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Evaluation of antidiabetic effect of nanoselenium in Streptozotocin induced diabetes in a rat model

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ABSTRACT

This study was designed to investigate the anti-diabetic effects of selenium nano-particles (SeNPs) at dose of 2 mg/kg body weight in streptozotocin (STZ) induced diabetes in a rat model at dose 50 mg/kg body weight. Rats were administered SeNPs orally in normal and experimentally induced diabetic rats for 35 days and glibenclamide (Glib) at the dose rate of 20 mg/kg, which was used as a reference drug. Blood samples and pancreatic tissue were collected at the end of experiment. Administration of SeNPs significantly decreased blood glucose levels and enhanced serum insulin concentration, the result showed also decrease in liver function enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST).All groups showed non-significant changes in serum creatinine levels, decrease in the cardiac function enzymes creatine kinase-MB (CK-MB). Cholesterol, triglyceride, and low-density lipoprotein (LDL) levels were significantly decreased and high-density lipoprotein (HDL) was significantly increased. Histological study revealed that SeNPs were able to prevent atrophy of island of Langerhans cells compared to diabetic group. However, Glib injection also exhibited a significant improvement in diabetic animals after 35 days of treatment. This study suggests that SeNPs capped with chitosan can be used as an antidiabetic showing synergistic effect in STZ-induced diabetic rats,.

1. INTRODUCTION

Diabetes is among the most dominant chronic diseases in the world, and is a metabolic disease caused by abnormal insulin action or secretion, as stated by the International Diabetes Federation (IDF). In 2017, approximately 5 million deaths worldwide while 451 million embody patients were concerned to diabetes mellitus (DM) (Cho et al., 2018). In Egypt, the prevalence of DM was 14.5% in 2017. IDF recorded Egypt among the world top 10 countries related the number of diabetic patients (Hegazi et al., 2015; IDF, 2017). Diabetes is a silently life-threatening condition that may lead to altered genetic susceptibility, cardiovascular and hemodynamic complications and metabolic complications (Forbes and Cooper, 2013). Different factors can causes that promote or prevent DM such as genetic, environmental, diet and exercise. Furthermore, Diabetes pathogenesis and the progression of its co- morbidities have been strongly associated with oxidative stress, while hyperglycemia associated with common symptoms, namely polyphagia, polyuria, and polydipsia (Beverley & Eschwège, 2003).

Biomedical application revealed that, Se nan-oparticles have anti-diabetic, antioxidant and anti-cancer properties (Hosnedlova et al., 2018) and lower toxicity in comparison to the selenite (SeO4−2) or selenite (SeO3 −2) counterpart (Benko et al., 2012; Shakibaie et al., 2012). Polymeric nano-structure and micro-particles have shown interesting commitment for protein transmission (Delie and Blanco-Príeto 2005). Nano-particulate hydrogels consisting of chitosan have been developed and tested over the past two decades (des Rieux et al., 2006; Galindo-Rodriguez et al., 2005). Furthermore, they have been applied as delivery systems for the controlled release of therapeutic ingredients and used their muco-adhesive characteristics via interactions between opposite charges. This specific feature can provide the ability of tissue binding for the aim of specific drug delivery (Li et al., 2014; Zhao et al., 2014).

Glib is an important drug for the management of hyperglycaemia in moderate DM (Caro, 1985). Recent studies have shown that Glib is a potent insulin-like agent that promotes glucose uptake, glucose oxidation and activation of glycogen synthase in rat liver and adipocytes (Altan et al., 1985; Atalay et al., 1994).

The present work aimed to evaluate the effect CTS-SeNPs to reduce the diabetic complications, toxicity and restore insulin resistance accompanied with diabetes compared to Glib as a pharmaceutical product used in treatment of diabetes in male rats.

2. MATERIAL AND METHODS

2.1. Experimental animals:

Forty-eight adult male waster rats with body weight 150 - 200 gm were obtained from the Nile Pharmaceutical Co., Cairo, Egypt. Rats were housed at the animal house at the National Center for Radiation Research and Technology (NCRRT) (Cairo, Egypt).The rats were housed in metal

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cages at a temperature of 25 ± 2ºC for 1 week for adaptation before the beginning of experiment. The study was conducted in accordance with international guidelines for animal experiments and approved by the Ethical Committee of the National Center for Radiation Research and Technology (NCRRT), Atomic Energy Authority, Cairo, Egypt.

