EFFECT OF PROTEIN AND CHROMIUM INTAKE ON SOME NUTRITIONAL AND BIOCHEMICAL STATUS IN DIABETIC RATS

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ABSTRACT

The present study was designed to investigate the effects of protein and chromium intake on some nutritional and biological status in streptozotocin induced diabetic rats. This study was conducted on forty -nine Sprague –Dawley adult male rats, weighting 190 ±15 g were classified into control and six treated groups with drug, chromium, high protein, low protein, high protein with chromium and low protein with chromium groups for ten weeks.

The obtained results revealed that the drug, chromium, high protein, high protein with chromium and low protein with chromium groups rat group had a significant higher values of body weight gain, feed efficiency ratio, insulin, hemoglobin, packed cell volume, GPX, SOD and globulin and a significant lower value in ALT, AST, AP,γGT ,creatinine and urea when compared with control group but a significant decrease in the value of glucose and MDA compared with drug group .

The high protein and high protein with chromium rat groups showed a significant increase in the value of total protein but high protein with chromium and low protein with chromium rat groups showed significant decrease in the value of albumin/ globulin ratio when compared with control group or with drug rat group.

The chromium, high protein, high protein with chromium and low protein with chromium rat groups showed significant decrease in the value of total bilirubin while the chromium, low protein and low protein with chromium rat groups showed significant decrease in the value of uric acid when compared with control group.

The low protein rat group showed a non significant difference in final weight, weight gain, feed intake, feed efficiency ratio, insulin, packed cell volume, SOD , MDA, ALT, AST, AP , γGT, total bilirubin and creatinine and a significant decrease in glucose and GPX but a significant increase in heamoglobin and globulin when compared with control group but showed a significant decrease in weight gain, feed efficiency ratio, insulin, packed cell volume, GPX, SOD and a significant increase in glucose, MDA, ALT, AST, AP ,γGT and creatinine when compared with drug group.

Key wards :(protein - chromium - diabetic rat).

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أثر تناول البروتين والكروميوم على الحالة الغذائية والبيوكيميائية في الفئران المصابة بالداء السكري

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الملخص العربي

تهدف هذه الدراسة إلى تقدير اثر تناول البروتين و الكروميوم على الحالة الغذائية والبيولوجية للفئران المصابة بالداء السكري ولذلك أجريت هذه الدراسة على تسعة و أربعون من فئران التجارب يتراوح أوزانهم بين ١٩٠ ± ١٥ جرام لمدة عشرة أسابيع من الإصابة بالداء السكري. وتم تقسيمهم إلى المجموعة الضابطة و ست مجموعات معالجة وهى المعالجة بالدواء الخافض السكر، و الكروميوم وعالية البروتين ومنخفضة البروتين وعالية البروتين مع الكروميوم ومنخفضة البروتين مع الكروميوم وقد أسفرت النتائج المتحصل عليها فيما يلي:

أن المجموعات المعالجة بالدواء و الكروميوم وعالية البروتين وعالية البروتين مع الكروميوم ومنخفضة البروتين مع الكروميوم أظهرت ارتفاع معنوي في الوزن المكتسب و نسبة كفاءة الطعام والأنسولين و الهيموجلوبين PCV و GPX, SOD والجلوبيولين وانخفاض معنوي في ALT, AST, AP, γGT والكرياتينين و اليوريا بالمقارنة بالمجموعة الضابطة وانخفاض معنوي في الجلوكوز و MDA بالمقارنة بالمجموعة المعالجة بالدواء.

أن مجموعتي عالية البروتين وعالية البروتين مع الكروميوم أظهرت ارتفاع معنوي في البروتين الكلي بينما مجموعتي عالية البروتين مع الكروميوم ومنخفضة البروتين مع الكروميوم أظهرت انخفاض معنوي في نسبة الالبيومين الى الجلوبيولين بالمقارنة بالمجموعة الضابطة و المعالجة بالدواء.

أن المجموعات الدوائية وعالية البروتين وعالية البروتين مع الكروميوم ومنخفضة البروتين مع الكروميوم أظهرت انخفاض معنوي في البيلروبين الكلي بينما مجموعات الكروميوم ومنخفضة البروتين ومنخفضة البروتين مع الكروميوم أظهرت انخفاض معنوي في حمض اليوريك بالمقارنة بالمجموعة الضابطة.

