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MOLECULAR STUDIES ON SELENIUM NANOPARTICLES IN DIABETES

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ABSTRACT

The fundamental characteristic of selenium nanoparticles have given it a great priority to the attention of scientists and researchers, particularly in their medical applications, selenium nanoparticles identified as an antioxidant and also to protect the body and get rid of the harmful damage of free molecules resulting from oxygen metabolism, A vital and important role where it has been proven in a laboratory that it shields the body from the danger from the risk of heart illness, diabetes and cancer tumors. Therefore, this study was concerned the possible protective effect of CTS-SeNPs and Actozone drug in Sprague Dawely male rats. The experiment was applied on fifty Sprague Dawely male rats (140-160 gm) were prepared for this experiment. They were brought from Laboratory Animal Farm of the Faculty of Veterinary Medicine, Zagazig University. Results revealed that CTS-SeNPs + Actozone group had the highest body weight than other groups. Diabetic group revealed the highest glucose, liver enzymes activities, lipid profile and G6PD values and group treated with CTS-SeNPs showed significant decrease in values compared to the diabetic group. Diabetic group was the lowest insulin reading, total protein, albumin, globulin, hepatic oxidant/antioxidant, Pdx1 and Ngn3 genes levels and group treated with CTS-SeNPs showed significant increase in values compared to the diabetic group. Histopathological examination of all liver & pancreas specimens diabetic treated with CTS_SeNPs + actozone group; liver & pancreas of rat showed almost normal histological picture except for mild vascular congestion. In conclusion the results of this investigation demonstrated that DM-induced severe biochemical and histopathological changes in the liver and pancreas and CTS-SeNPs have a protective effect and minimize the risk of diabetic complications.

KEYWORDS: *Diabetes Mellitus, CTS-SeNPs, Actozone, Streptozotocin.*

INTRODUCTION

Diabetes Mellitus (DM) is a metabolic disease characterized by hyperglycemia and disability to preserve on blood glucose level homeostasis.[1] The International Diabetes Federation (IDF) documented that Egypt is in between the world top 10 countries in the number of patients with diabetes. It is predictable that the number of patients with diabetes in the Middle East and North Africa region to grow by 96% from year 2013 to 2035 or from34.6 million to 67.9 million.[2]

Nanotechnology defined as the engineering and manufacturing of materials at the atomic and molecular scale leading to the construction of materials in the nanometer scale size range (often 100 nm or smaller), without changing ultimate characters. The physical and chemical properties of materials can considerably improve or change as their size is decreased into small clusters of atoms. Small size defined as diverse arrangements and spacing for surface atoms, and these govern the object's physics and chemistry.[3]

Se is an important element with a high nutritional value and very essential role for most of physiological interactions in human body.[4] It is obvious that selenium nanoparticles (SeNPs) have been known as promising tool in drug therapies of type 2 diabetes; this is due to its low toxicity and high therapeutic characters.[5]

Chitosan, the N-deacetylated of chitin, is a rich natural polysaccharide.[6] Chitosan is studied to showed great bioactivities, such as antitumor, antibacterial, hypocholesterolemic, and antihypertensive characters. It has been investigated that chitosan-based agents are highly powerful nutraceuticals for treatment and inhibit of diabetes and its complications.[7] It has also been documented that CTS could be a good stabilizer for SeNPs.[8]

Pioglitazone is a TZD which stimulate insulin sensitivity, decline blood glucose and hemoglobin A1C levels, hinder adipose tissue lipolysis, decrease microalbuminuria, inhibit inflammation, and decrease blood pressure in experimental animals and in TZD treated patients.[9]

Streptozotocin (STZ) was initially isolated from Streptomyces achromogenes in 1960, with its diabetogenic characters this action was explained by Kataoka et al.[10] showing that the diabetogenic properties are due to selective destruction of pancreatic islet β-cells. The majority of diabetic patients suffer from type 2 diabetes (T2D) so rats were injected by nicotinamide before STZ to partially protect the β-cells against STZ. This compound combination produces a model of insulin deficient, but not insulin resistant.[11]

Pancreatic and duodenal homeo box gene 1 (Pdx1) in the adult pancreas, is essential in preserving β-cell function. It activates transcription of several genes involved in glucose homeostasis such as insulin, glucokinase and glucose transporter GLUT2.[12] Neurogenin 3 (Ngn3) acts as essential function in the synthesis of pancreatic endocrine precursors.[13]

Therefore, the present work aimed to investigate the anti-hyperglycemic activity of chitosan-stabilized selenium nanoparticles (CTS-SeNPs) compared to Actozone drug in streptozotocin induced diabetic rats and noted difference in body weight all over the experiment, determination of some biochemical parameters, gene expression levels for Pdx1 and Ngn3 genes of pancreatic beta cells and histopathological examination from pancreas and liver tissues.

MATERIALS AND PROCEDURES

Experimental animals

Fifty Sprague Dawely male rats (8 weeks of age), weighting from (140-160) gm were purchased from the Laboratory Animal Farm at Zagazig University. All rats were kept in cages made of stainless steel in a pathogen free environment at a controlled temperature (21–24ºC) with a relative humidity of (50–60%) and a 12 hour light–dark cycle. The rats were adapted 2 weeks before to use in any study. All experimental steps were applied according to the NIH general guidelines for the Use and Care of the Laboratory Animals in scientific investigations.

Chemicals

1-2-1 Nano–sized particles of CTS-SeNPs were done by a adjusted steps.[14]

I-2-2 Actozone oral drug: Each tablet contains Pioglitazone as (hydrochloride) 30 mg (Amoun Pharmaceutical Company, Egypt).