2.2. Chemicals:
STZ, Sodium selenite (Na2SeO3), Acetic acid, Chitosan (CTS), were purchasedfrom Sigma Chemical, St. Louis, USA, Glib tablets (Dianil® 5 mg) dietary supplement was purchased from Sanofi- Egypt.

2.3. Synthesis of CTS-SeNPs:
CTS-SeNPs was synthesized by a modified process according to (Chen et al., 2008). CTS was dissolved in 4% Acetic acid (1: 100, w:v), 5 ml of CTS was added to 5ml, 0.01 M of Se selenite. The mix was mixed using magnetic stirring, and heated to 70 ^{O}C, The mixture was exposed to ultrasonic for 15 min., then exposed to gamma radiation at 50 kGy to reduce the nano-particle size and took the red color as in fig (1).

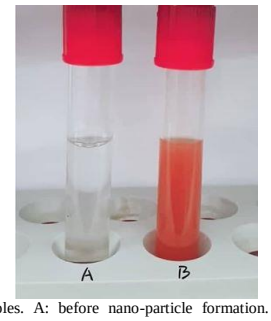

Fig.1CTS-SeNPs samples. A: before nano-particle formation. B: after nano-particle formation showing red color.

2.4. Characterization of SeNPs:
Synthesized CTS-SeNPs was characterized by using transmission electron microscope (TEM) of JOEL JEM-2100 (Nanotech Company, Egypt), microscope with an accelerating voltage of 200 kV, connected to Gatan Digital Camera, Model Erlangshen ES500, Dynamic light scattering (DLS), UV absorbance, Fourier transform infrared spectroscopy (FTIR).

2.5. Induction of diabetes:
Diabetes was induced in rats by a single intraperitoneal injection (50 mg/kg bw.) dissolved in sodium citrate buffer (0.1 M, pH 4.2– 4.5). After three days later, glycemia was determined in blood sample obtained by tail prick using glucose strips (Accu-Chek, Roche). Rats with blood glucose levels >200 mg/dl were considered diabetic according to Gupta and Gupta, (2009).

2.6. Experimental design:
Rats were divided into six groups (8 rats each) placed and kept in individual cages as follow:
Group I: (Non- diabetic control group): Rats were given 1 ml saline daily, by oral intubations using gavages needle.
Group II: (CTS-SeNPs group): Rates were administered with CTS-SeNP at dose (2 mg Se/kg bw, in 1 ml saline) daily, by oral intubations using gavages needle (Zeng et al., 2018).
Group III: (Glib group): Rates were administered with Glibat dose(20 mg/kg bw, in 1 ml saline) daily, by oral intubations using gavage needle (Zeng et al., 2018).
Group IV: (STZ group): Rats received a single intraperitoneal injection of STZ (50 mg/kg bw)once at the first day of experiment.
Group V: (STZ+CTS-SeNPs): Rats received a single intraperitoneal injection of STZ as group IV and after 3 days were administered with CTS-SeNPs at dose (As group II) daily, by oral intubations using gavage needle.
Group VI: (STZ+Glib):Rats received a single intraperitoneal injection of STZasgroupIV and after 3 days were administered with Glibat dose (as group III).
All animals were sacrificed after 35 days of treatment.

2.7. Sampling:
2.7.1. Blood samples:
Blood sample was collected from retro-orbital plexus of eyes puncture. Blood was allowed to clot then centrifuged for 15 minutes at 3,000 rpm. Sera were separated in dry sterile tubes by automatic pipette, and then stored at -20 °C in a deep freezer until determination.
2.7.2. Tissue samples:
Pancreatic tissues were collected and Fixed in 10 % neutral buffered formalin for histological study.

