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Evaluation of the activity of two barley hybrids in reduction of blood glucose levels in diabetic albino rats

Marwa A. Desoukey; S.M.M. Saad*; F.F.A. Foda*; Gamal N. Gabrial** and S.S. Mohamed****
* Agri. Biochem. Dept., Fac. Agri., Benha Uni. Egypt.
** Nutrition and Food Science Dep., National Research Centre, Dokki, Giza, Egypt.

Abstract

Type 2 diabetes mellitus is the most common form of diabetes. Many efforts are made to control this disease. Diet therapy is a very powerful mean to complete and enhance drug therapy to control blood glucose levels in diabetic patients. Barley is a wonderful cereal grain. It is considered as a rich source of antioxidant and magnesium that acts as a cofactor for many enzymes involved in the glucose metabolism and insulin secretion. So, the present work aims to investigate the effect of new barley cultivars (barley Gemmiza & barley New valley) on some physiological parameters on streptozotocin-induced diabetic rats. STZ diabetic rats which were fed on diet mixed with 30% barley of the two hybrids for 30 days. Results showed that the use of 30% barley resulted in marked decrements in serum glucose, total cholesterol, triglycerides, LDL cholesterol and increased HDL cholesterol. More over the liver and kidney markers were also improved in the groups that treated with barley relative to its diabetic group. The obtained results might recommend that barley can be utilized as an essential part of the diabetic patient’s diet.

Key words: barley- STZ- Diabetic rats -biological parameters- cholesterol.

Introduction

Diabetes mellitus (DM) is a metabolic disorder characterized by elevation of the blood glucose concentration as well as glucosuria, this elevation in glucose levels is either due to the inability of cells to utilize glucose or insufficient insulin production by pancreatic cells, and metabolic abnormalities in fat and protein metabolisms which increases the risks of oxidative stress and ketosis. And hence a series of hazardous effects can occur that include retinopathy, nephropathy and atherosclerosis **(Wjtowicz *et al.*, 2004 and Hamden *et al*., 2008)**.

There was a good evidence that consumption of whole grains may reduce the risk of diabetes **(Montonen *et al*., 2003)**. Cereal grains make up a major part of human food. Barley is a part of most people’s diets in many countries. **(Slavin *et al*.,1999)** described as "whole grain” if all components of the kernel (the bran, germ and endosperm) are still present in their natural proportion. Barley is a wonderful cereal grain that was used by ancient civilizations as a food for humans and animals, as well as to make alcoholic beverages. In addition, barley water has been used for various medicinal purposes. Barley played an important role in ancient Greek culture as an important food for athletes, who attributed much of their strength to their barley-containing training diets **(Francois 1996)**.

Barley is a rich source of magnesium, a mineral that acts as a co-factor for more than 300 enzymes, including enzymes involved in the body’s use of glucose and insulin secretion. it is also a very good source of fibers, selenium and a good source of phosphorus and copper. It has been found that frequent consumption of whole grains reduced the risk of type 2 diabetes by 31% indicating that whole grains offer special benefits in promoting healthy blood sugar control **(Van Dam *et al*., 2006).** (**Nilsson *et al*., 2006)** mentioned that eating whole grain barley can regulate blood sugar for up to 10 hours after consumption, what seems to have been responsible for barley’s effectiveness in regulating blood glucose is barley’s soluble fiber content **(Cade *et al*.,2007). (Liu 2004**) reported that a cup of cooked barley provides 52% of the daily value for selenium, which is an essential component of several major metabolic pathways antioxidant defense system. Selenium is incorporated at the active site of many proteins, including glutathione peroxidase, which is one of the body’s most powerful antioxidant enzymes. The present study aims to evaluate the effect of new barley hybrids on biological value of streptozotcin –induced diabetic rats.

Material and Methods

1. Materials

a. Source of samples

Barley (*Horduem Vulgare L*.) cultivars(barley Gemmeza and barley New Valley) were obtained from barley Research Department, Field crops Research Institute, Agriculture Research Center, Giza-Egypt.

The barley hybrids were grow under different environmental conditions.

b. Chemicals

Streptozocin (STZ), sodium citrate, casein, vitamins, cholesterol, minerals and cellulose were obtained from El Gomhoriya Company for pharmaceutical industries. Cairo- Egypt.

C .Animals

Male albino adult rats 24 rats, weight about (150g) each were obtained from the animal house the Department of Food Science and Nutrition in the National Research Center, Dokki, Cairo, Egypt.
The animals were kept under normal healthy laboratory conditions.

