28.60 ± 1.71	30.30 ± 1.77	35.0 ± 2.11
% of change 1		↓42.93	↓29.03	↓24.81	↓13.15
% of change 2			↑24.35	↑31.74	↑52.17
p_1		<0.001*	<0.001*	<0.001*	0.003*
p_2			0.001*	<0.001*	<0.001*
Sig. between groups.				P_3=0.723,p_4<0.001*,p_5=0.010*	
LDL-Cholesterol (mg/dL)	39.60 ± 12.87	123.9 ± 8.13	101.1 ± 7.62	106.4 ± 8.89	79.56 ± 6.74
% of change 1		↑212.88	↑155.30	↑168.69	↑100.91
% of change 2			↓18.40	↓14.12	↓35.79
p_1		<0.001*	<0.001*	<0.001*	<0.001*
p_2			<0.001*	0.001*	<0.001*
Sig. bet. grps.				p_3=0.685,p_4<0.001*,p_5<0.001*	

Pairwise comparison between each 2 groups was done using Post Hoc Test (Tukey) for ANOVA test
p_1: p value for comparing between each group and Group I
p_2: p value for comparing between each group and Group II
p_3: p value for comparing between Group III and Group IV
p_4: p value for comparing between Group III and Group V
p_5: p value for comparing between Group IV and Group V
*: Statistically significant at $p \le 0.05$

Evaluation of MDA level:

Table (4) shows a statistically significant increase (*p*<0.001) in MDA concentration in all studied groups compared to control group. The highest MDA was observed in untreated experimental group (II) 6.05±0.25 nmol/ml, followed by clinical group 4.11± 0.12 nmol/ml, while the lowest concentration was obtained in combined experimental group 3.51± 0.14 nmol/ml.

Table (4): Comparison between the different studied groups according to mean blood MDA level after 8 weeks of baseline

	Group I (n=10) Mean ± SD.	Group II (n=10) Mean ± SD.	Group III (n=10) Mean ± SD.	Group IV (n=10) Mean ± SD.	Group V (n=10) Mean ± SD.
MDA (nmol/ml)	3.21 ± 0.05	6.05 ± 0.25	3.81 ± 0.12	4.11 ± 0.12	3.51 ± 0.14
% of change 1		↑88.47	↑18.69	↑28.04	↑9.35
% of change 2			↓37.02	↓32.07	↓41.98
p_1		<0.001*	<0.001*	<0.001*	<0.001*
p_2			<0.001*	<0.001*	<0.001*
Sig. bet. grps.				p_3<0.001*,p_4=0.001*,p_5<0.001*	

Pairwise comparison between groups was done using Post Hoc Test (Tukey) for ANOVA test
p_1: p value for comparing between each group and Group I
p_2: p value for comparing between each group and Group II
p_3: p value for comparing between Group III and Group IV
p_4: p value for comparing between Group III and Group V
p_5: p value for comparing between Group IV and Group V
*: Statistically significant at $p \le 0.05$

The Histopathological Examination:
Microscopic observations of pancreas:

In the present study, the histological appearance of the control pancreas "GI", revealed a typical pancreatic structure (acini and Islet of Langerhans). Thin connective tissue septa dividing the organ into lobules of variable sizes and shapes. Within the lobules, a compound acinar gland represented the exocrine portion of the pancreas. Each acinus consists of a single layer of pyramid-shaped cells with the narrow apical ends bordering the lumens and the broad basal portions resting on a basal lamina and scant reticular

stroma (Fig. 3A). The nucleus is located in the basal region of the acinar cell. Many of the acinar cells in rats have two nuclei. In normal rats, the acinar tissue adjacent to some islets of Langerhans has enlarged cells that stain more densely eosinophilic in hematoxylin- and eosin-stained sections. Pancreatic endocrine cells (Islet of Langerhan's) (round or oval-shaped cell mass) are scattered between the acini, the islets appeared lightly stained (appeared faintly stained with H&E) than the surrounding acinar cells, they are pale pink oval or rounded areas. The islet of Langerhans cells arranged in a trabecular and acinar pattern with abundant eosinophilic cytoplasm and central small nucleus, separated by thin loose connective tissue with thin vessels. The Islets of Langerhans have a large number of β- cells that have a normal round shape with well distinctly round nuclei (Fig.3A).

