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Anti-Diabetic Effect of Chitosan in Alloxan Induced Diabetic Rats.

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ABSTRACT

Diabetes mellitus (DM) is a complex and multi-various groups of disorders that disturb the metabolism. Chitosan as anti-diabetic agent was studied in diabetic rats. Five groups of rats (Sprague-Dawley) (8 rats for each group) were used, group 1 used as normal control fed on a standard diet whereas groups 2, 3, 4 and 5 were diabetic rats, group 2 was diabetic control. Which fed on a standard diet group 3 rats fed on semi modified Chitosan 5% in diet treatment but group 4 rats ingested 5g chitosan suspended in water by stomach tube (twice weekly) and group 5 rats were drinking drug in water. Feeding period continued for 8 weeks. At the end of the experiment fasting blood samples were obtained from animals in all groups and analyzed for several biochemical parameters. The results showed that rats in groups 3, 4 and 5 attained more body weight than group 2. Serum glucose, malondialdhyde, total lipids, total cholesterol, LDL cholesterol and triglycerides were all increased in diabetic control group. The total antioxidant capacity and HDL cholesterol were decreased. Also liver and kidney functions were elevated. Addition of either chitosan to the diet or diamicron drug in water caused a marked improvement of all three parameters and returned back to near normal values. The conclusion is chitosan supplementation can protect against health hazards exerted due to diabetic mellitus.

Keywords: diabetes mellitus, alloxan, chitosan, drug (diamicron), liver function, kidney functions.

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INTRODUCTION

In recent years many reports have focused on chitosan and its variety of biological activities such as antidiabetic, antihyperlipidemic, antioxidant, antimicrobial, anti inflammatory, anticancer, and immune-stimulating effects; chitosan offers a number of uses in food, cosmic, biomedical, and pharmaceutical industries.

Many diseases contribute major public health problems throughout the world. Among these diseases are diabetes mellitus and cardiovascular diseases. Chitosan is safe and friendly substance for the human organism. Natural polymer composed of randomly distributed β-(1-4) - linked glucosamine residues. It has been used in several areas such as biomedical, pharmaceutical, biotechnological fields as well as in the food industry and textile finishing industrials [28].

Diabetes mellitus (DM) is a chronic disease with complex underlying etiologies. It is a group of metabolic disorder characterized by elevated blood glucose level resulting from the defects in insulin secretion, insulin action or both. The incidences of diabetic mellitus are on the rise world wise. Based on the health organization [25] report, the number of diabetic patients is expected to increase from 171 million in year 2000 to 366 million or more by the year 2030.

The International Diabetic Foundation estimates that 366 million adults aged 20-79 were affected by diabetes worldwide in 2011 and this figure is expected to rise 522 million by the year 2030 with most of the increase coming from developing countries.

Furthermore, a statistical survey from the regional office of World Health Organization for the Eastern Mediterranean Countries [25] indicated that the prevalence of percentage total death diabetes mellitus in Egypt was 3% of the part population, Bahrain 12%, Iraq 1%, Jordan 7%, Saudi Arabia 6%, Syria 3%, Iran 2%, and Kuwait 4%, group aged (all ages).

Type-1 diabetes [insulin-dependent diabetes mellitus (IDDM)] is characterized by autoimmune-mediated destruction of pancreatic β cells culminating in absolute insulin deficiency. It has been predicted that between 2005 and 2020, new cases of type-1 diabetes in European children younger than 5 years will double and that the prevalence of cased in those younger than 15 years will increase by 70%.

People with type-1 diabetes require daily insulin treatment to sustain life. The treatment by injection of insulin usually incurs pain and may negate the quality of life if diabetic patients. The alternative routes for insulin delivery, such as oral and nasal pathways have been explored over the years.

Type-2 diabetes mellitus, also known as non insulin dependent diabetes mellitus (NIDDM), develops in middle or later life and affects 2-6% of adults in most Western societies. World Health Organization [24] estimates that more than 220 million people worldwide have diabetes and this number is likely to double by 2030.