D. Kits

Kits of determination of aspartate transaminase (AST), alanine transaminase (ALT), Total protein (TP), High density lipoprotein cholesterol (HDL-cholesterol),
Low density lipoprotein cholesterol (LDL-cholesterol), urea, creatinine, total cholesterol(TC) and triglycerides (TG) were obtained from Biodiagnostic El- Dokki-Giza.

2. Methods

a. Chemical composition of barley grains:

chemical compositions (ash, total lipids, crude protein and crude fiber) were determined according to the method cited in **A.O.A.C (2010)** and total carbohydrate were calculated according to **FAO (1982)** by difference as follows:
Carbohydrates%= 100-(protein% + ash% + lipid % +fibers %)

b .Experimental design:

The animals were fed on basal diet (table 1) for one week to adaptation in the experimental animal cage.
After the adaptation period, the animals (24 rats) were divided into groups
Group 1: negative control fed on basal diet (G-)
Group 2: positive control diabetic group fed on basal diet (G+)
Group 3: injected of STZ group fed on diet containing barley Gemmiza (30%)
Group 4: injected of STZ group fed on diet containing barley New Valley (30%).

Table 1. Composition of diet of different groups (g/100g diet):

Groups / Ingredients	G1	G2	G3	G4
Casein	15	15	10	10
Starch	65	65	42	42
Oil	10	10	10	10
Cellulose	4	4	3	3
Salt mix	4	4	4	4
Vit. Mix	1	1	1	1
Barley	-	-	30	30

The diet composition was designed according to the chemical composition of the tested barley grains.

Induction of diabetes

The experimental animals were fasted for 12h and then diabetes was induced by a single intraperitoneal injection of streptozotocin (STZ), at a dose 45mg/kg body weight dissolved in a freshly prepared citrate buffer (0.1M, PH 4.5) **(Burcelin *et al.,* 1995)**.

After injection, all animals were returned to their cages and given free access to food and water. The animals were given 10% sucrose solution to prevent hypoglycemia. After 3 days, for the blood-glucose levels were measured from tail blood samples by using Element Blood Glucose Monitoring system for self-testing.
Animals with blood-glucose levels more than 200mg/dl were considered diabetic and used for the experiment.

Biochemical parameters:

Fasting blood –glucose levels were measured in the whole blood after 12h fasting.

Blood samples were collected by withdrawing from vena cava of normal rat's eyes, as well as diabetic ones, blood samples were centrifuged at 300rpm to obtain serum, and serum blood glucose levels were determined. Total cholesterol, triglycerides, high density lipoprotein HDL, low density lipoprotein LDL, aspartate amino transferase (AST), alanine amino transferase (ALT), urea and creatinine were measured in the blood serum.

Statistical analysis:

Statistical analysis was carried out according to **Fisher (1970)**

Results and Discussion

The chemical composition of the tested barley grains:

The analysis of the barley grains from both Gemmiza and New Valley revealed that the protein content varied between 11.4% &10.6% and these results are in good harmony with those reported by as **(Czuchajowska *et al.,* 1998)** and **(Virkki *et al.,* 2005)** who found that the protein content was 11.8% in barley grain. While **(Limberger-Bayer *et al.,* 2014)** found that it was (10.6%). The carbohydrate content which was the major component of barley grains as it constituted (80.44%) in the Gemmiza and (81.3%) in the New Valley grains and that is higher than the reports of **(Sakhatwat *et al.,* 2014)** who reported it to be **(**58.38%) and (67.30%) respectively.

Table 2. The chemical composition and proximate analysis of the tested cultivars:

Composition	Gemmiza	New valley
Protein	11.4%	10.6%
Carbohydrate	80.44%	81.3%
Fat	0.76%	0.61%
Ash	3.2%	2.4%
Fibers	4.2%	5.1%

The fat content was found to vary between 0.76%-0.61% in Gemmiza and New Valley respectively which was found to be a little pit lower than the results reported by **(Sakhatwat *et al.*, 2014)** to be (1.8%) and **(Limberger-Bayer *et al.*,2014)** who found that it was (2.44%) and (**Virkki *et al*., 2005)** who reported that the fat content was found to be 4.4%. The fibers content was in the same range reported by **(Sakhawat *et al*., 2014)** as it varied between 4.2%-5.1% In the present study ash content of barley cultivars was found To be between 3.2%- 2.4% in Gemmiza and New Valley respectively and previous value in the ash content was reported by (**Sakhawat *et al*.,2014)** who found it to be 2.5% and (**Limberger-Bayer *et al.*, 2014)** found that it was 1.32% and (**Li *et al.*,2001)** found that the ash content was 1.8%. These differences in the chemical composition could mainly be attributed to the genetic difference in the tested verities as well as the difference in the growth conditions and agriculture processes.