Light microscopic examination of pancreatic sections of untreated experimental group fed on rodent pellets "GII", revealed obvious alterations and deteriorations in pancreatic architecture (changes of both exocrine and endocrine components) (Fig. 3B). In addition, focal necrosis and dilated acini, Atrophy of the exocrine pancreatic acinar cells and diffusely basophilic necrotic areas.

The cytoplasm becomes disorganized, cellular membranes become indistinct; the cytoplasm becomes disorganized, cellular membranes become indistinct were detected. Regarding the islets appeared highly irregular shape, decreased in size, and β- cells are almost entirely lost and degenerated entering connective tissue sheet (Fig. 3C). Moreover, Microscopic examination of pancreatic sections of experimental untreated group, fed on oats (G III) showed no pathology with normal architecture similar to that of the control rats. In the present study, the pancreatic acini of this group appeared typically pyramidal cells with distinct cell margins and well-defined cytoplasm containing basal rounded nuclei. The islets of Langerhans are rounded or oval in the configuration (Fig.3D). In the present study, microscopic investigation of diabetic pancreatic sections (GIV) of rats in experimental group fed on rodent pellets in combination with metformin treatment were exhibited the normal appearance of acini and islets of Langerhans (Fig. 3E). Light micrographs of diabetic pancreatic sections of experimental group "GV", in which rats fed on oats in combination with metformin treatment, revealed normal architecture of the pancreatic tissue which was similar to that of the control group "GI".
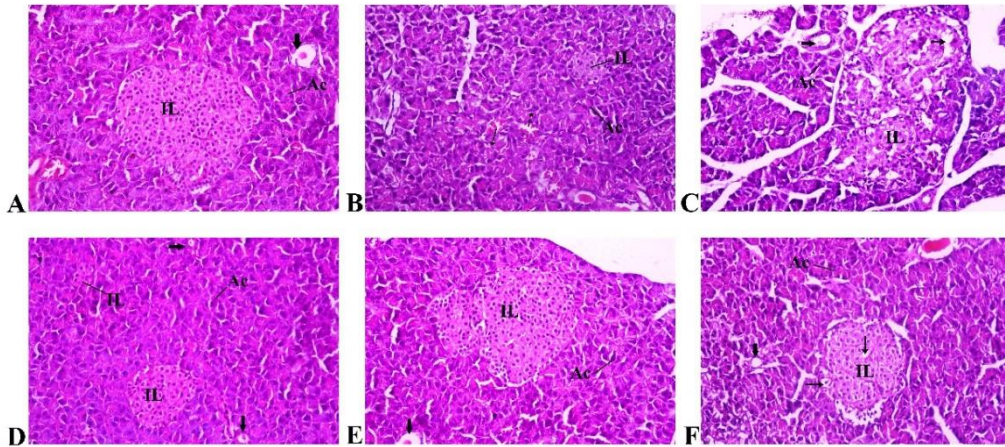

Figure (3): (A-F) Photomicrographs of the histological status of pancreatic tissue in rat stained by haematoxylin and eosin.