I-2-3 STZ was dissolved in freshly prepared citrate buffer 0.71g of sodium dibasic phosphate in 100 ml D.W. titrated against 0.96 g of acetic acid in 100 ml till extent the pH of 4.5 then injected I/P (Sigma-Aldrich, Chemical Cp. St. Louis, Mo, USA).[15]

1-2-4 Nicotinamide is a vitamin B3 (niacin) derivate with antioxidant capacity which reduces cytotoxic actions of STZ that dissolved in normal saline and is usually administrated I/P.[16]

Synthesis of CTS-SeNPs

According to Chen et al.[14] CTS-SeNPs were synthesized as following 5 ml CTS solution (CTS dissolved in 4% HAc) was mixed with ascorbic acid (5 ml, 0.01 M) under magnetic stirring, and then, ultrasonic application. Followed by adding freshly prepared ascorbic acid solution (5 ml) and then we add NaOH and HAc in order to adjust the pH. The residual Na2SeO3 was removed by dialysis against Milli-Q water at 4 °C, we detect by inductively coupled plasma-mass spectrometry (ICP-MS, Perkin Elmer Nexion 300, USA) analysis, the absence of Se in the outer solutions it means that the liquid phase of CTS- SeNPs was prepared eventually. Furthermore, the way to synthesis SeNPs was the same to above method, but there was a need to replace aqueous solution of CTS with the equal volume of Milli-Q water.

Characterization and measurements

The CTS-SeNPs synthesized were characterized via several methods. We detected the morphology of the SeNPs in the presence and in the absence of CTS by TEM (Zeiss Libra 200FE, Germany) and SEM (FEI-MLA Express 2, America). DLS analysis with a Nano ZS instrument (Nano- Zs90, UK) was used to determine of the mean diameter.

Experimental induction of diabetes

Overnight fasted adult albino rats (n=40) were rendered diabetic via administration of 100-500 mg/kg of Nicotinamide that injected I/P 15 min before conduction a single I/P injection of STZ (60 mg / Kg) dissolved in cold citrate buffer (pH 4.5). The rats were permitted to drink 5 % glucose solution all overnight to defeat drug-induced hypoglycemia. Levels of blood glucose were evaluated 2 consecutive days after STZ injection by making an injury to the tip of the tail, squeezing it carefully and by the glucose oxidase procedure. Rats were believed to be diabetic if glycaemia was 200 mg/dl or more, with monitoring initial and final glycaemia during wound healing period. Rats in the control group (n=20) were injected with the saline alone (0.01 M citrate buffer, pH 4.5).[17]

Experimental design

50 albino male rats were divided into five groups each group contains 10 rats , at which 10 rats administrated normal saline only, while 40 rats administrated streptozotocin.

Group 1: a normal control group in which the rats received normal saline

Group 2: a diabetic control group in which the rats were diabetic using streptozotocin (60 mg /kg).[18]

Group 3: STZ-induced diabetic rats which treated orally with CTS-SeNPs (2 mg/kg).[19]

Group 4: STZ-induced diabetic rats which treated with a standard oral hypoglycemic agent, Actozone drug from

(Amoun Pharmaceutical Company, Egypt) (20 mg/kg b.wt).[20]
Group 5: STZ-induced diabetic rats which treated with Actozone drug 20 mg/kg b.wt) and CTS-SeNPs (2 mg/kg) (combination group).[19]

Sampling collection

Blood Sample

Blood samples were obtained from orbital venous plexus were obtained into screw capped tubes containing sodium fluoride for glucose test and tubes without anticoagulant for the rest of the tests then separated by centrifugation at 3000 r.p.m for 15 minutes. Then stored at 2-8^{o}C until used.[21]

Tissue specimens

At the end of experiment, a part of pancreatic tissue were coiled in aluminum foil and put immediately in liquid nitrogen container to do snap-freezing of tissue and reduce the action of endogenous RNase, for real time-PCR analysis analysis.

Another section of pancreatic & liver tissues were fixed at 10% buffered formalin solution at room temperature for 24 hour and dehydrated in ascending alcohol series and emerged in paraffin wax. Approximately 5 µm thick sections were stained with hematoxylin-eosin (H&E) for general morphology and histopathological evaluation.

Preparation of liver homogenate: one gram of the liver tissue rinsed in distilled water then put in homogenized buffer and homogenized alone using a Dounce homogenizer at 4 ^{o}C. The crude tissue homogenate then centrifuged at 3000 r.p.m for 15 min at 4^{o}C and the supernatant was collected and stored at -20^{o}C till estimation of malondialdehyde (MDA), reduced glutathione (GSH), glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT).

Evaluation of body weight

The rats were individually weighed at first day of age to obtain the initial body weight, and then the body weight was recorded every week to calculate the body weight development of the rats of each group.

Biochemical determinations

Quantitative determination of glucose

Glucose oxidase (GOD) turn glucose into gluconic acid. Hydrogen peroxide (H2O2) formed and evaluated by achromogenic oxygen acceptor, phenol, 4 – aminophenazone (4-AP) in the presence of peroxidase (POD). The intensity of the color formed is proportional to the glucose concentration in the sample.[22]

Determination of Insulin in serum (INS)

Insulin was evaluated according to Unger et al.[23]

Determination of liver functions

Evaluation of liver enzyme activities: Alanine aminotransferase (ALT) and Asparate aminotransferase (AST) were evaluated colorimetrically by Reitman and Frankel,[24] Alkaline phosphatase activity (ALP) determined according to Belfield and Glodberg.[25]

Determination of protein profile

Evaluation of total protein according to Henry,[26] Serum albumin and globulin were evaluated according to Doumas et al.[27]

Determination of lipid profile

Evaluation of serum cholesterol level was done according to colorimetric procedure that described by White et al.[28] Serum triglycerides (TG) level was done according to colorimetric procedure that described by Wahlefeld and bergmeyer,[29] High Density Lipoprotein cholesterol (HDL-C) was evaluated according to the procedure described by Sugiuchi et al.[30] Low Density Lipoprotein cholesterol (LDL-C) and Very Low Density Lipoprotein (VLDL) were evaluated according to the procedure described by Giurgea et al.[31]

Determination of antioxidant enzymes

Determination of GPx activity determined according to the procedure described by Paglia and Valentine,[32] Oxidized glutathione (GSSG) produced by peroxidase activity is couple to a reaction catalyzed by glutathione reductase.