Furthermore, a statistical survey from the regional office of World Health Organization for the Eastern Mediterranean Countries [24] indicated that the prevalence of hypercholesterolemia in Egypt was 19.4% of the whole population, Bahrain 40.6%, Iraq 37.5%, Jordan 36%, Saudi Arabia 19.15% Syria 34%, Iran 43.6% and Kuwait 38.6%, group aged (14-64 years).

MATERIALS AND METHODS

Materials:

- Shrimp shells were obtained from Obour market; the wastes were washed and drayed then, packed in plastic bags and stored at 20-25°C until using.
- Reagents: all kits were purchased from bio-diagnostic company, Egypt.

Methods:

Chitosan preparation:

Isolation of chitin and preparation were prepared according to the method described as the following.

A- Deproteinisation with 3% NaOH for 30 min at 121°C and a solid/solvent ratio of 1:10 (w/v).Then, Demineralization by: 1 N HCl for 30 min at room temperature and a solid/solvent ratio of 1:10 (w/v) according by [5].
B- Deacetylation conditions:45 % NaOH for 30 min and a solid/solvent ratio of 1:10 (w/v) then, dried after washing according by [6].

Experimental animals:

Forty Sprague-Dawley male albino rats weighing 130±10g were obtained from laboratory animal house National Research Centre, Egypt. The animals were housed in stainless steel cages in a controlled environment (25±4 °C, 50-60% relative humidity and 12-hour light-dark cycle). The animals were fed with a basal diet and water for two weeks (adaptation), and were then randomly assigned to 5 groups (8 rats each). Animal experiments were conducted according to the guidelines of Animal Care and Ethics Committee of the Department of Biochemistry, Faculty of agriculture Cairo University, Egypt.

Induction of hyperglycemia in rats:

Hyperglycemia was induced in the rats by intra-peritoneal injection of 5% solution of alloxan in saline (125mg/kg body weight) stated that by [7]. Before induction of hyperglycemia fasting blood samples were taken from rats for estimation the levels of blood glucose.

After two days of the induction blood sample was taken for analysis of fasting blood glucose which follow up for 3 weeks to insure the induction of hyperglycemia. For recorded the initial reading blood samples were taken for estimation of serum total lipid, triglycerides, total cholesterol, HDL-cholesterol, ALT, AST, ALP activity, creatnine and urea.

Experimental design:

Thirty two of diabetic rats were divided into 4 groups each contain 8 rats. In addition of 8 normal rates were fed basal diet without any treatment (normal control). All the rats were kept in stainless steel cages. The diets and water were given free for 8 weeks. The body weight and food consumption during the experimental period were followed weekly.

Group 1: (normal control): normal rats fed on basal diet consisting of corn starch 65%, casein 15%, and corn oil 10%, salt mixture 4%, vitamins mixture 1% and cellulose 5% described [2].
Group 2: (diabetic control): diabetic rats fed on basal diet.
Group 3, 4 and 5 were diabetic rats fed on basal diet. Group 3 fed on semi- modified chitosan 5% in diet. Group 4 rats ingested chitosan 5g suspended in water by stomach tube (twice weekly), twice weekly into diabetic rats. Group 5 using drug (diamicron) 1 tablet 30 mg daily was added to drinking water.

Blood sampling:

After 8 weeks (experimental period), the rats were fasted overnight. All the animals were scarified by decapitation. Blood samples were collected from each rat from the retro-orbital vein and were received into clean dry centrifuge tubes. Serum was separated by centrifugation at 3000 rpm for 15 minutes and kept in deep-freezer at -20°C until used for estimation of glucose level, total lipid, triglycerides, total cholesterol, HDL-cholesterol, LDL-cholesterol, serum ALT, AST, ALP activity, creatinine, urea, malondialdhyde and total antioxidant capacity.

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Methods:

Body weight gain and food consumption were measured according to [9]. Using the following equation:

BWG = final body weight – initial body weight
Feed efficiency ratio (FER) = weight gain (g)/Food intake (g)

Serum glucose was determined according to [22]. Serum total lipids (TL), total cholesterol (TC) and high density lipoprotein cholesterol (HDL-C) were determined according to [12-1] and [3] respectively. Low density lipoprotein cholesterol (LDL-C) and very low density lipoprotein cholesterol (VDL-C) was done by [15].