Microscopic observations of liver:

In the present study, the histological appearance of the control liver "GI" was investigated to offer an accurate study and better evaluation of any alterations which might occur in the liver of experimental animals. The basic structural component of the liver is hepatocyte radiated from the central vein as hepatic lobules. Each lobule consisted of regular cords of hepatocytes radiate outwards from a central vein to the periphery of the lobule. These cords of hepatocytes are separated by irregular sinusoids. Hepatocytes appeared large

polygonal cells have acidophilic cytoplasm and possess centrally located, one or more prominent round nucleoli. Regarding the nuclei of the hepatocytes, the micrographs revealed that the nuclei appeared spherical, central in position (Fig. 4A). Hepatocytes appeared large polygonal cells have acidophilic cytoplasm and possess centrally located, one or more prominent round nucleoli. The chromatins appeared as a thin layer of dense granular bodies along the nuclear membrane and as masses scattered in the

nucleoplasm (Fig. 4B). Additionally, Kupffer cells occurred at various points along the sinusoids. These cells presented a distinctive appearance frequently occupied a large part of the sinusoidal lumen. At the periphery of each hepatic lobule, the portal area of connective tissue presents in which branch of bile duct, branch of hepatic artery and branch of hepatic vein were seen (Fig. 4C).
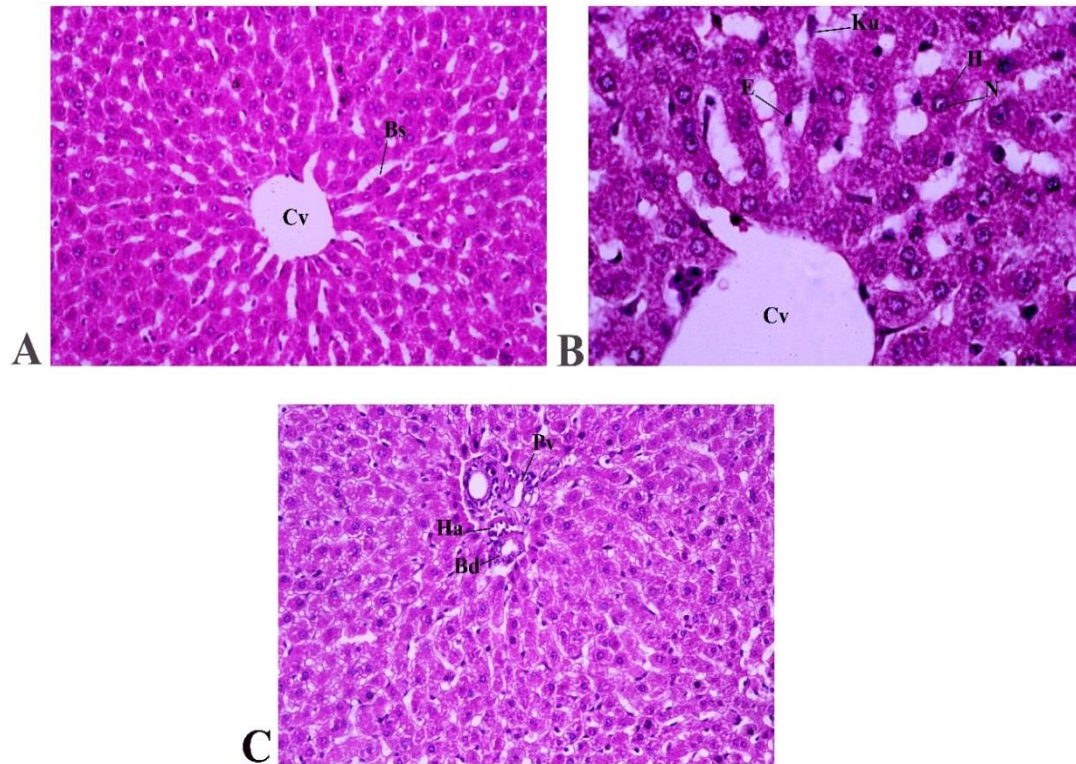

Figure (4): (A-C) Photomicrographs of the histological status of liver tissue of nondiabetic control rat (group I) stained by haematoxylin and eosin.

Light microscopic examination of liver sections of untreated experimental group fed on rodent pellets "GII" showed hepatocellular injury represented by the loss of the normal architecture of the live. The light microscopic evidence of cellular injury is loss of normal staining intensity of the cytoplasm of the hepatocytes. Besides, cellular pleomorphism, lost their typical shape and size were also notice. A marked dilation of most of the central veins was observed. Most of the hepatocytes displayed a marked degeneration characterized by highly vacuolated cytoplasm and pyknotic nuclei (condensed nuclei and intensely stained with hematoxylin). Some hepatocytes contained nuclei with variable size and shape and others contained binucleated ones (Figs. 5A&B).