2.8. Analysis:
2.8.1. Biochemical analyses:
Blood glucose levels was measured according to Trinder, (1969) using a glucose assay kit (Spectrum-Diagnostics, Cairo, Egypt) by the glucose oxidase method. Serum insulin concentrations were analyzed according to Unger et al., (1961).Alanine aminotransferase and aspartate aminotransferase were determined by using Enzymatic Kinetic method (Bergmeyer et al., 1985). Creatinine was assayed using Kit (Cat. No-ab65340) according to Husdan and Rapoport (1968). The functioning of cardiac studied by measurement of creatine kinase (CK-MB) in serum according to Wu & Bowers, (1982).Cholesterol, triglyceride, HDL, LDL, and very low-density lipoproteins (VLDL) were determined by the methods of Friedwaid et al., (1972).
2.8.2.Histopathological examination:
According to Banchroft et al., (1996) Fixed pancreatic tissue in 10% buffered neutral formalin solution. Tissues paraffin blocks were prepared and sectioned at about 3-5 µm thickness and then stained with hematoxylin and eosin stain.

2.8.1. Statistical analysis:
Results were expressed as mean ± SE using SPSS (13.0 software, 2009). Data were analyzed using one-way ANOVA followed by Duncan's test. Values were statistically significant at $p < 0.05$.

3. RESULTS

3.1. Characterization of the prepared SeNPs:
NPs morphology such as particle size and shape was inspected via TEM analysis.CTS-SeNPs showed size around 50 - 130 nm with a semi-spherical shape.
DLS results showed that CTS-SeNPs sizes ranged from 39.4 to 265.6 nm with high percentage of sizes 52.85, 61.2, 82.09 and 95.07 (14.5, 16.4, 12.9 and 10.6 %).
CTS-SeNPsformation was approved by the appearance of a peak in the UV visible region at 270.5 nm characterize for CTS-SeNPs formation.

3.2. Biochemical analysis:

Data were presented in fig. (1 and 2), glucose and insulin levels didn't changed during the experimental period in the control, CTS-SeNPs and Glib groups (P>0.05). However, a remarkable increase in glucose level was noted in the STZ groups confirming that they became diabetic. In addition, after administering CTS-SeNPs and Glib to diabetic rats, a marked reduction in glucose level was noted in comparison with the STZ group (p≤ 0.05). Simultaneously, insulin levels drastically declined in STZ groups compared to the control group, but treating with CTS-SeNPs and Glib restored the level of serum insulin in STZ+CTS-SeNPs and STZ+Glib group.

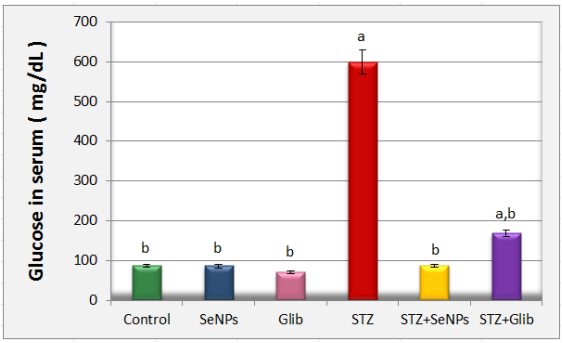

Fig. 2 Effect of CTS-SeNPs treatment on serum glucose level.

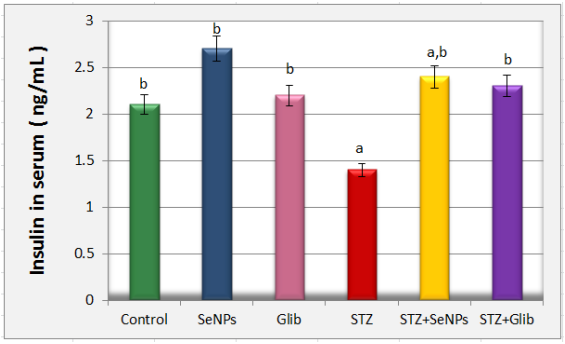

Fig. 3 Effect of CTS-SeNPs treatment on serum insulin level.

The results presented in table (2) Showed that Cholesterol, Triglyceride, HDL and LDL levels were nearly similar to control group in CTS-SeNPs and Glib group (P>0.05). STZ group showed a significant increases in serum cholesterol, triglyceride, LDL and decrease in HDL levels compared to control rats. Treating diabetic rats with CTS-SeNPs or Glib showed significant decrease (p≤ 0.05) in cholesterol, triglyceride, LDL and increase in HDL levels in STZ + CTS-SeNPs and STZ + Glib groups compared to STZ group.