Biochemical activity of incorporating barley in the artificially induced diabetic rats:

Around the world, the incidence of type 2 diabetes mellitus is increasing rapidly. Changing the diet may help to prevent development of type2 diabetes and to control blood-glucose levels.

The results indicated various changes in the biological activities between the normal and diabetic rats as well as the rats receiving barley as a treatment.

Table 3. Effect of barley on serum glucose levels

Groups	Glucose concentration mg/dl
G1	94.98±1.10
G2	369.50±31.90
G3	161.67±6.05
G4	169.50±7.91

G1: negative control G2: positive control G3: Gemmiza barley G4: New valley barley

Fig (1). Effect of barley on serum glucose levels (mg/dl) of STZ-induced diabetic rats after 4 week

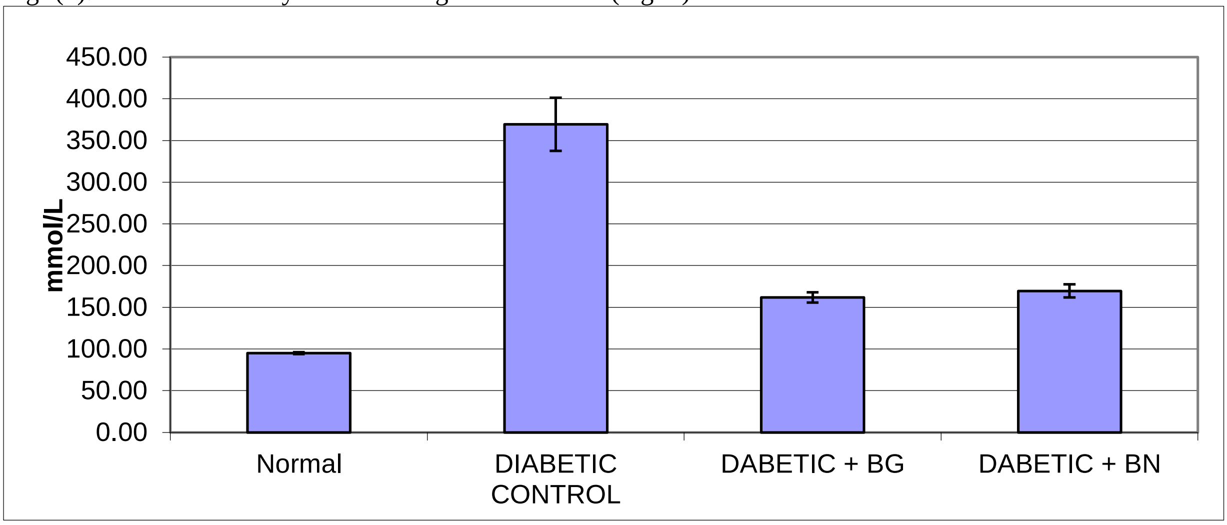

Fig (1) Glucose levels after receiving barley diet for 30 days

Results in table (3) and fig. (1) indicates the change in serum glucose levels after 4 weeks of treatments using 30% barley incorporated in the diet of the hyperglycemic rats and as shown in the diabetic group the serum blood glucose was elevated extremely from the control that recorded 94.98±1.10 and reached 369.50±31.90 and that elevation was reduced to some extent by using 30% barley either from Gemmiza or from New valley to reach 161.67±6.05 and 169.50±7.91 respectively. These results indicates the ability of barley to reduce serum glucose levels and these data are in accordance with those reported by with (**Naismith *et al.*, 1991)** and **(Mahdi and Naismith 1991)** who reported that diet-containing barley had a modulating effect on the diabetes symptoms. The obtained results are also in agreement with the findings of **(Mokhtar *et al.*, 2006)** who also reported the hypoglycemic activity of barley water against diabetic rats. The hypoglycemic effect of barley has been attributed to the effects of the β-glucan fibers that reduces glucose levels by increasing the viscosity of the contents of the stomach and small intestine, which decreases the absorption of digested nutrients from the small intestine. This finding is consistent with **(Battilana *et al.*, 2001)** who stated that a possible mechanism to explain the hypoglycemic effect of barley might involve delayed or reduced carbohydrate absorption from the gut. The effectiveness of barley β- glucan fibers in barley food products in lowering blood cholesterol and glycemic index has been reported in numerous publications **(Cavallero *et al.*, 2002 and Behall *et al.*, 2004).** Products prepared from barley flour enriched with β-glucan fibers exhibit favorable responses on glucose metabolism, and particularly on insulinemic responses **(Casiraghi *et al.*, 2006).**