Furthermore, examinations revealed that the diffusion and periportal inflammatory leucocytic infiltrations were seen. Most of the hepatocytes displayed a marked degeneration characterized by highly vacuolated cytoplasm and pyknotic nuclei. The nuclear changes were evident and the nuclei appeared as darkly stained compact masses of chromatin. Some hepatocytes contained nuclei with variable size and shape and others contained binucleated ones (Fig.5C). The portal areas were characterized by dilated and congested portal veins and numerous branches of bile ducts. In addition, the sinusoids, it showed abnormality in the margins architecture with activated Kupffer cells were observed (Fig. 5D). The histopathological changes were graded and summarized in (Table 5).

Table (5): Histopathological changes in liver tissues of all experimental groups

histopathological changes	control (GI)	Diabetic untreated (GII)	Diabetic liver + Oat grains (GIII)	Diabetic liver + metformin (GIV)	Diabetic liver + Oat grains + metformin (GV)
Irregular architecture	–	++	–	–	–
Central vein dilation	–	+++	–	–	–
Leukocytic infiltration	–	++	–	–	–
Pyknotic nuclei	–	++	+	+	–
necrotic changes	–	+	–	–	–
Portal vein dilation	–	++	–	–	–
Hepatic artery dilation	–	++	–	–	–
thickening of the wall of the blood vessels	–	+++	–	–	–

Severity of liver histological changes using scores on a scale of none (–), mild (+), moderate (++), and severe (+++) damage.
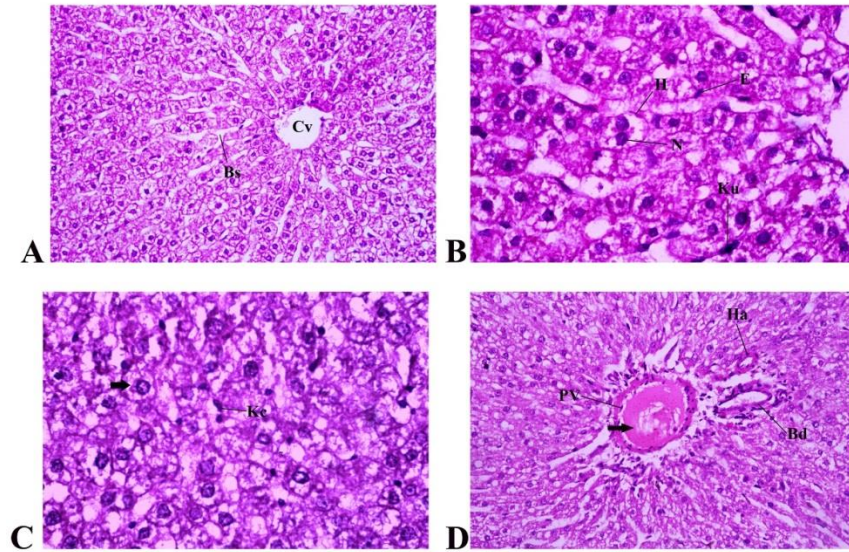

Figure (5): (A-D) Photomicrographs of the histological status of liver tissue of diabetic non-treated rats (group II) stained by haematoxylin and eosin.