GSH evaluated according to the procedure of Beutler et al.[33]

Estimation of MDA concentration as a marker of lipid peroxidation according the procedure adapted by Esterbauer et al.[34]

Estimation of SOD activity in liver homogenate. According to the procedure adapted by Misra and Fridovich.[35]

Estimation of CAT activity in liver homogenate according the procedure adapted by Sinha.[36]

Determination of glucose-6-phosphate dehydrogenase (G6PD)

G6PD estimated in liver homogenate according the procedure adapted by Tian et al.[37]

Molecular determinations

Total RNA was extracted using PureLink® RNA Mini Kit purchased from Ambion by life technologies by Thermo Scientific, Catalog numbers: 12183018A and using the manufacture instructions. The purity of RNA samples were checked using NanoDrop® ND-1000 Spectrophotometer, NanoDrop Technologies Wilmington, Delaware, USA. The Synthesis of cDNA was occurred by using High Capacity cDNA Reverse Transcription Kit purchased from Thermo Scientific, code 4374966. Real time PCR amplification was performed using Maxima SYBR Green qPCR Master Mix (2X) kit purchased from Thermo scientific, cataloug

#K0251, to detect the gene expression of PDX1 and Ngn3. The amount of target gene expression levels were quantified using the formula of $2^{-\Delta\Delta ct}$.[38] The primer sequences of the desired genes, was designed according to Table 1.

Table 1: Primers sequences used in preparations.[39]

Gene	Sequence	Frag. length (bp)	Ann. Temp. °C
PDX1	5'- TCC CAT GGA TGA AGT CTA CC-3'	246	60
	5'- TGT CCT CCTCCT TTT TCC AC-3'		
Ngn3	5'- CGC CGG TAG AAA GGA TGA C-3'	316	60
	5'- GAG TTG AGG TTG TGC ATT CG -3'		
GAPDH	5' -AGA AGG CTG GGG CTC ATT TG-3'	258	60
	5' -AGG GCC ATC CAC AGT CTT C-3'		

Histopathological examination of the liver and pancreas

Specimens from liver and pancreas tissues were obtained immediately after sacrificing animals from all groups and fixed in 10% neutral formalin before to routine processing in paraffin wax. Sections (5 um thick) were cut and stained using H&E and examined microscopically according to Suvarna et al.[40]

Statistical analysis

Data were described as mean ±SE. Arsine transformation performed for proportion type parameters (SOD and GSH) while square root transformation performed on MDA parameter values, to normalize data. One way ANOVA was applied to compare among means of groups (Control, Diabetic, Diabetic with CTS_SeNPs, Diabetic with Actozone drug and Diabetic with CTS_SeNPs + actozone drug). P-value < 0.05 considered statistically significant. Duncan's multiple range test was applied as Post hock test after significant ANOVA results to determine differences among groups at P < 0.05. Data were analyzed by SPSS version 24.[41]

RESULTS

Effect of CTS-SeNPs (2 mg/kg/b.wt) and Actozone drug (20 mg/kg/b.wt) in normal and induced diabetic rats on body weight (g).

Table (1) and figure (1) revealed the effect of CTS-SeNPs (2 mg/kg/b.wt) and Actozone drug (20 mg/kg/b.wt) in normal and induced diabetic rats on body weight. Our results revealed that there was highly statistically difference in body weight at 1st, 2d, 3rd, and 4th week of experiments (P < 0.01**). At all weeks of experiment the group treated with CTS-SeNPs + Actozone had the highest body weight followed by normal group that showed non-significant change in body weight compared to group treated with Actozone. Group treated with CTS-SeNPs showed non-significant change in body weight at 1 st week only compared to that treated with Actozone. Diabetic group showed the weakest (lowest) body weight among groups.

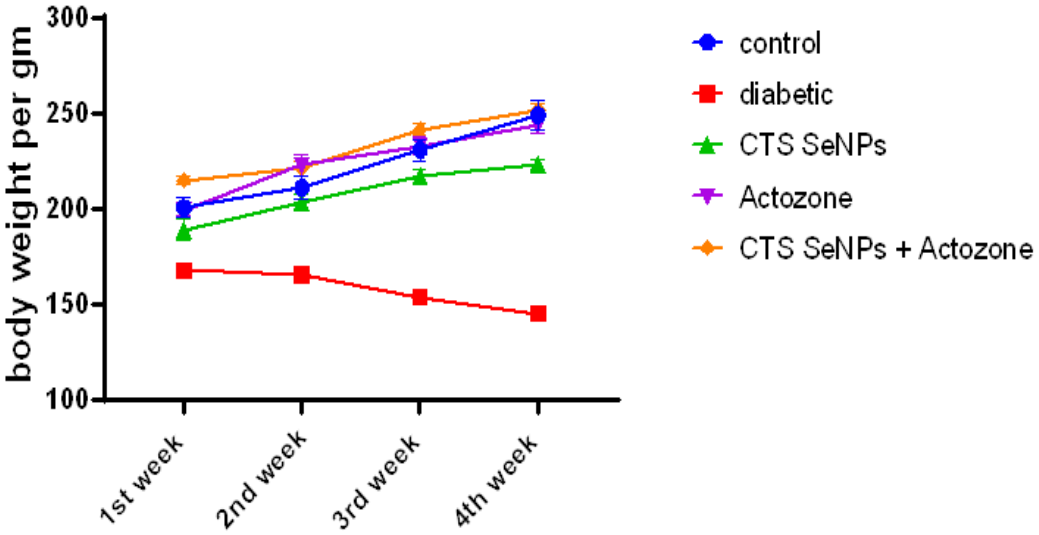

Figure (1): Effect of CTS-SeNPs (2 mg/kg/b.wt) and Actozone drug (20 mg/kg/b.wt) in normal and induced diabetic rats on body weight.