أن المجموعة منخفضة البروتين أظهرت عدم وجود اختلاف معنوي في الوزن النهائي والمكتسب و المتناول من الطعام ونسبة كفاءة الطعام والأنسولين و PCV و γGT, AP, AST, ALT, SOD MDA و البيلروبين الكلي و الكرياتينين و انخفاض معنوي في الجلوكوز و GPX بينما أظهرت ارتفاع معنوي في الهيموجلوبين و الجلوبيولين بالمقارنة بالمجموعة الضابطة وقد أظهرت أيضا انخفاض معنوي في الوزن المكتسب ونسبة كفاءة الطعام والأنسولين و PCV و ارتفاع معنوي في الجلوكوز MDA, ALT, AST, AP, γGT والكرياتينين بالمجموعة المعالجة بالدواء.

و توصى الدراسة بضرورة زيادة نسبة البروتين وتدعيمه بالكروميوم في الوجبة لتحسين الحالة الصحية لمرضى الداء السكري

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INTRODUCTION

Diabetes mellitus is a metabolic disease characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. There are two important characteristics of this disease, one is insulin resistance, which means the failure of peripheral tissues such as liver, muscle and adipose tissue to respond to physiologic doses of insulin, and the other one is dysfunction of pancreatic beta cell to properly secrete insulin in response to elevated blood glucose level (Wattanakit et al., 2005 and Ziegler, 2005).

Protein is certainly an essential nutrient which plays many key roles in the way our bodies function (Hoffman and Flavo 2004). Proteins are the natural choice of the body when faced with diabetes. In uncontrolled diabetes, muscle protein is broken down into amino acids to be converted into glucose by the liver. Since proteins have to supply enough energy to substitute for carbohydrates, proteins are broken down faster than they are made. Excess protein has been linked with osteoporosis, kidney disease, calcium stones in the urinary tract, and some cancers. Low-protein diets ameliorate the symptoms of some diabetic patients, possibly by improving insulin sensitivity and alleviating hyperinsulinemia (Rigalleau et al., 1998). The excess of amino acids attenuates insulin-stimulated glucose transport *in vitro*, while amino acid deficiency enhances it (Takano et al., 2001 and Tremblay and Marette 2001). In addition, dietary protein has been shown to modulate some insulin-dependent processes, such as the regulation of enzymes for gluconeogenesis (Rossetti et al., 1989) .

Dietary supplements are widely used not only by the general public but also by diabetic patients. The adequate intake of foods rich in chromium should be considered as part of the nutritional to diabetic patients. Chromium is an essential trace element typically found in foods, such as egg yolks, coffee, green beans, nuts, and whole-grain products. Chromium assists in weight loss, proper insulin functioning, and blood sugar

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regulation. It may also help decrease appetite and sugar cravings, increase muscle mass, regulate cholesterol, and help break down carbohydrates, fats, and protein (Kalman, 2003)

Chromium is required for the maintenance of normal glucose and fat metabolism and used for the treatment of diabetes and high blood sugar levels. Additionally, it may also help regulate blood sugar levels in patients with low blood sugar disorders. Chromium is the glucose tolerance factor which potentiates the action of insulin and energetic metabolism, without serious adverse effects (Cefalu and Hu 2004 and Fernando and Martha 2005).

People with diabetes are frequently given advice about protein and chromium that has no scientific basis and need adequate and accurate information about protein on which to base their food decisions. The present study was designed to investigate the effect of the protein either low or high with or without chromium on the nutritional and some biochemical parameters on streptozotocin induced diabetic rats.

MATERIALS AND METHODS

A – Materials:

1-Streptozotocin:

Streptozotocin is procured from Sigma, St. Louis, MO, USA. It is often used for preparing type 1 diabetes animal model because of its differential wreck action to beta cells of pancreas. It can also be used for preparing non-insulin dependent diabetes animal model (Alain et al., 1969 and Arulmozhi et al., 2004).

2- Antidiabetic drug:

Amaryl drug is produced by Saofi –Avents Egypt under licence of Saofi –Avents Germany. It is antidiabetic sulfonylurea. Each tablet contains 2mg glimepiride. The human therapeutic dose is 4 mg daily. The human therapeutic dose was converted to rat dose according to Paget and Barnes, (1964) that was o.36 mg/kg body weight daily which dissolved in distilled water and given to rats by oral intubation.