Determination of liver function biomarkers:

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were measured calorimetrically in serum according to the method described by [18]. Alkaline phosphatase (ALP) activity in serum was determined according to [17].

Determination of kidney biomarkers:

Urea content was determined according to the method described by [16]. Cratinine level content determined according to the method described by [13]. Determination of lipid peroxide (Malondialdehyde) Lipid peroxide was determined according to the method described by [20].

Statistical analysis:

Statistical analysis, data were obtained for different variables analyzed using ANOVA analysis which stated by [4].

RESULTS AND DISCUSSION

Body weight gain, food intake and feed efficiency ratio:

Mean values of body weight gain, food intake and feed efficiency ratio of the different experimental groups are shown in Table (1).

Table 1: body weight gain, food intake and feed efficiency ratio of the different experimental groups.

Parameters Treatments	Body weight gain BWG)(g)	Food intake (F1) (g)	Food efficiency ratio(FER)
Normal control	70.54±5.11 a	534.96±40.12 a	0.12±0.011 a
Diabetic control	58.43±3.71 b	565.60±51.22 a	0.01±0.001 b
Chitosan 5%	74.34±3.11 a	553.73±44.71 a	0.12±0.012 a
Ingestion	73.50±4.97 a	556,00±39.99 a	0.12±0.010 a
Drug	73.74±4.21 a	563.11±49.21 a	0.12±0.011 a

All values are represented as mean ±S.E. Means with different letters are significantly different (P<0.05).

Tabulated data show that diabetic rats (diabetic control) had significant lower gain in body weight BW (58.43±3.71g) as compared to normal control (70.54±5.11g). Taking chitosan by different ways (chitosan 5% in diet and ingestion by stomach tube 5g chitosan suspended in water) and drug in drinking water (diabetic groups 3, 4 and 5) had significant high body weight gain (74.34±3.11, 73.50±4.97 and 73.74±4.21 g respectively) as compared to diabetic control group. However here were no significant differences between the three diabetic treated groups. Concerning food intake (F1) results revealed that all groups showed no significant differences between each other during the experimental period (8weeks). The present results showed that addition of chitosan significantly increased feed efficiency ratio (FER) as compared to diabetic control group. Chitosan significantly improved BWG and FER of the diabetic animals (3 .4 and 5) as compared to diseased control group.

Diabetes is characterized by lower body weight gain and that was observed in the present study and this result is in agreement with [14] chitosan be acted by reducing the absorption of cholesterol and fat, subsequently increasing total fecal weight, fecal fat excretion and fecal water excretion, thereby reducing body weight gain. Chitosan oligosaccharides (CO) were proved to inhibit the differentiation of adipocytes. It has an anti-diabetic effect on mice, by means of differential proteomic analysis of plasma. This was followed by immunoblotting, and gene expression in adipose tissue to clarify the molecular mechanism. Chitosan might act as a potent down-regulator of obesity-related gene expression in mice that may normalize altered plasma proteins to overcome metabolic disorders of obesity [8].

Serum glucose (mg/dl), total antioxidant capacity (mM/l) and malondialdehyde (nmol/ml) of the different experimental groups in Table (2).

Blood glucose, total antioxidant capacity and malondialdehyde:

Data in Table (2) show mean values of serum glucose, total antioxidant capacity and malondialdehyde of the experimental rats. Tabulated data illustrated that serum glucose level of normal control (G1) was significantly lower (P<0.05) when compared with diabetic control G2 were which 63.21±4.33 and 106.07 ±6.12mg/dl respectively. On the other hand treated diabetic groups G3, G4 and G5 which fed on semi-modified diet containing 5% chitosan and ingested chitosan as well as drug showed significant decrease (P<0.05) in serum glucose level as compared to the diabetic control group (106.07±6.12, 66.63±5.99, 64.43±5.71 and 76.28±4.44 mg/dl respectively). The best response for serum glucose was obtained in groups 4(ingested chitosan) followed by group 3 fed on chitosan 5% and group 5(drug treatment) respectively. With regard to the effect of feeding chitosan on serum level of total antioxidant capacity (TAC) in diabetic rats, the present data demonstrated that diabetic control group had significant lower value in total antioxidant capacity. The previous results show that the treatments with chitosan and drug diseased for rats significantly improved serum glucose level.