Effect of CTS-SeNPs (2 mg/kg/b.wt) and Actozone drug (20 mg/kg/b.wt) on the mean value of serum blood glucose and insulin levels of rats in different treated groups

Diabetic group revealed the highest glucose reading (383.7 ± 41.3[a]) and group treated with CTS-SeNPs showed significant decrease in glucose value compared to the diabetic group (237.78±2. 72[b]). The group that was treated with CTS-SeNPs + Actozone revealed non-significant difference compared with the group treated with Actozone, (133.7 ± 9.65[cd]) while there was significant decrease in glucose value compared to diabetic group (+ve control) and no difference compared to (–ve control) group.

While the diabetic group was the lowest insulin reading (6.63±0.01[e]) and group treated with CTS-SeNPs showed significant increase in insulin value compared to the diabetic group (13.25±0.007[d]). The group that was treated with CTS-SeNPs + Actozone revealed significant difference with the group treated with Actozone (26.25±0.01[b]) and there was significant increase in insulin value compared to diabetic group (+ve control) and also there was significant difference compared to (–ve control) group.

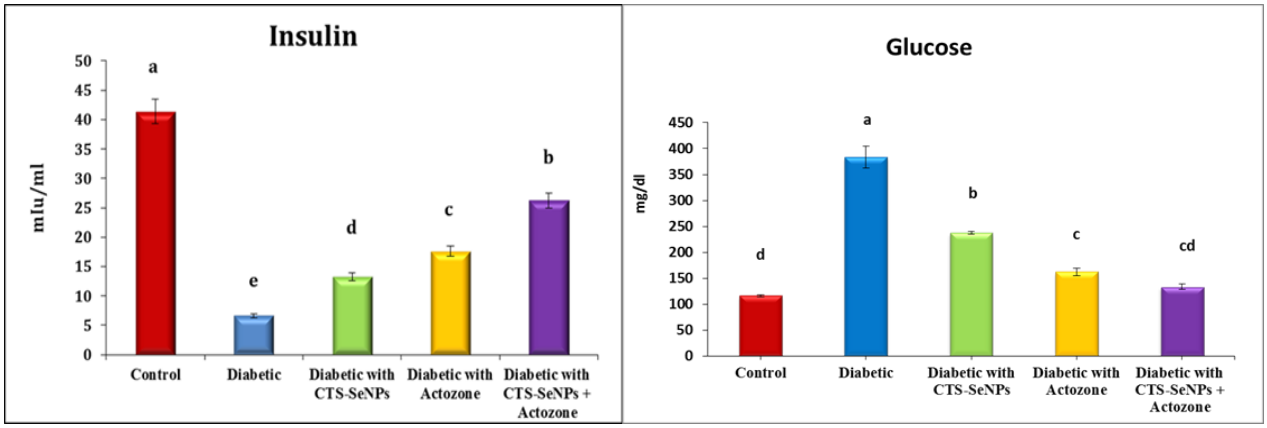

Figure (2): Effect of CTS-SeNPs (2 mg/kg/b.wt) and Actozone drug (20 mg/kg/b.wt) in normal and induced diabetic rats on serum blood glucose & insulin levels.

Effect of CTS-SeNPs (2 mg/kg/b.wt) and Actozone drug (20 mg/kg/b.wt) on the mean value of liver functions of rats in different treated groups

Our results showed that diabetic group is the highest in liver enzymes activities (ALT, AST and ALP), and group treated with CTS-SeNPs showed non-significant decrease in ALT enzyme only compared to the diabetic group. The group that was treated with CTS-SeNPs + Actozone revealed non-significant difference with the group treated with Actozone in AST enzyme only, while there was significant decrease in liver enzymes values compared to diabetic group (+ve control) and no difference compared to (–ve control) group.

Table (2): Effect of CTS-SeNPs (2 mg/kg/b.wt) and Actozone drug (20 mg/kg/b.wt) in normal and induced diabetic rats in liver enzymes levels.

Treatment	AST (µ/L)	ALT (µ/L)	ALP (µ/L)
Control	**150.93±4.15[c]**	**79.25±5.23[cd]**	**110.43±5.39[cd]**
Diabetic	**195.33±3.78[a]**	**109.18±7.50[a]**	**185.10±7.7[a]**
Diabetic with CTS-SeNPs	**175.36±3.21[b]**	**103.23±3.88[ab]**	**153.15±3.39[b]**
Diabetic with Actozone	**159.58±3.03[c]**	**90.33±5.15[bc]**	**121.25±5.81[c]**
Diabetic with CTS-SeNPs + Actozone	**151.20±4.92[c]**	**71.28±3.4[d]**	**94.99±3.04[d]**
P-value	**< 0.001****		**< 0.0001*****

**P< 0.001 and ***P< 0.0001 (highly significant difference).

Means with different superscript are statistically different according to Duncan's multiple range test at P< 0.05. Our results indicated that diabetic group is the lowest in total protein, albumin and globulin levels, and group treated with CTS-SeNPs showed significant increase in total protein and globulin levels values only compared to the diabetic group. The group that was treated with CTS-SeNPs + Actozone revealed non-significant difference with the group treated with Actozone, while there was significant increase in total protein and globulin levels values only compared to diabetic group (+ve control) and no difference compared to (-ve control) group.

Table (3): Effect of CTS-SeNPs (2 mg/kg b.wt) and Actozone drug (20 mg/kg b.wt) in normal and induced diabetic rats on proteinogram.