3-Chromium drug:

Chromium drug is produced by Arab Company for Pharmaceuticals and Medicinal Plants (MEPACO) Egypt. Each capsule contains 200 mcg

chromium picolinate. The human therapeutic dose of chromium is 400 mcg daily. The human therapeutic dose was converted to rat dose according to Paget and Barnes, (1964) that was 36 mcg/100 g body weights daily which dissolved in distilled water and given to rats by oral intubation.

3- Protein:

High protein and low protein diet was prepared by ± 50% the normal level of casein in standard diet.

4-Experimental animals:

A total of forty-nine Sprague –Dawley adult male rats were purchased from the Agricultural Research Center, Giza, Egypt. The average weight was 190 ±15 g. The animals were kept under observation for five days before experiment and supplied with standard diet and water *ad libitum*. The standard diet comprised of casein (200g/kg), corn starch (497g/kg), sucrose (100g/kg) , cellulose (30 g/kg) ,corn oil (50g/kg), mineral mixture (100g/kg) , vitamin mixture (20g/kg) and DL-methionine (3g/kg).The standard diet was performed according to NRC (1995).

B-Methods:

The rats were randomly divided into seven groups (7 rats /group). The rats were injected with a single intraperitoneal dose of streptozotocin (55mg/kg body weight) in 0.1 M citrate buffer of pH 4.5 then supplied with 5% glucose solution for 48 h after injection in order to prevent hypoglycemia (Peschke et al .,2000). After four days, blood samples were taken from orbital plexus for estimation of glucose. The rats having persistent hyperglycemia were considered as diabetic rats and used for the experiment. The diabetic rats were classified into control and treated six groups with drug, chromium, high protein, low protein, high protein with chromium and low protein with chromium groups. The feed intake was calculated daily and the body weight gain was recorded weekly. At the end of the experiment (ten weeks), the rats were fasted overnight then sacrificed to obtain blood samples.

First part of blood was heparenized for estimation of hemoglobin and packed cell volume (PCV) (Drabkin, 1949 and Mc Inory 1954). Second part of blood was collected in tubes containing potassium oxalate and sodium fluoride for the estimation of glucose by *O*-toluidine method (Sasaki et al., 1972). Third part of blood was left to coagulate then centrifuged at

3000 rpm for 15 minutes to obtain serum. Serum insulin, alanine and aspartate aminotransferase (ALT&AST), alkaline (AP) and gamma glutamyl phosphate (γ GT) activity were estimated according to Wilson and Miles (1977), Reitman and Frankel (1957), Kind and King (1954) and Henry, (1974), respectively. In addition, total protein, albumin, total bilirubin, creatinine, urea and uric acid were estimated according to Weichselbaum (1946) and Bartholomev and Delany (1966) ,Jendrassik (1938) ,Bonsens and Taussky,(1984), Patton and Crouch , (1977) and Fossati et al., (1980) , respectively. Liver Glutathione peroxidase(GPX) , superoxide dismutase (SOD) and malondialdehyde (MDA) activity was determined as described by the method of Beutler et al., (1963), Dechatelet et al. (1974) and Stocks and Donnandy (1971) , respectively.

Calculation of some parameters was occurred as following:

* Feed efficiency ratio (FER) = weight gain (g)/ feed intake (g) (Chapman et al., 1950).

*Serum globulin (G) = total proteins- albumin (Coles, 1974).

*A/G ratio = albumin / globulin values for each individual sample .

C-Statistical analysis:

All the obtained data were statistically analyzed by SPSS computer soft ware. The calculated occurred by analysis of variance ANOVA and follow up test LSD (Artimage and Berry 1987).

RESULTS

1-Nutritional results:

The statistical date in table (1) denoted that, the drug, chromium, high protein, low protein, high protein with chromium and low protein with chromium rat groups had a significant higher values of body weight gain and feed efficiency ratio (p<0.01) when compared with control group.

The chromium, high protein, high protein with chromium and low protein with chromium rat groups showed non significant difference in final body weight, weight gain, feed intake and feed efficiency ratio in compared with drug rat group. The low protein rat group showed a significant decrease in weight gain and feed efficiency ratio in compared with drug rat group.