2. Effect of barley on lipid profiles of diabetic rats:

Table 4. Effect of barley on lipid profiles of diabetic rats (Total cholesterol, triglycerides, HDL-C and LDL-C levels):

Groups	T. cholesterol	Triglycerides	HDL-C	LDL-C
G1	113.63±3.63	111.50±4.52	57.15±1.05	31.90±3.30
G2	156.55±4.30	152.70±5.25	37.80±3.58	119.93±11.78
G3	111.53±2.90	108.40±5.19	51.08±2.08	39.01±0.82
G4	116.25±3.61	108.66±3.88	54.36±1.47	41.61±2.32

The serum lipid parameters, TC, TG, and LDL-C in the STZ diabetic rats were increased while HDL-C was decreased compared with the normal control group (Table 4). Both G3 and G4 groups improved their lipid status as the levels of TC, TG, and LDL-C decreased while there was marked increase in the HDL

It was reported that hypertriglyceridemia, hypercholesterolemia and reduced HDL level were commonly seen in diabetes **(Newairy *et al.*,2002 and Colca *et al*.,1991)** It has been suggested that either the removal of triglycerides from the circulation or its entry into the circulation or both was impaired in non-insulin-dependent diabetics **(Sharma and Raghuram., 1990)**. Also the increased mobilization of free fatty acid (FFA) from peripheral depot **(Krishnaveni *et al.*, 2010)**.

The present results showed remarkable increases in plasma TG, cholesterol, and LDL, also a decrease in HDL level (Table 4) and fig (2:5), and these results are in agreement with those found in diabetic rats by **(Newairy *et al.*,*2002*)**. Barley is rich in soluble fiber beta-glucan and it is contributing to lowering blood cholesterol level **(Wursch and Pi-Synner.,1997)**. The mechanism involved in reduction of diabetic hypercholesterolemia by treatment of barley has yet to be elucidated although several hypotheses have been advanced. There is strong evidence that the soluble polysaccharide beta-glucan present in barley was related to hypocholesterolemic effect by many mechanisms. The soluble beta-glucan is bind bile acids in the small intestine, these bile acids are synthesized in the liver from cholesterol and secreted into the small intestine. The fiber-bile acids complex prevents bile acids from being reabsorbed from the small intestine, enhancing the secretion of bile acids, to replace the lost acids; cholesterol is drawn from the circulation for the production of bile acids, thereby reducing the blood cholesterol levels. Other proposed mechanism for the reduction of blood cholesterol by the fibers include, the soluble polysaccharides were fermented in the colon. This molecule is absorbed and taken to the liver where it has inhibitory effect on the activity of hydroxymethylglutaryl-CoA-reductase, thus reducing de novo cholesterol synthesis **(Chen and Anderson.,1986)**. Also, tocols (tocopherols and tocotrienols) have been identified as another minor component in barley with value-added potential**.** **(Weber *et al.*,1991)** reported that tocotrienols had ability to lower serum cholesterol. Other suggestion that the hypolipidemic effect of barley may contributed to presence of some saponins. **(Sidhu *et al.,1987)*** reported that saponins increased the cholesterol secretion to 65.8%, and stimulated lipoprotein lipase activity and might stimulate the enzymes relating to the metabolism of lipid including cholesterol. Other studies have found that saponins in the high cholesterol diet of rats reversed the hypercholesteremia and increased both the rate of bile acid secretion and the fecal excretion of bile acids and neutral sterols **(Sauvaire *et al.*,1991).**