Treatment	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A/G
Control	6.64±0.14[a]	3.54±0.13[a]	3.1±0.05[a]	1.14±0.05[b]
Diabetic	5.45±0.10[c]	3.31±0.07[a]	2.14±0.11[c]	1.56±0.09[a]
Diabetic with CTS-SeNPs	5.83±0.09[b]	3.34±0.09[a]	2.49±0.16[b]	1.37±0.12[ab]
Diabetic with Actozone	6.45±0.09[a]	3.6±0.06[a]	2.85±0.13[a]	1.27±0.07[b]
Diabetic with CTS-SeNPs + Actozone	6.69±0.06[a]	3.53±0.11[a]	3.17±0.09[a]	1.12±0.06[b]
P-value	< 0.0001***	> 0.05[NS]	< 0.001**	< 0.05*
**P< 0.001 and ***P< 0.0001 (highly significant difference) and NS: non-significant difference *P > 0.05.				
Means with different superscript are statistically different according to Duncan's multiple range test at P< 0.05.				

Effect of CTS-SeNPs (2 mg/kg/b.wt) and Actozone drug (20 mg/kg/b.wt) on the mean value of lipid profile of rats in different treated groups

Diabetic group revealed the highest lipid profile reading except HDL is the lowest, group treated with CTS-SeNPs showed significant decrease in TG and VLDL values only compared to the diabetic group except for HDL value which showed significant increase compared to diabetic group. The group that was treated with CTS-SeNPs + Actozone revealed non-significant difference in Cholesterol level only with the group treated with Actozone, while there was significant difference in lipid profile value compared to diabetic group (+ve control) and there was significant difference in TG and VLDL values only compared to control) group.

Table (4): Effect of CTS-SeNPs (2 mg/kg/b.wt) and Actozone drug (20 mg/kg/b.wt) in normal and induced diabetic rats on lipid profile.

Treatment	Cholesterol (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Control	107.65±6.61[b]	101.45±7.26[c]	54.71±0.96[a]	32.65±5.89[bc]	20.29±1.45[c]
Diabetic	170.10±10.81[a]	181.65±11.51[a]	42.77±1.11[c]	96.01±3.96[a]	35.99±2.47[a]
Diabetic with CTS-SeNPs	160.28±7.29[a]	153.08±9.47[b]	46.18±0.63[b]	83.48±5.89[a]	30.62±1.89[b]
Diabetic with Actozone	111.43±4.08[b]	106.0±6.77[c]	46.82±1.39[b]	43.41±4.94[b]	21.20±1.35[c]
Diabetic with CTS-SeNPs + Actozone	90.93±2.18[b]	73.41±3.52[d]	52.11±0.55[a]	24.14±2.47[c]	14.68±0.71[d]
P-value	< 0.0001***	< 0.0001***	< 0.0001***	< 0.0001***	< 0.0001***
*P< 0.05 (there is significant difference) and ***P< 0.0001 (highly significant difference).					
Means with different superscript are statistically different according to Duncan's multiple range test at P< 0.05.					

Effect of CTS-SeNPs (2 mg/kg/b.wt) and Actozone drug (20 mg/kg/b.wt) on the mean value of hepatic oxidant/antioxidant & Glucose-6-phosphate dehydrogenase (G6PD) status of rats in different treated groups

Diabetic group reveal the lowest hepatic oxidant/antioxidant & G6PD reading except for MDA it is the highest and group treated with CTS-SeNPs showed significant increase in hepatic oxidant/antioxidant & G6PD values compared to the diabetic group except for MDA it showed decrease in value compared with diabetic group. The group that was treated with CTS-SeNPs + Actozone revealed non-significant difference with the group treated with Actozone only but in MDA showed significant decrease in value compared with Actozone only, while there was significant increase in hepatic oxidant/antioxidant t& G6PD values compared to diabetic group (+ve control) except for MDA which showed decrease in value in Actozone only compared to diabetic group and no difference compared to (-ve control) group table (5).

Table (5): Effect of CTS-SeNPs (2 mg/kg/b.wt) and Actozone drug (20 mg/kg/b.wt) in normal and induced diabetic rats on hepatic oxidant/antioxidant & G6PD levels.

Treatment	Mean ± SE					
	GPx	SOD[1]	GSH[1]	CAT[1]	MDA[2]	G6PD
Control	34.0±0.009[a]	42.3±0.02[a]	11.2±0.004[a]	68.4±.03[a]	30.6±.02[d]	11.87±0.01[a]
Diabetic	16.0±0.003[e]	11.8±0.02[e]	1.4±.0005[e]	21.8±.02[d]	165.0±.07[a]	2.83±0.002[c]
Diabetic with CTS-SeNPs	18.1±0.004[d]	23.3±0.01[d]	3.6±.001[d]	41.9±.02[c]	90.3±.04[b]	6.06±0.007[b]
Diabetic with Actozone	23.65±0.01[c]	30.3±0.01[c]	5.4±0.003[c]	60.9±.03[b]	52.0±.02[c]	4.16±0.003[bc]

Diabetic with CTS-SeNPs + Actozone	29.0±0.004[b]	40.0±0.02[b]	7.1±0.005[b]	67.6±.05[a]	35.5±.03[d]	12.06±0.01[a]
P-value	< 0.0001***	< 0.0001***	< 0.0001***	< 0.0001***	< 0.0001***	< 0.0001***
	***P< 0.0001 (highly significant difference).					
	Means with different superscript are statistically different according to Duncan's multiple range test at P< 0.05.					
	[1]Arsine transformed parameters; [2]Square root transformed parameter.					

Effect of CTS-SeNPs (2 mg/kg/b.wt) and Actozone drug (20 mg/kg/b.wt) in normal and induced diabetic rats on the level of gene expression of Ngn3 & Pdx1 by using real time PCR

Our results revealed that diabetic group is the lowest and group treated with CTS-SeNPs showed significant increase compared to the diabetic group. The group that was treated with CTS-SeNPs + Actozone revealed significant difference with the group treated with Actozone only, while there was significant increase compared to diabetic group (+ve control) and no difference compared to (–ve control) group figure (4).