3-Biochemical results:

Data presented in table (2) showed that the value of glucose was significantly decreased at p <0.001 in drug, chromium, high protein, high protein with chromium and low protein with chromium rat groups and at p <0.05 in low protein rat group when compared with control group. The value of insulin was significantly increased at p <0.001 in drug, chromium, high protein and high protein with chromium rat groups and at p <0.01 in low protein with chromium rat group when compared with control group. The mean value of hemoglobin and packed cell volume was significantly increased at p <0.01 in drug, chromium, high protein, high protein with chromium rat groups and low protein with chromium when compared with control group. The low protein rat group showed significant increase in hemoglobin at p <0.05 in when compared with control group.

The chromium, high protein, high protein with chromium and low protein with chromium rat groups showed non significant difference in in the mean value of glucose, insulin, hemoglobin and packed cell volume compared with drug rat group. The low protein rat group showed a significant increase in glucose and a significant decrease in insulin, packed cell volume compared with drug rat group.

Data in table (3) revealed that the value of GPX was significantly increased at p <0.001 in drug, chromium, high protein, high protein with chromium and low protein with chromium rat groups but significant decrease at p <0.05 in low protein rat group when compared with control group. The value of SOD was significantly increased at p <0.001 in chromium and high protein with chromium rat group and at p <0.01 in drug, high protein, and low protein with chromium rat groups when compared with control group. The value of MDA was significantly decreased at p <0.001 in chromium, high protein, high protein with chromium and low protein with chromium rat groups and at p <0.01 in drug rat group when compared with control group.

The low protein rat group showed a significant decrease in GPX and SOD but significant increase in MDA compared with drug rat group.

Data recorded in table (4) showed that the values of ALT and γGT were significantly increased at p <0.001 in drug and high protein with chromium rat groups and at p <0.01 in chromium, high protein and low protein with chromium rat groups when compared with control group. The

value of AST was significantly decreased at p <0.001 in drug, chromium, high protein, high protein with chromium rat groups and at p <0.01 in low protein with chromium rat group when compared with control group. The value of AP was significantly decreased at p <0.01 in at chromium, high protein, high protein with chromium and low protein with chromium rat groups and at p <0.05 in drug when compared with control group.

The low protein rat group showed a non significant difference in ALT, AST, AP and γGT compared with control rat group but significant increase compared with drug rat group.

From the data in table (5), it could be observed that the value of total protein was significantly increased in high protein and high protein with chromium rat groups at p <0.05 while the value of globulin was significantly increased at p<0.001 in high protein with chromium rat group and at p<0.01 in drug, chromium, high protein, and low protein with chromium rat groups but at p<0.05 in low protein rat group when compared with control group. The value of albumin/ globulin ratio was significantly decreased at p<0.05 in high protein with chromium and low protein with chromium rat groups when compared with control group.

The value of total protein was significantly increased in high protein and high protein with chromium rat groups while the value of albumin/ globulin ratio was significantly decreased in high protein with chromium and low protein with chromium rat groups compared with drug rat group.

The data in table (6), it could be observed that the value of total bilirubin was significantly decreased in chromium, high protein, high protein with chromium and low protein with chromium rat groups at p <0.01 while the value of creatinine was significantly decreased at p<0.01 in drug, chromium, high protein rat groups and at p<0.05 in high protein with chromium and low protein with chromium rat groups when compared with control group. The value of urea was significantly decreased in drug , chromium , high protein, low protein ,high protein with chromium and low protein with chromium rat groups at p<0.01 while the value of uric acid was significantly decreased in chromium , low protein and low protein with chromium rat groups at p<0.05 when compared with control group.

The values of total bilirubin, creatinine, urea and uric acid were in non significant difference in chromium, high protein, low protein, high protein with chromium and low protein with chromium rat groups except

creatinine in low protein rat group which showed significant increase when compared with drug rat group.

DISCUSSION

Pathological alterations in non-enzymatic glycosylation of proteins and lipids can interfere with their normal function, cellular protein kinase C activation and oxidative stress in diabetic animal and human . Hyperglycemia depletes natural anti-oxidants and facilitates the production of free radicals (Abd El Ghany, and Khaled, 2000 and Johansen et al., 2005).