Table 2: Serum glucose (mg/dl), total antioxidant capacity (mM/l) and malondialdehyde (nmol/ml) of the different experimental groups.

Parameters Treatments	Glucose (mg/dl)	Total Antioxidant capacity (mM/L)	Malonaldhyde (nmol/ml)
Normal control	63.21±4.33 c	1.40±0.10 a	3.10±0.20 b
Diabetic control	106.07±6.12 a	1.20±0.08 b	4.10±0.24 a
Chitosan 5%	66.63±-5.99 c	1.30±0.10 ab	1.69±0.17 d
Ingestion	64.43±5.71 c	1.45±0.11 a	2.30±0.19 c
Drug	76.28±4.44 b	1.46±0.11 a	2.33±0.20 c

All values are represented as mean ±S.E. Means with different letters are significantly different (P<0.05).

These results are in agreement with [23] who treated diabetes with gliclazide (diamicron drug) which is a second generation of hypoglycemic sulfonylurea and acts selectively on pancreatic beta cell to control diabetes mellitus. Chitosan beads were produced by dispersion technique using tripolyphosphate (TPP) as gelating agent. The effects of process variables including chitosan molecular weight, concentration of chitosan can make the blood glucose lowering effect in streptozotocin-diabetic rats. [26] Reported that chitosan did not affect plasma in normal rats. Significantly decreased plasma glucose was observed in diabetic rats fed with high molecular weight (MW) chitosan diet than animals fed with cellulose diet. However, no statistical significant difference in plasma glucose and total cholesterol was observed in diabetic rats fed with low MW chitosan.

The same table show the antioxidant capacity (1.40±0.10 m M/L) of normal control was higher than that of the diabetic control (1.20±0.08mM/L) chitosan treatments found that group3, 4 and5 show significant differences (1.20±0.08,1.30±0.10,1.45±0.11 and1.45±0.11 mM/L respectively) between each other compared with normal control. Concerning malondialdehyde level (MDA), data revealed that diabetic control group showed significant increase in MDA level as compared to the normal control (4.10±0.24 and 3.10±0.20nmol/ml, respectively). Diabetic groups 3, 4 and 5 treated with chitosan and drug showed significantly decreased to the level of MDA 1.69±0.17, 2.30±0.19 and 2.33±0.20nmol/ml respectively as compared to the diabetic control (4.10±0.24nmol/ml). The similar results also, showed in study of [27] the

antioxidant effect of chitosan (COSs) on pancreatic islet cells was detected under optical microscopy and with colorimetric assay and gel electrophoresis. The activities of glutathione peroxidase and superoxide dismutase, total antioxidant capacity, and content of malondialdehyde in serum and tissue slices of pancreas were examined after 60 days to determine the effect of COSs in streptozotocin-induced diabetes in rats. The results showed that COSs can prohibit the apoptosis of pancreatic islet cells. All concentrations of COSs can improve the capability of total antioxidant capacity and activity of superoxide dismutase and decrease the content of malondialdehyde drastically. Morphological investigation in the pancreas show that COSs have resulted in the reduction of islets, loss of pancreatic cells, and nuclear pyknosis of pancreatic cells [26].

Lipid profile:

The values of serum total lipid profile, total cholesterol, total cholesterol and triglycerides and lipoprotein fraction (LDL-C, HDL-C and vLDL-C) of the different groups are shown in table (3).

Table 3: Mean values of lipid profile and lipoprotein fractions of different experimental groups.

Parameters Treatments	T.lipid (mg/dl)	T.cholesterol (mg/dl)	Triglyceride (mg/l) [vLDL-C]	LDL-C	HDL-C
Normal control	612.66±41.01 c	188.12±10.01 b	202.41±12.21 b	35.703±3.00 b	39.816±2.01 a
Diabetic control	935.25±59.12 a	246.84±14.27 a	249.90±17.21 a	47.817±3.01 a	31.584±2.11 b
Chitosan 5%	729.54±41.11 b	190.8±11.11 b	144.69±10.22 d	32.895±2.35 b	44.6±2.99 a
Ingestion	736.98±39.12 b	179.22±10.23 b	169.08±9.99 c	33.867±1.99 b	40.008±3.01 a
Drug	648.78±41.12 c	183.3±11.00 b	137.43±7.89 d	30.168±1.11 b	43.904±2.77 a

All values are represented as mean ±S.E. Means with different letters are significantly different (P<0.05).