In addition, microscopic examination of liver sections of fed on oats without metformin treatment (G III) revealed normal architecture of the liver tissue which was similar to that of the control rats. Concerning the hepatocytes, the hepatocytes of this group "III" appeared well stained, radiating normally around the central vein which appeared typically polygonal with distinct cell margins and well-defined cytoplasm (Figs. 6A&B).
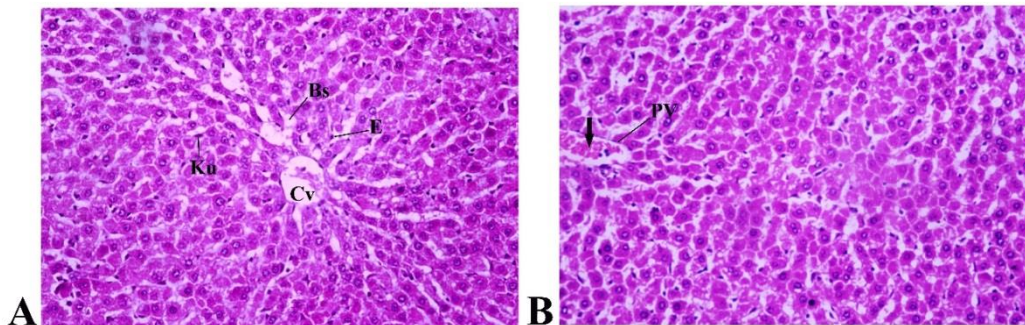

Figure (6): (A-B) Photomicrographs of the histological status of liver tissue of diabetic rats fed on oat (Group III) stained by haematoxylin and eosin.

Moreover, diabetic liver sections of group "G IV", of rats in which animals fed on rodent pellets + metformin 200 mg/Kg exhibited a marked recovery. The hepatocytes appeared normal in shape (polygonal) and size, with well-defined cytoplasm and distinct cells margins (cell membranes) (Figs. 7A&B) respectively. The histopathological changes were graded and summarized in table (4).
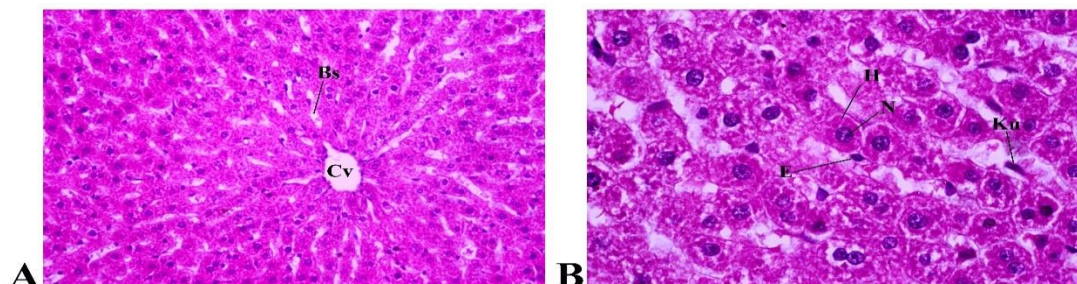

Figure (7): (A-B) Photomicrographs of the histological status of liver tissue of diabetic rats treated with metformin (group IV) stained by haematoxylin and eosin.

In the present study, Light micrographs of diabetic liver sections of group "GV", in the group fed on oats + metformin 200 mg/Kg". The hepatocytes appeared more or less normal in shape (polygonal) and size, with well-defined cytoplasm and distinct cells margins (cell membranes). Regarding the nuclei of the hepatocytes, the micrographs revealed that the nuclei appeared spherical, central in position and well stained violet (Fig. 8A). Moreover, the nuclei exhibited normal chromatin material dispersed within nucleoplasm and prominent centric and eccentric nucleoli. Nuclear membranes of the envelope appeared distinct and normal. Further observation, revealed considerable number of Kupffer with regular triangular centric nuclei and endothelial cells along the sinusoidal capillaries with intensely stained regular nuclei. Whereas, the sinusoids appeared normal (Fig. 8B).
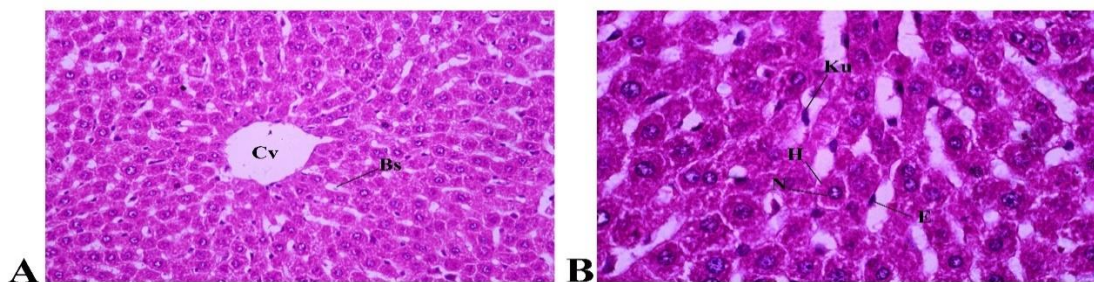