Histopathological results on liver and pancreas tissues

In figure 5 normal control group liver section of control group showing normal hepatic parenchyma (A). Diabetic group liver of rat showing severe portal congestion, and portal fibrosis with mononuclear cell infiltration and marked cytoplasmic vacuolation (B). Diabetic with CTS_SeNPs group liver of rat showing portal fibrosis with mononuclear cell infiltration and hydropic degeneration of hepatocytes and focal leukocytic aggregation (C). Diabetic with Actozone group liver of rat showing vacuolar degeneration of some hepatocytes (D). Diabetic with CTS_SeNPs + actozone group Liver of rat showing almost normal histological picture except for mild vascular congestion (E).

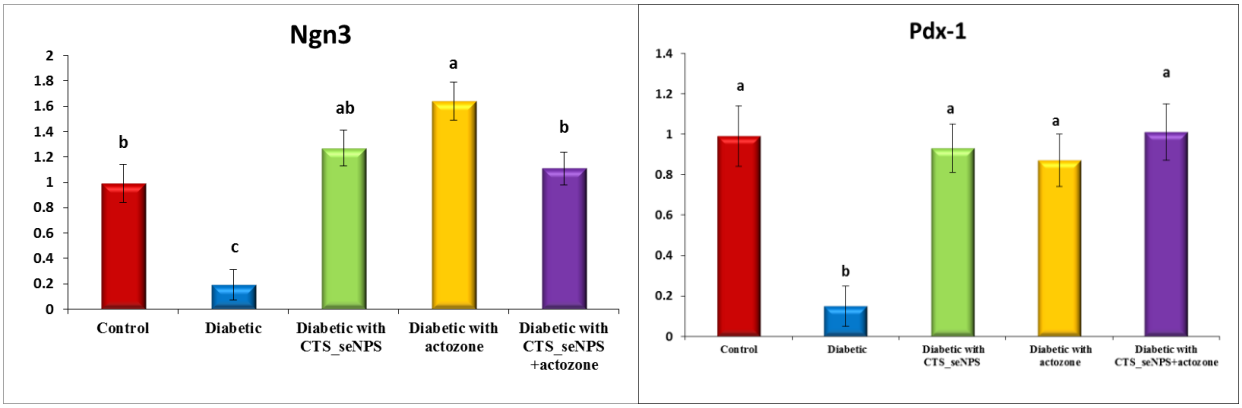

Figure (4): Effect of CTS-SeNPs (2 mg/kg/b.wt) and Actozone drug (20 mg/kg/b.wt) in normal and induced diabetic rats on Ngn3 & pdx1 level.

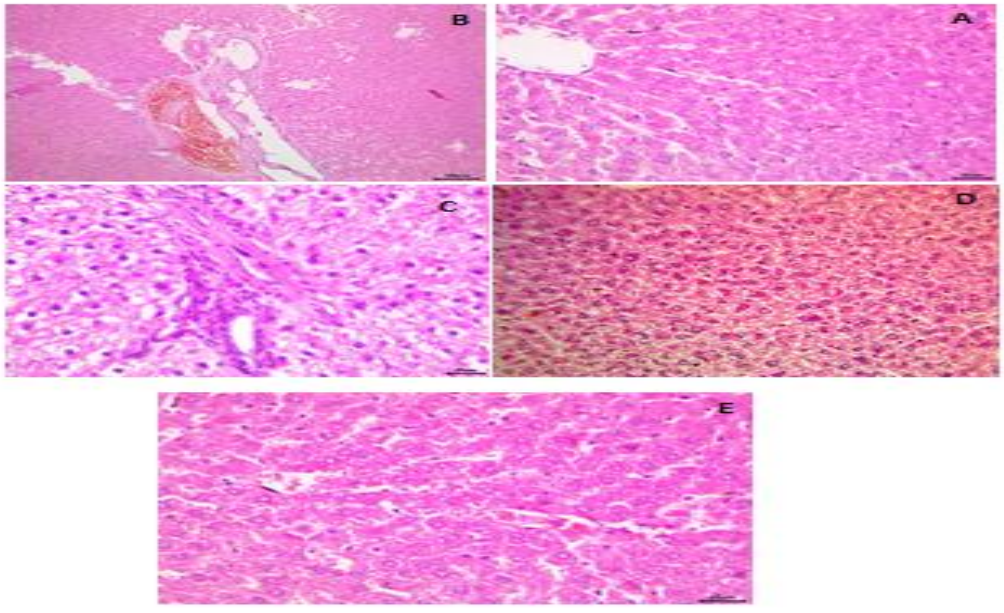

Figure (5): Liver of rat histological picture (H&E), with (x400).

In figure 6 normal rats' pancreas revealed normality in both exocrine and endocrine counterparts, with a healthy acinar epithelium and its secretory granules (A). STZ-induced Diabetic rats serial sections from the pancreatic tissues revealed decrease in densities of the islet cells and degenerative changes in the β-cells of the islets that mainly occur as cloudy swelling, hydropic degenerations, necrotic and apoptotic changes (B). Diabetic with CTS_SeNPs group revealed a moderate increase in the densities of the islets of Langerhans and in the population of their cellular contents. Other islets structures including the capillary net-work and delta cells (δ-cells) were apparently normal (C). Diabetic with Actozone group revealed a remarkable increase in the densities of the islets of Langerhans and in the population of their cellular contents. Neither degenerative nor apoptotic changes were seen in the examined sections (D). Diabetic with CTS_SeNPs + actozone group revealed marked characteristic increase in the densities of the islets of Langerhans and in the population of their cellular contents. The characteristic feature was the presence of some peripheral cells with a metamorphic changes, they may be a transforming cells from other islets or non-islets cells (aciner cells), (E).