Effect of barley on the lipid profile (TC; TG; LDL-C and HDL-C) in diabetic rats

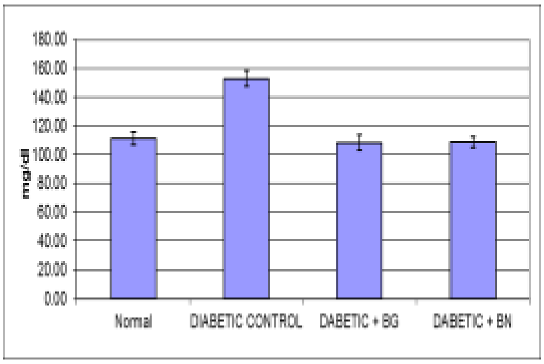

Fig (2). Effect of barley on serum total cholesterol of diabetic rats

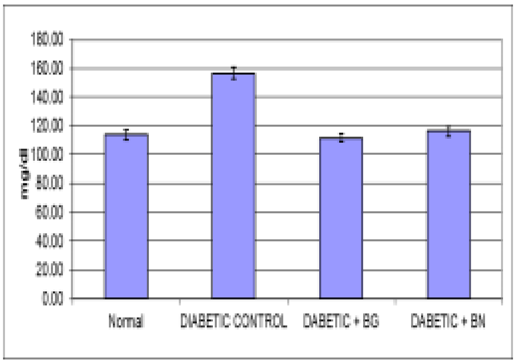

Fig (3). Effect o f barley on triglycerides of diabetic rats

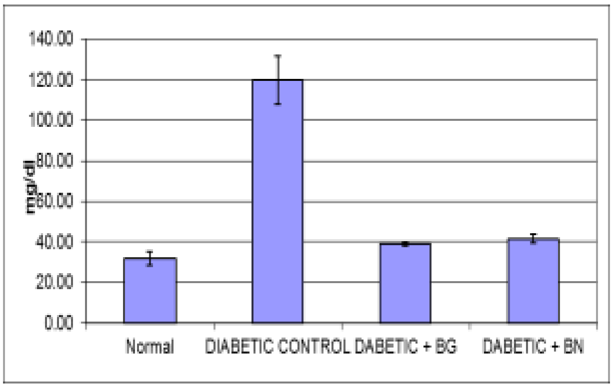

Fig (5). Effect of barley on LDL-C of diabetic rats

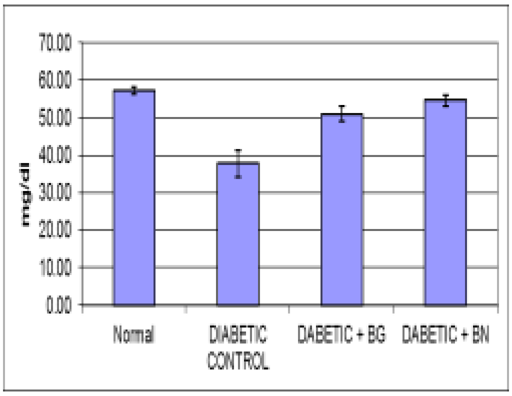

Fig (4). Effect of barley on HDL-C of diabetic rats

3. Effect of different diets on kidney functions of diabetic rats:

Table 5. Effect of barley on the Urea and creatinine levels (mg/dl) of diabetic rats

Groups	Urea	Creatinine
G1	18.77±0.72	0.69±0.02
G2	54.21±3.98	1.61±0.09
G3	29.45±1.01	0.68±0.02
G4	28.02±1.13	0.39±0.02

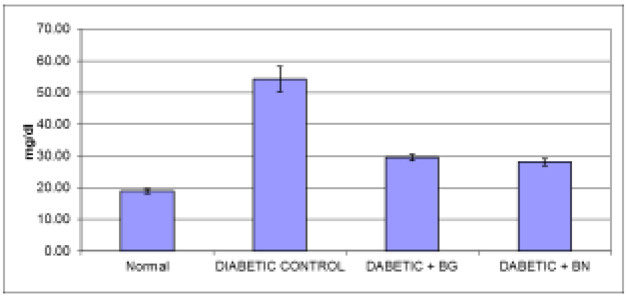

Figure (6) demonstrates the change in Urea levels after receiving barley diet

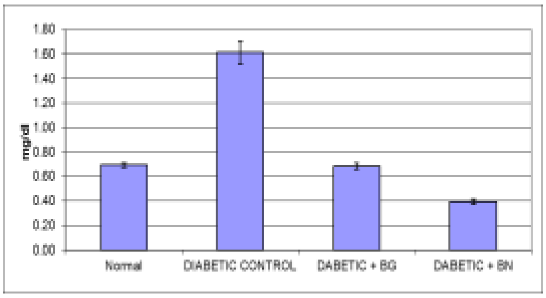

Fig (7). Effect of different diets from barley on creatinine of diabetic rats

Urea is the end product of protein catabolism and is excreted through urine. Creatinine is an end product of creatine metabolism. Creatine is synthesized in the liver and Passes into the circulation. Urea and creatinine are regarded as reliable markers of renal function **(Adelman *et al.*, 1981)**. The concentrations of urea and creatinine were higher in diabetic rats when compared to the normal rats.