Figure (8): (A-B) Photomicrographs of the histological status of liver tissue of diabetic rats treated with metformin (group V) stained by haematoxylin and eosin.

DISCUSSION

Results of chemical analysis of oat grains in the present study revealed that it's a rich source of dietary fibers, fat, protein and carbohydrates (9.33, 8.75, 14.12 and 53.22%) respectively, which agreed with the study done by Rasane et al.,[17] who reported that the oat protein ranged from (11–15%), fat (5-9%), crude dietary fibers (2.3–8.5%) and carbohydrates are around (60%). On other hand the analysis confirmed the high dietary fibers content of oats in comparison with the rodent pellets (9.33, 0.67%) respectively; that helps in our study to assess its effect on the diabetic rats. The experimental rats that fed on oats for 4 weeks showed body weight maintenance in comparison with the groups fed on the rodent pellets. According to the researches this body improvement was due to the effect of dietary fibers of oats as a physical regulator of nutrients absorption in the body gut. This was in agreement with a study that confirmed the dietary fibers help in prolonging the gastric emptying time, and retarding the absorption of nutrients. Both are dependent on the physicochemical form of the dietary fibers, and in particular on its influence on digesta viscosity that produced a colloidal gel matrix in the intestinal lumen, causing a regulation of the nutrients absorption.[18]

The dietary fibers could efficiently adsorb glucose, delay glucose diffusion and its release from starch and inhibit the activity of α-amylase to different extents. These mechanisms results in decrease the postprandial serum glucose level.[19] These data agreed

with this study that illustrates the experimental group fed on oats and treated with metformin for 4 weeks has the lowest FBG level in comparison with the other experimental groups. On other hand the treatment with metformin appeared an additional positive effect on the FBG level. This was in agreement with the study of Kamenova (2020),[20] that confirmed the positive role of metformin in regulating the blood glucose level, as the metformin suppresses the hepatic gluconeogenesis and hepatic glucose production, increases glucose uptake and glucose utilization in peripheral tissues such as muscle and adipose tissue.

The study illustrate that the diabetic group fed on oats and treated with metformin for 4 weeks was the lowest serum insulin level 12.44 ± 0.33 in comparison with the experimental untreated group (group II) 20.52 ± 0.21, this was in line with the study of Marc P, it illustrated that the oat cereal fibers can reduce the occurrence of type 2 diabetes, the mechanism of action may be accomplished with a reduction in fasting blood insulin level. This occurs because oats have water-soluble gel-forming fibers such as β-glucan. These fibers produce a viscous solution in the small intestine, that decreases the contact and mixing of macronutrients with digestive enzymes, which in turn postponement the absorption of glucose, therefore reduces the insulin level.[21]

On other hand the study of Mbara et al. (2021)[22] illustrated the positive effect of metformin treatment, as it reduces the insulin requirements., which in the same line of this study, that showed the diabetic group fed on oat and treated with metformin for 4 weeks was the lowest serum insulin level in comparison with the other experimental groups.

Results of the present study illustrate that the experimental rats fed on oats and treated with metformin for 4 weeks had the lowest HOMA IR value 3.92 ± 0.95 in comparison with the other experimental groups. This was in line with the study of Ma et al. (2013),[23] who revealed that the oat intake significantly reduced the HOMA-IR value in comparison with the base line. Also, the result was in agreement with the study of Bahriz et al. (2020),[24] illustrated that the all-treated rats with metformin resulted in decrease in HOMA-IR index.