The values reported that serum total lipid was 612.66±41.01mg/dl for normal control group but was 935.25±54.12 mg/dl for diabetic control group. Diabetic rats fed on chitosan diet 5 % (G3). Ingested Chitosan (G4) and group received the drug with drinking water (G5) as diabetic treatments had improved value relative to both control. Serum total cholesterol was 188.12±10.01 mg/dl in normal control which was increased to 246.84±14.27mg/dl for diabetic rats (diabetic control) then, reduced to values around that of normal which were control 190.80±11.11, 179.22±10.23 and 183.30±11.00mg/dl for diabetic treated groups 3, 4 and 5 respectively. LDL-C and VLDL-c had the same trend, but HDL-C showed significant decrease value for diabetic control (31.58±2.11) relative to normal control (39.82±2.01). The different treatment improved the abnormal values of diabetic rats.

The values reported for serum triglycerides were 202.41±12.21, 249.90±17.21, 144.69±10.22, 169.08±9.99 and 137.43±7.89 mg/dl for the normal control and groups 2, 3, 4 and 5 respectively. It can be noticed that all lipid parameters except HDL-cholesterol significantly elevated in diabetic control group as compared to normal control. After using chitosan and drug treatments the alternation in lipid metabolism was partially modulated as evidenced by decrease levels of serum total lipid, total cholesterol triglycerides, LDL-cholesterol and vLDL-C levels and at the same time increased level of HDL-cholesterol in diabetic groups. Although there were no significant differences in the results of lipid parameters between groups 3, 4 and 5. This data are agreement with these of explanted by [26] who reported that, there is significant decreases in total cholesterol and increased in HDL cholesterol and fecal cholesterol excretion were observed in diabetic rats fed with high MW chitosan diet than animals fed with cellulose diet. However, no statistical significant difference in total cholesterol was observed in diabetic rats fed with low MW chitosan. Also, [19] reported that chitosan can favorably modulate plasma lipids. The author evaluated the effect of chitosan on plasma lipids and lipoproteins in 28 patients with plasma triglyceride levels >150 mg/dL (mean age: 63+/-12 years), not taking other lipid-lowering agents. All patients received a chitosan derived from fungal Mycelium (Xantonet, Bromatech, Italy) at a fixed dose of 125 mg/day in addition to their current medications for 4 months. Polyacrylamide gel electrophoresis was used to measure low-density lipoprotein (LDL) subclasses. After treatment, total cholesterol reduced by 8%, LDL cholesterol by 2%, and triglycerides by 19%, with a concomitant 14% increase in high-density lipoprotein cholesterol. They also found a beneficial effect of chitosan on LDL subclasses Also, [10] reported, the effects on chitooligosaccharides (COS) for the management of alloxan induced diabetes in mice. For the carbohydrate metabolism in diabetes by the COS, the amount of glucose in blood along with quantification of glycogen in liver were measured and noted a significant recovery

in respect to diabetic control group. As hyperlipidemia and oxidative stress are the disorders of diabetes so, they have also assessed the serum levels of total cholesterol (TC), triglyceride (TG), low density lipoprotein cholesterol (LDLc), very low density lipoprotein cholesterol (vLDLc) and high density lipoprotein cholesterol (HDLc). For the recovery of oxidative stress the SOD, MDA and catalase in liver and GOT and GPT activities in serum were measured. The COS results a significant recovery in the levels of above mentioned biosensors of lipid profile when treated to experimentally induce diabetic mice. The effect of COS at the dose of 10 mg/kg body weight was found to be a potent agent for diabetes and complication associated with this disease. The COS has no toxic effect in general which has been focused here by the monitoring of COS dose in normal healthy mice.