Hence it was the indication that the STZ diabetes may lead to renal dysfunction. Figure (6) demonstrates the change in urea levels after receiving barley diet from 2 different governorates, the diabetic group had an average of 54.21 which was reduced dramatically after receiving barley diet to 29.45 in the group receiving barley from Gemmiza and to 28.02 in the New Valley group. The treatment of STZ induced diabetic rats with barley reduced the urea level in serum when compared to the diabetic group. Similarly, the elevation of creatinine level in serum was caused by diabetes (figure 7). The diabetic group had an average creatinine level of 1.61 which was reduced after administration barley diet to 0.68 in the group receiving barley from Gemmiza and to 0.39 in the New Valley group.

4. Effect of different diets on liver function of diabetic rats:

Table 6. Effect of different barley diets on ALT, AST (IU/I) and Total protein (g/dl) of diabetic rats

Groups	AST	ALT	Total protein
G1	24.70±0.77	21.08±1.12	9.02±0.65
G2	60.96±1.49	52.25±3.14	7.13±0.73
G3	43.69±1.46	38.61±1.61	8.86±0.54
G4	47.06±1.33	38.87±2.00	8.53±0.72

The serum levels of ALT and AST were reduced in the barley treated groups Figures (8-9). demonstrates the change in AST and ALT levels after receiving barley diet from 2 different governorates. The diabetic group had an average of 60.96 and 52.24, respectively, which was reduced dramatically after receiving barley diet to 43.68 and 38.61, respectively in the group receiving barley from Gemmiza and to 47.05 and 38.87, respectively in the New valley group. Alanine amino transferase (ALT) and aspartate amino transferase (AST) are excellent markers for diagnostic purpose, which play a main role in the conversion of amino acid to ketoacid. AST was found in many tissues like liver, kidney, heart, brain and skeletal muscle it is not specific liver enzyme; but ALT is more specific liver enzyme found in large amount in liver, when compared to other tissues. Serum ALP is a sensitive detector for intra-hepatic and extra-hepatic bile obstruction; the presence of bile obstruction and all bone diseases **(Adaramoye *et al* 2008)**. Moreover, ALT, AST and ALP are marker enzymes for liver function and integrity **(Edwards *et al* 1995)**, which are liberated into the serum whenever liver cells are damaged and the serum enzyme activity is increased. The increment level of ALT, AST and ALP were observed in the diabetic control, indicating that diabetes may induce hepatic dysfunction in rats. These elevations are also associated with cell necrosis of some tissues. The Increase in the activities of AST, ALT in serum may be due to the leakage of these enzymes from liver cytosol to blood stream which gives an indication on the hepatotoxic effect of STZ.

Total protein concentration was also decreased in the serum of STZ induced diabetic rats. The reduction of total protein is due to an increased conversion glycogenic amino acid to CO2 and H2O (**Mortimore and Manton 1970**). Diabetic rats treated with parley attained the protein level near to normal.

The obtained results are in agreement with **Mohammed and Nassier 2013** who found the intake of barley diet partially decreased the GGT, AST, ALT and ALP levels.