The lowest levels of TC and LDL-C in the study were shown in the experimental groups fed on oat, which were in comparison with the other groups that fed on rodent pellets. This was in agreement with the study done Li et al. (2020)[6] it revealed that administration of oats to the diabetic type 2 rats induced highly significant decreasing in the serum TC and LDL-cholesterol levels in comparison with the untreated experimental group, attributed this result to the high dietary fibers content of the oat grains.

With concern of the blood HDL-cholesterol level (the good Cholesterol part), the results show that the highest HDL-cholesterol level between the experimental groups was in the group that fed on oat grains. That is may be due to the effect of the high dietary fibers of the oat that regulate the nutrients absorption in the body gut, where low dietary glycemic index and low glycemic load are associated with a higher concentration of plasma HDL.[25]

The present study showed that triglyceride was slightly high in the experimental groups fed on the oats compared to the other groups fed on the rodent pellets. This result attributed to the high oil content of the oat grains diet in comparison with the rodent pellets. According to the study of Krasilnikova V et al, who stated that oat grains oil content, makes it possible to consider as a potential oil-bearing crop as its composition range from 5.91 to 7.87%. Oats' oil content is composed of unsaturated fatty acids, which considered as a healthy essential nutritional factor. Oats doesn't raise the rats' serum Total cholesterol (TC) and LDL-cholesterol levels in comparison with the other groups fed on rodent diet. Oats' oil includes 6 family of essential fatty acids, these fatty acids are important as a precursor of hormonal compounds.[26] On other hand the triglycerides level of rats fed on oats and treated with metformin was lower than rats fed on oat grains without metformin treatment. This difference could be due to the effect of metformin treatment that was in line with the study of Garimella et al.,[27] who showed in his study a significant reductions in serum TG following treatment with metformin.

With concern of the serum MDA concentration, the result appeared that the rats fed on oats had the lowest levels of MDA, this result was in agreement with the study of Risfianty et al. (2016)[28] who confirmed that the intake of the high dietary fibers diet reducing the serum MDA concentration in diabetic rats. Also this results was in line with the study of King Abdulaziz University that revealed the administration of oat in diabetic type 2 rats resulted in a dose-dependent decrease of MDA concentration.[29]

The histopathological examination of the pancreas and liver showed that its tissues in the experimental groups fed on oats were the lowest distortion architecture.

These data were in agreement with the study of Marmouzi et al. (2017),[30] this study confirmed the healthy effect of the dietary fibers on the organ tissues. On the other hand, improvement in microscopic examination of tissues in groups fed on oat have a protective role against these histological alterations due to their higher content of antioxidant substance, mainly beta-glucan which has powerful antioxidant attributes as its molecules help to prevent cell damage, working in association with enzymes resulting in the increase in the GSH "Anti-Oxidant Glutathione" product by the organs.

CONCLUSION AND RECOMMENDATIONS

The study revealed that the oat which was cultivated in Egypt well balanced in its main nutrients as proteins, fats and carbohydrates. Moreover, represent a good source of healthy dietary fibers. On other hand, the study showed that the oats diet has a healthy effect on the diabetic type 2 rats. Its dietary fiber content help in improving the body weight, decreased FBG, TC, LDL, HOMA, and increase serum HDL level. Likewise, oats has a healthy effect on the pancreas and liver tissues of the diabetic rats as it decrease the distortion in its tissue architecture. From the results of this study, it is recommended for diabetic patients with type 2 DM to consume regularly the high dietary fibers diet as oats, in the same way with metformin treatment to help in decrease the diabetes symptoms and to regulate lipid profile in a safe way with minimal side effects.

ETHICAL CONSIDERATIONS

ACKNOWLEDGEMENT

CONFLICT OF INTEREST

None

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