Liver and kidney function:

Results of liver and kidney functions of the different experimental groups are shown in table (4). Data in table 4 showed a significant increase of aspartate amino transferase (AST) activity in the diabetic control (52.93±3.77 IU/L) as compared to normal control group (39.72± 2.99 IU/l). Treatments with chitosan and drug lead to alternation of AST activity in these groups. AST activity significantly decreased in all groups compared with diabetic control. The most decrease was obtained in group 4 (38.91± 2.78 IU/l), followed by group 5 (41.48± 2.99 IU/L) and group 3 (43.47± 3.01 IU/L). With regard to alanine amino transeferase level (ALT) data show significant increase in diabetic control group (57.88± 4.44 IU/L) as compared to normal control (37.66± 2.97 IU/L). In our experiment, (G 3 of 2.58 ±1.98, 4G of 41.74 ±.3.00 and 5G of 33.20± 5.00 IU/L) exhibited significant decrease in ALT activity as compared to diabetic control group.

Table4: Liver and kidney functions of the different experimental groups.

Parameters Treatments	AST (IU/L)	ALT (IU/L)	ALP (mg/L)	Urea (mg/dl)	Creatinine (mg/dl)
Normal control	39.72±2.99 b	37.66±2.97 bc	87.54±5.21 b	27.46±1.97 b	0.10±0.008 c
Diabetic control	52.93±3.77 a	57.88±4.44 a	142.09±11.11 a	52.81±3.21 a	1.75±0.11 a
Chitosan 5%	43.47±3.01 b	22.58±1.92 d	76.92±4.01 b	26.23±2.0 b	1.00±0.07 b
Ingestion	38.91±2.78 b	41.74±3.00 b	83.80±5.21 b	30.21±2.10 b	1.10±0.09 b
Drug	41.48±2.99 b	33.20±2.13 c	81.33±5.00 b	27.42±2.01 b	0.95±0.05 b

All values are represented as mean ±S.E Means with different letters are significantly different (P<0.05).

Alkaline phosphatase (ALP):

The enzyme activity shows a significant increase in diabetic control group (142.09±11.11 IU/L) as compared to normal control group (87.54I±5.21 IU/L). Treatments with chitosan and drug for diabetic rats significantly decreased ALP activity which were 76.92±4.01, 83.80±5.21and 81.33±5.00 IU/L respectively for groups 3G,4G and 5G as compared to diabetic control, the changes in activity of AST, ALT, ALP are directly related to metabolism in which the enzymes are involved. [11] studied the develop and characterize a chitosan gel/gelatin microsphere (MSs) dual delivery system for sequential release of bone morphogenetic protein-2 (BMP-2) and insulin-like growth factor-1 (IGF-1) to enhance osteoblast differentiation and also, They found that significantly greater alkaline phosphatase activity was found in cells treated with the sequential delivery system compared with other treatments (P<0.05) after a week of culture.

Data concerning kidney function are shown also in the same table (4). Serum urea level in diabetic rats (52.81±3.21) was very high when compared with normal rats 27.46±1097 and diabetic rats treated with chitosan. Showed significant decrease for group 3G was 26.23±2.00 and was 4G 30.21±2.01 but G5 of drug was 27.42±2.01 urea. Creatinine values were decreased in diabetic rats treated by chitosan and drug as compared to the diabetic control (1.753±0.11).There was no significant between 3G (1.00±0.07), 4G (1.10±0.09) and 5G (0.95±0.05) treatments but the values still more than that of normal control. [21] Showed effects of dietary supplementation of chitosan (COS) and galacto-mannan-oligosaccharides (GMOS) on some serum biochemical indices .Dietary supplementation of COS and GMOS increased (P<0.05) the serum growth hormone (GH) and insulin like growth factor-1 (IGF-I) levels along with enhanced hepatic and the muscle IGF-I mRNA abundance. Dietary supplementation of oligosaccharides such as COS and GMOS may improve growth and feed conversion efficiency by increasing plasma GH and IGF-I levels, in the early-weaned piglets.

CONCLUSION

Our data have shown that chitosan can improve blood glucose level, lipid profile, liver and kidney functions and decreasing oxidative stress.

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