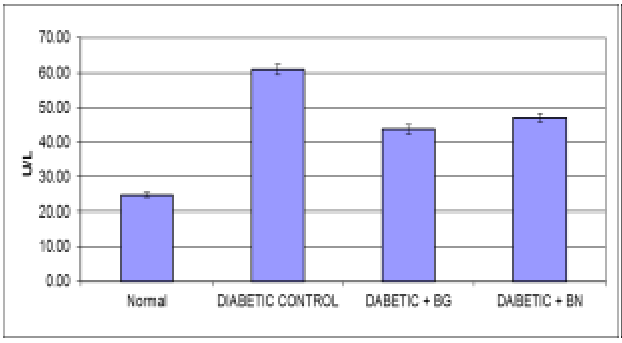

Fig (8). Effect of different diets from barley on AST of diabetic rats

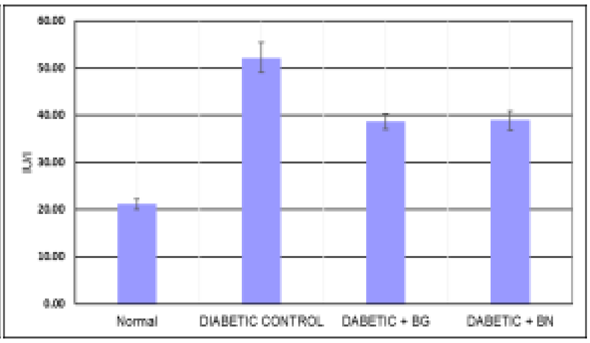

Fig (9). Effect of different diets from barley on ALT of diabetic rats

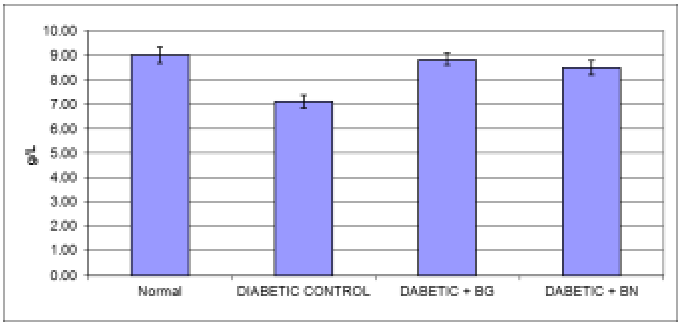

Fig (10). Effect of diets from barley on total protein of diabetic rats

Generally, from the accomplished results, it might recommended that barley cultivars i.e. Gemmiza and New Valley cultivars can be utilized as an essential part of the diabetic patient's diet.

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تقييم قدرة نوعين من هجن الشعير على خفض مستويات سكر الدم في الجرذان البيضاء المصابة بمرض السكري

مروة احمد دسوقى**، صلاح مصطفى سعد*، فرحات فوده على فوده*، جمال نوب غبريال** و شريف صلاح محمد**

*قسم الكيمياء الحيوية الزراعية- كلية الزراعة – جامعة بنها – مصر

**قسم التغذية و علوم الاطعمة- المركز القومى للبحوث- الدقى- جيزة- مصر

تعد الاصابة بمرض السكري من النوع الثاني هي الاكثر شيوعا و هناك العديد من االجهود المبذولة لمحاولة التحكم في هذا المرض .
تعتبر النظم الغذائية من أهم الوسائل المكملة و المساعدة للعلاج الدوائي للتحكم في مستويات السكر في الدم في مرضي داء السكري.
يعتبر الشعير من الحبوب عالية القيمة الغذائية حيث انه يعد مصدرا غنيبا لمضادات الاكسدة وكذلك بعض المعادن و اهمها الماغنسيوم الذى يلعب دورا هاما كعامل مساعد للعديد من الانزيمات التى تدخل فى تكسير الجلكوز وايضا فى افراز هرمون الانسولين.
لذا تهدف هذه الدراسة الى معرفة تاثير نوعين من هجن الشعير الحديثة (شعير الجميزة و شعير الوادى الجديد) على بعض القياسات الفسيولوجية فى الفئران المصابة بالسكر معمليا عن طريق الحقن بالستربتوزوتوسين(STZ) ثم تغذية الفئران المصابة معمليا بمرض السكرى على طعام يحتوى على 30% شعير من سلالتين (شعير الجميزة و شعير الوادى الجديد) لمدة 30 يوم .
اظهرت النتائج ان اضافة 30% شعير ينتج عنها نقص ملحوظ فى مستويات سكر الدم وكذلك فى مستويات كل من مستوى الدهون فى الدم و وظائف الكبد و وظائف الكلى فى الدم مع زيادة فى مستوى كوليسترول الدهون عالية الكثافة وهو النوع المفيد من الكوليسترول (HDL)
كذلك وجد ان هناك تحسنا فى مؤشرات كل من وظائف الكبد و الكلى فى المجموعات التى تم معالجتها بالشعير مقارنة بالمجموعة المصابة بمرض السكر و هذه النتائج تضيف الى التوصيات باستخدام الشعير كجزء اساسى من تغذية مرضى السكر.ى للمساعدة فى العلاج الدوائى لهذا المرض.