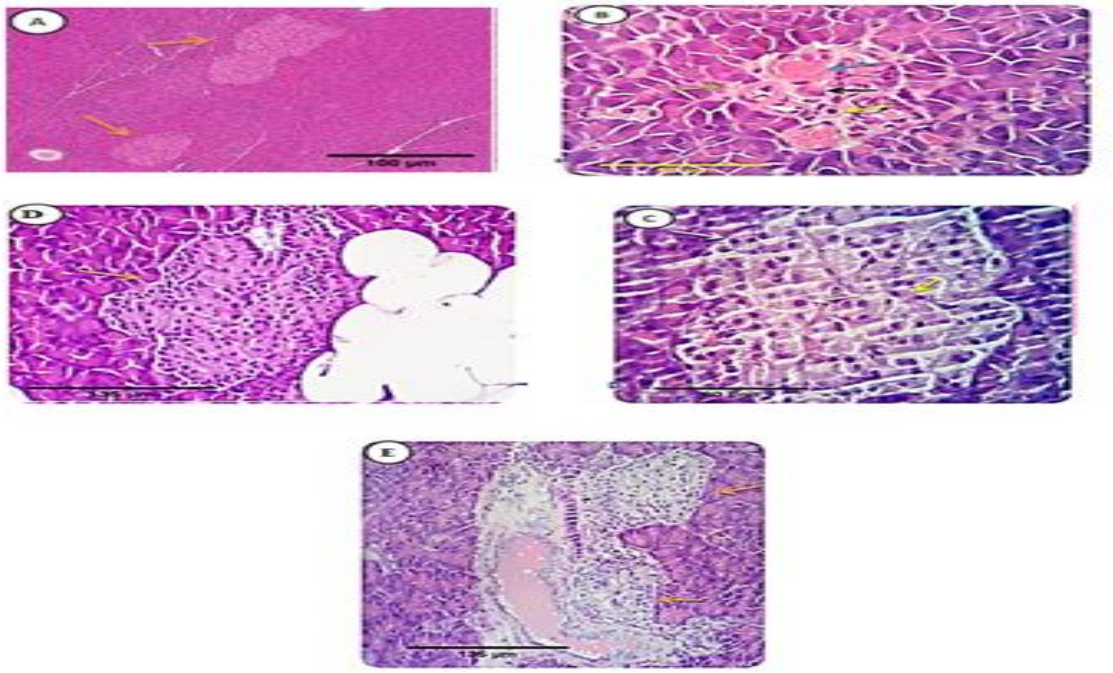

Figure (6): Pancreas of rat histological picture (H&E), with (x400).

DISCUSSION

In the present investigation, it has been plentifully revealed that there was highly statistically difference in body weight at 1st, 2d, 3rd, and 4th week of experiments. At all weeks of experiment the group treated with CTS-SeNPs + Actozone had the highest body weight followed by normal group that showed non-significant change in body weight compared to group treated with Actozone only. Group treated with CTS-SeNPs showed non-significant change in body weight compared to that treated with Actozone body weight for CTS-SeNPs and Actozone groups respectively. Diabetic group showed the weakest (lowest) body weight among groups.

Body weight loss is the main properties of diabetes, which may be because of the protein wasting in a situation of unavailability of carbohydrate for utilization as an energy source. A study reported the effects of CTS-SeNPs on body weight in STZ-induced diabetic mice. The diabetic mice were shown to have significantly lower initial body weights than the normal mice. It was worth noting that CTS-SeNPs showed no significant difference from glibenclamide, a kind of anti-diabetic drug, in restoring the body weight of diabetic mice.[19]

Another study documented that there was an enhancement in body weight but no clear change in blood glucose levels when treated diabetic animals with SeNPs (0.5 mg Se/kg).[42]

Our findings revealed that diabetic group reveal the highest glucose reading and group treated with CTS-SeNPs showed significant decrease in glucose value compared to the diabetic group except insulin which showed the opposite. The group that was treated with CTS-SeNPs + Actozone revealed non-significant difference with the group treated with Actozone only in both glucose and insulin while there was significant decrease in both values compared to diabetic group (+ve control) and no difference compared to (-ve control) group, the following reports supported our results and confirmed it.

Insulin resistance causing DM and inefficient functioning pancreatic beta cells, leading to the inability

of the body to produce insulin in type 1 diabetes and further complications in type 2 diabetes. Decreased insulin secretion increases lipolysis, which ultimately causes elevated serum lipids levels, i.e., dyslipidemia.[43]

Sunil et al.[44] noted that I/P supplementation of STZ (55 mg/kg) selectively destroy some of the pancreatic β-cells, leading to insulin deficiency and thus type 2-diabetes.

The hypoglycemic effect of Se could, however, be confirmed by other mechanisms, as an decline in intestinal glucose transport, which has been noted in vitro in the case of selenite.[45] Se has also been shown to induce renal glucose excretion in rats.[46]

Previous studies have been performed in isolated rat adipocytes, revealed that sodium selenate induced glucose uptake by translocating glucose transporters to the plasma membrane and activating serine/threonine kinases, including the p70 S6 kinase.[47]

In the present study it is evident that diabetic group is the highest in liver enzymes activities (ALT, AST and ALP), and group treated with CTS-SeNPs showed significant decrease in liver enzymes activities in comparison of the diabetic group. The group that was treated with CTS-SeNPs + Actozone revealed non-significant difference with the group treated with Actozone only, while there was significant decrease in liver enzymes values compared to diabetic group (+ve control) and no difference compared to (-ve control) group and the following studies confirm our results.

Maiti et al.[48] have documented that such increases in the activities of ALT, AST, and ALP in the serum of diabetic rats due to leakage of these enzymes from liver cytosol into the bloodstream because of hepatic injury associated with STZ. The increased levels of ALT and AST associated with insulin deficiency has been related to increased gluconeogenesis during diabetes.[49]

Treatment with SeNPs lead to restore the activities of ALT, AST, and ALP toward normal levels. This finding could be explained due to the radical scavenging property of Se and the importance of Se in protecting the integrity and the functions of tissues.[50]

The present investigation revealed that diabetic group revealed the highest lipid profile reading, group treated with CTS-SeNPs showed significant decrease in lipid profile value compared to the diabetic group. The group that was treated with CTS-SeNPs + Actozone revealed non-significant difference with the group treated with Actozone only, while there was significant decrease in lipid profile value than diabetic group (+ve control) and no difference than (–ve control) group and this in agreements with other studies.