It is well known that streptozotocin induced diabetes is linked to the production of reactive nitrogen species and oxygen species which damages DNA of pancreatic cells, leading to cell necrosis and loss of insulin Streptozotocin diabetic rats groups showed loss in body weight resulting of protein wasting due to unavailability of carbohydrate for utilization as an energy source (Szkudelski, 2001). Gastroparesis is a form of diabetic neuropathy that can affect the digestion of food and retard glucose absorption. Symptoms of gastropathy can include earlysatiety, decreased appetite, bloating, abdominal discomfort, nausea, vomiting and unexplained hypo/hyperglycemia. Gastropathy can result in postprandial hypoglycemia that is later followed by higher than normal blood glucose rises (Valentine et al .,1998).

The reduction in hemoglobin and packed cell volume may be attributed to an increasing glycation of a number of proteins including hemoglobin. Glycated hemoglobin is considered as one of the markers of degree of oxidative stress in diabetes mellitus (Whiteside, 2005). Streptozotocin molecules might increase the production of oxygen free radicals that play a crucial role in determining tissue injury as exert direct or indirect effects on islet endothelium and mediate fragmentation of nuclear DNA in beta cells (Hui et al .,2007) . Persons with diabetes are several times more prone to kidney disease than the general population. Hyperglycemia is the primary risk factor associated with development of both micro- and macrovascular complications. Hyperglycemia produces its deleterious effects is the production of free reactive oxygen species (ROS). Thus, oxidative stress, defined as a persistent imbalance between the excessive production of ROS and/or defects in antioxidant defense. The mitochondrial process involved in the glucose-mediated insulin secretion is particularly

affected by oxidative stress. Increase in mitochondrial ROS, a consequence of increased glucose levels (American Diabetes Association, 2004).

Sulphonylurea drug is known to lower the blood glucose level by stimulating B-cells to release insulin (Bethesda 2001).

Current evidence indicates people with diabetes have similar protein requirements to those of the general population. Although protein plays a role in stimulating insulin secretion, excessive intake should be avoided as it may contribute to the pathogenesis of diabetic nephropathy (Brenneret al., 1982 and Franz, 1997). Restricting protein intake to less than requirements has no additional benefit on the progression of renal disease and may result in inadequate intake of essential amino acids. However, there is some evidence that the use of non-meat protein sources may be preferable to animal protein sources in the management of renal disease (Pedrini et al., 1996).

Protein nutrition influences the signaling pathway of insulin. Upon activation by insulin, the insulin receptor (IR) binds to various types of substrate proteins, including adapters, docking proteins and enzymes; the family of IR substrate (IRS) proteins constitutes the major group (White 2002). Dietary protein is a very strong regulator of serine phosphorylation of IRS-1 in muscle. This change is accompanied by an increased sensitivity of IRS-1 to insulin. This increase may contribute to elevated insulin responsiveness by manipulations of dietary protein (Toyoshima et al., 2004).

The low protein rats gained weight at a considerably lower rate than normal. This was obtained in the absence of any decrease of spontaneous food intake but basal plasma insulin levels were decreased by 40% in low protein rats than normal. The basal hepatic glucose production in the low protein rats was similar to that in the normal rats. The modest improvement of glucose tolerance which is revealed in the low protein rats results from changes in the insulin action upon the target tissues because both the insulin-mediated glucose uptake by peripheral tissues and the ability of insulin to suppress hepatic glucose output are enhanced (Swenne et al ., 1988 and Crace et al .,1990).

Hyperuricemia can be the consequence of increased uric acid production or decreased excretion. Any cause for decreased glomerular filtration, tubular excretion or increased reabsorption would result in an

elevated serum uric acid. Increased serum uric acid has been found to predict the development of renal insufficiency in individuals with normal renal function (Schmidt-Nielsen et al., 1985). High fat and high protein food may also cause increased oxidative stress in diabetes, resulting in further injury to pancreatic cells.

Chromium deficiency can result in decreased glucose tolerance but is believed to be rare. Chromium picolinate significantly improved glycemic control in people with diabetes (Anderson, 1997 and Albarracin et al., 2008). Chromium improves the action of insulin by improving tyrosine kinase activity on the insulin receptor. Tyrosine kinase, the enzyme required for phosphorylation, is chromium dependent, and phosphotyrosine phosphatase, an enzyme that inactivates the insulin receptor, is inhibited by chromium. Chromium exerts a powerful cellular antioxidant action and decreases the hepatic extraction of plasma insulin (Davis et al., 1997 and Anderson et al., 2001).