Diabetes mellitus is often associated with abnormalities in lipid. Thus, strategies aimed at inhibiting of dyslipidemia are essential for the treatment of DM. Generally, dyslipidemia is characterized with elevated TC, TG, LDL-C levels and decreased HDL-C level in serum.[51]

Fazilati et al.[52] demonstrated that nano particles of selenium with powerful antioxidant and raising activity of antioxidant effect better disease in comparison with the drug glibenclamide in reducing blood glucose, triglycerides, cholesterol, T3, and increasing HDL.

Our results investigated that diabetic group reveal the highest oxidant/antioxidant reading and group treated with CTS-SeNPs showed significant decrease in oxidant/antioxidant value compared to the diabetic group The group that was treated with CTS-SeNPs + Actozone revealed non-significant difference with the group treated with Actozone only, while there was significant decrease in antioxidant value compared to diabetic group (+ve control) and no difference compared to(-ve control) group while MDA values showed opposite results.

Significantly decline in antioxidant enzymes activities and induce of MDA levels were noted in STZ-induced diabetic mice, indicated the enhanced oxidative stress in liver and kidney. Oral adminstration of CTS-SeNPs leading to significantly increase of CAT, SOD and GSH-Px activities in liver and kidney compared to the diabetic mice ($P < 0.05$), indicating the improved scavenging toxic ROS ability.[19]

Campbell et al.[53] studied the specific effects of selenium on pancreatic beta-cell gene expression and islet cell function. Min6 cells were stimulated for 3 h with 30 nmol/l Na2SeO3 and whole cell extracts assayed for gluthathione peroxidase (GPx) activity. Results revealed that Na2SeO3 addition stimulated a significant 5-fold increase in GPx activity ($P < 0.05$), indicating that Min6 beta-cells are significantly seleno-sensitive.

We measured the level of G6PD and Results revealed that diabetic group is the highest in G6PD and group treated with CTS-SeNPs showed significant decrease in G6PD compared to the diabetic group. The group that was treated with CTS-SeNPs + Actozone revealed non-significant difference with the group treated with Actozone only, while there was significant decrease in G6PD value than diabetic group (+ve control) and no difference than (–ve control) group. Following reports supported our results and confirmed it.

In another study a significant reduction ($P<0.05$) in the activities of malic, HK, and G6PD was documented in STZ-induced diabetic animals, along with a significant increase ($P<0.05$) in glucose-6-phosphatase activity in hepatic tissue in comparison with the untreated control. After treatment with SeNPs, the diabetic rats shows a significant enhancement ($P<0.05$) in the activities of HK,

glucose-6-phosphatase, and G6PD compared with the diabetic control.[45]

Studies have noted that Pdx1 is the most essential transcription element in the process of directional differentiation and maturation of the pancreas. It is important for pancreatic bud synthesis during the embryonic period and the subsequent differentiation of islet β cells. Pdx1 bind directly to the insulin gene promoter region which is consistent with our results.[54]

Plus to Pdx1, epithelial cells that have advanced differentiated into pancreatic β cells require the interaction of a several transcription factors such as Ngn3 and Pax6 to lead to maturation into islet β cells.[55]

Ngn3 is not expressed in pancreatic progenitor cells, but becomes expressed in endocrine progenitor cells.[56] It is known that Ngn3 function as a switch in regulating the pancreatic development procedure. Wang et al.[57] noted that in rat embryonic development and adult pancreatic islet repair, the expression of Ngn3 declined. Moreover, the expression of related genes (NeuroD1, Nkx2.2, and Pax6), which are found at downstream of Ngn3 and regulate islet cell differentiation and maturation, will also be declined, and finally lead to islet endocrine dysfunction.

Our histopathological finding showed that liver and pancreas of control rat were showing normal histological structure but in diabetic group, diabetic with CTS_SeNPs group and Actozone group liver of rat showing vacuolar degeneration of some hepatocytes. While in diabetic with CTS_SeNPs + actozone group liver of rat showing almost normal histological picture except for mild vascular congestion while in pancreas rats, showing decreased densities of islets cells, apoptotic and necrotic changes in diabetic group. In rest groups' rats showing indiction in the densities of the islets of Langerhans and this confirmed with the following studies.

Lin et al.[58] also investigated the histological analysis of the liver and pancreas by H&E staining showed a notable difference between Nano-Se-B. longum treated and control mice. The hepatic cells showed to be edematous and the cytoplasm was loose in the tissue in the STZ-treated groups, the progression of liver and pancreas pathological damage was slowed after Nano-Se-B. longum treatment.

Al-Quraishy et al.[45] documented the impact of SeNPs liver and pancreas at which in untreated diabetic rats, degenerative and necrotic changes were consistently noted, such as shrunken islets of Langerhans, hydropic degeneration, and degranulation in the cytoplasm and lymphocyte infiltration. The nucleus of the necrotic cells revealed marginal hyperchromasia or either pyknosis. In the SeNPs, insulin, or SeNPs and insulin treatment groups, however, most of the cells of the islets of Langerhans were preserved, although some hydropic degeneration, degranulation, and necrosis was still noted, and the diameter of the islets of Langerhans area remained decreased. And pathological lesions were dramatically ameliorated in the liver in diabetic rats treated with SeNPs, insulin, or SeNPs and insulin, where the sections showed hepatic lobules in form of radiating plates of strands of hepatocytes, and the central vein.

CONCLUSION

The results of this investigation demonstrated that DM-induced severe biochemical and histopathological changes in the liver and pancreas and CTS-SeNPs have a protective effect and minimize the risk of diabetic complications.

Conflict of interest

The author declares that he has no conflicts of interests.

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