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Table (1): Mean values ± SD of body weight gain, feed intake and feed efficiency ratio (FER) of the experimental rats groups.

Group \ Variables	Control	Antidiabetic drug	Chromium	High protein	Low protein	High protein with Chromium	Low protein with Chromium
Initial weight (g)	190.31 ± 13.61[a]	191.11 ± 14.22[a]	190.44 ± 13.17[a]	192.35± 14.21[a]	189.99± 14.61[a]	189.59± 14.33[a]	190.66 ± 13.77[a]
Final weight(g)	232.45 ± 25.18[a]	252.43 ± 33.14[a]	249.75 ± 30.85[a]	255.49± 42.91[a]	216.16± 34.71[a]	257.80± 43.01[a]	250.77 ± 38.11[a]
Weight gain (g)	33.14 ± 6.40[b]	61.32 ± 8.33[a**]	59.31 ± 6.22[a**]	63.14 ± 7.10[a**]	26.17 ± 3.20[b]	68.21 ± 7.30[a**]	60.1 1± 7.42[a**]
Feed intake (g/day)	18.65 ± 2.10[a]	19.11 ± 3.11[a]	18.95 ± 2.01[a]	19.35 ± 2.14[a]	18.30 ± 1.99[a]	18.30 ± 1.89[a]	18.20 ± 1.29[a]
FER	0.029 ± 0.002[b]	0.053 ± 0.001[a**]	0.052 ± 0.005[a**]	0.054 ± 0.005[a**]	0.023 ± 0.002[b]	0.062 ± 0.004[a**]	0.055 ± 0.001[a**]

Significant with control group * P<0.05 **   P<0.01 *** P<0.001

Mean values in each raw having different superscript (a, b, c) denote significant difference.

Table (2): Mean values ± SD of glucose, insulin, hemoglobin and packed cell volume of the experimental rats groups.

Groups \ Variables	Control	Antidiabetic drug	Chromium	High protein	Low protein	High protein with Chromium	Low protein with Chromium
Glucose (mg/dl)	301.91 ± 39.71 a	140.17 ± 11.22 c***	152.61 ± 13.24 c***	155.61 ± 15.41c***	211.41± 20.17 b*	136.71 ± 11.24 c***	161.11 ± 13.76 c***
Insulin (μ/l)	7.31 ± 1.14 b	13.11 ± 2.15 a***	12.01 ± 2.17 a***	12.31 ± 7.18 a***	8.61 ± 1.03 b	13.01 ± 2.38 a***	10.14 ± 1.41 a**
Hemoglobin (gm/dl)	8.17 ± 0.88 b	12.16 ± 1.14 a**	11.99 ± 1.87 a**	12.33 ± 1. 41 a**	10.33 ± 1.71 a*	12.91 ± 2.14 a**	11.41 ± 1.33 a**
Packed cell volume	25.77 ± 3.45b	35.14 ± 4.71 a**	36.14 ± 4.82 a**	37.81 ± 3.51 a**	29.14 ± 3.99 b	37. 41 ± 3.71 a**	35.93 ± 4.01 a**

Significant with control group * P<0.05 **   P<0.01 *** P<0.001

Mean values in each raw having different superscript (a, b, c) denote significant difference.

Table (3): Mean values ± SD of liver GPX, SOD and MDA of the experimental rats groups.

Group / Variables	Control	Antidiabetic drug	Chromium	High protein	Low protein	High protein with Chromium	Low protein with Chromium
GPX (nmol/ mg tissue)	90.44± 5.77 a	130.17 ± 11.28a***	135.32 ± 12.46 a***	139.41 ± 13.71a***	68.91 ± 8.16 c*	141.31 ± 10.11a***	137.14 ± 12.40 a***
SOD (µ/mg protein)	66.99± 8.11 b	101.71 ± 10.11 a**	111.71 ± 11.21a***	103.14 ± 12.41 a**	65.67 ± 6.71 b	120.33 ± 11.32 a***	109.15 ± 9.99 a**
MDA (nmol/ mg tissue)	26.45± 2.41a	15.13 ± 1.33b**	11.14 ± 1.15 b***	12.41 ± 1.80 b***	21.98 ± 3.40a	10. 84 ± 1.14 b***	13.41 ± 1.91 b**

Significant with control group * P<0.05 **   P<0.01 *** P<0.001

Mean values in each raw having different superscript (a, b, c) denote significant difference.

Table (4): Mean values ± SD of serum AST, ALT, AP and γGT ratio of the experimental rats groups.

Groups / Variables	Control	Antidiabetic drug	Chromium	High protein	Low protein	High protein with Chromium	Low protein with Chromium
ALT (µ /ml)	45.22 ± 5.32a	27.96 ± 5.11 b***	33.14 ± 4.19b**	30.21 ± 3.42 b**	40.33 ± 5.17a	28. 14 ± 4.22 b***	32.40 ± 3.61b**
AST (µ /ml)	59.17 ± 6.14a	38.14 ± 4.22 b***	37.88 ± 3.13 b***	35.11 ± 3.61 b***	55.71 ± 6.17a	34.14 ± 3.20 b***	39.14 ± 3.17 b**
AP (µ /ml)	99.33 ± 10.13 a	79.33 ± 8.61 b*	66.22 ± 7.11 b**	65.14 ± 7.14 b**	87.32 ± 9.12 a	61.17 ± 6.01 b**	67.45 ± 7.20 b**
γGT (µ /ml)	7.31 ± 1.53a	4.51 ± 0.88 b***	5.32 ± 1.11 b**	4.99 ± 1.01 b**	6.91 ± 1.31 a	4.81 ± 0.69 b***	4.71 ± 0.78 b***

Significant with control group * P<0.05 **   P<0.01 *** P<0.001

Mean values in each raw having different superscript (a, b, c) denote significant difference.

Table (5) Mean values ± SD of serum total protein, albumin, globulin and A/G ratio of the experimental rats groups.

Variables \ Groups	Control	Antidiabetic drug	Chromium	High protein	Low protein	High protein with Chromium	Low protein with Chromium
Total protein (g/dl)	5.11 ± 1.02b	6.80 ± 1.13b	6.81 ± 1.21b	7.61 ± 1.30 a*	5.23 ± 1.10b	7.78 ± 1.31a*	6.55 ± 1.01b
Albumin(A) (g/dl)	3.20 ± 0.45a	3.41 ± 0.44a	3.61 ± 0.56a	3.81 0.77 a	3.11 ± 0.60a	3.45 0.55a	3.21 ± 0.33a
Globulin(G) (g/dl)	1.91 ± 0.33b	3.39 ± 0.56a**	3.20 ± 0.77a**	3.80 ± 0.87a**	2.12 ± 0.16 a*	4.33 ± 0.34a***	3.34 ± 0.35a**
A/G ratio	1.67 ± 0.15a	1.01 ± 0.13a	1.12 ± 0.11a	1.01 ± 0.03a	1.46 ± 0.21 a	0.79 ± 0.20b*	0.96 ± 0.11b*

Significant with control group * P<0.05 **   P<0.01 *** P<0.001

Mean values in each raw having different superscript (a, b, c) denote significant difference.

Table (6) Mean values ± SD of total bilirubin, creatinine, urea and uric acid of the experimental rats groups.

Variables \ Groups	Control	Antidiabetic drug	Chromium	High protein	Low protein	High protein with Chromium	Low protein with Chromium
Total bilirubin (mg/dl)	1.53 ± 0.22a	1.11 ± 0.02 ab	0.99 ± 0.11b**	0.98 ± 0.12 b**	1.41 ± 0.18 a	0.97 ± 0.17b**	0.98 ± 0.33b**
Creatinine (mg/dl)	1.14 0.01a	0.55 ± 0.03b**	0.65 ± 0.04b**	0.67 ± 0.01b**	0.98 ± 0.03a	0.59 ± 0.04 b*	0.71 ± 0.05b*
Urea (mg/dl)	39.77 ± 4.01a	25.67 ± 2.31b**	29.11 ± 2.41b**	28.31 ± 2.81b**	27.11 ± 2.77 b**	26.33± 4.25b**	25.76± 3.44a
Uric acid (mg/dl)	4.57 ± 1.01a	3.01 ± 0.99ab	2.99 ± 0.88b*	3.14± 1.11ab	2.66 ± 0.87 b*	3.11 ± 0.80ab	2.71 ± 0.21b*

Significant with control group * P<0.05 **   P<0.01 *** P<0.001

Mean values in each raw having different superscript (a, b, c) denote significant difference.