Table 1 Effect of CTS-SeNPs on serum ALT , AST , creatinine and CK-MB levels

Group	ALT (U/L)	AST (U/L)	Creatinine (mg/dl)	CK MB (U/ml)
Control	32.7 ± 4.0^{b}	60.6 ± 2.7^{b}	0.7 ± 0.13	113.7 ± 2.7^{b}
CTS-SeNPs	34.5 ± 5.0^{b}	59.3 ± 3.4^{b}	0.6 ± 0.08^{b}	110.7 ± 2.6^{b}
Glib	38.2 ± 7.3^{b}	58.2 ± 5.7^{b}	0.7 ± 0.14	111.8 ± 2.1^{b}
STZ	86.8 ± 3.7^{a}	94.8 ± 7.0^{a}	0.8 ± 0.09	210.8 ± 6.6^{a}
STZ+CTS-SeNPs	39.2 ± 4.4^{b}	60.5 ± 6.0^{b}	0.6 ± 0.15^{b}	144.5 ± 7.9^{a,b}
STZ+Glib	37.0 ± 4.5^{b}	69.7 ± 4.8^{a,b}	0.6 ± 0.15^{b}	164.5 ± 11.9^{a}

Data are presented: (Mean ± S.E). S.E = Standard error. Mean values with different superscript letters in the same column are significantly different at (P≤0.05).

Table 2 Effect of CTS-SeNPs on serum lipid profiles

Group	Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
Control	104.5 ± 3.6^{b}	67.5 ± 2.7^{b}	38.7 ± 1.2^{b}	59.0 ± 2.4^{b}
CTS-SeNPs	102.3 ± 4.6^{b}	67.8 ± 4.5^{b}	39.0 ± 1.3^{b}	55.3 ± 3.8^{b}
Glib	88.5 ± 39^{b}	67.5 ± 5.4^{b}	38.1 ± 1.5^{b}	49.2 ± 3.1^{a,b}
STZ	206.5 ± 11.1^{a}	179.0 ± 5.3^{a}	18.2 ± 0.98^{a}	157.7 ± 9.2^{a}
STZ+CTS-SeNPs	102.7 ± 4.2^{b}	66.3 ± 3.0^{b}	37.8 ± 1.9^{b}	56.0 ± 4.2^{b}
STZ+Glib	104.7 ± 7.5^{b}	63.8 ± 3.2^{b}	38.3 ± 1.5^{b}	55.2 ± 5.6^{b}

Data are presented: (Mean ± S.E). S.E = Standard error. Mean values with different superscript letters in the same column are significantly different at (P≤0.05).

3.3. Histopathology findings:

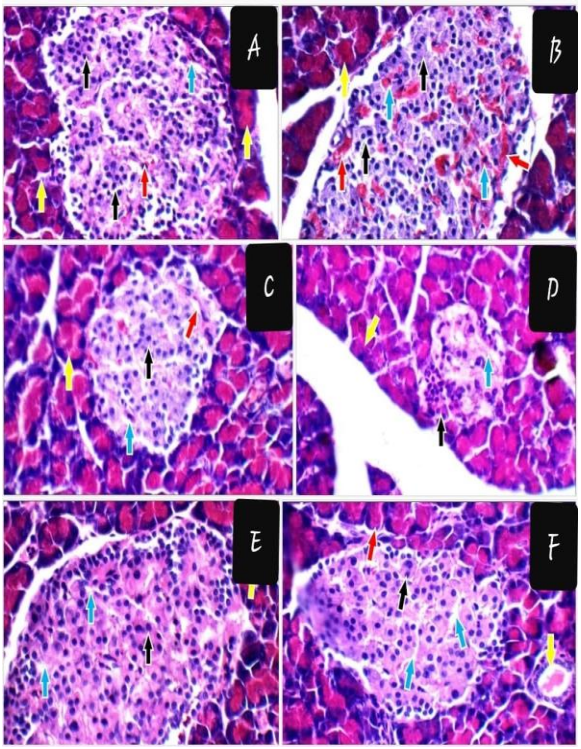

Fig. 3 Showing average-sized pale-staining islets of Langerhans (black arrows), average exocrine areas (blue arrows), average ducts (red arrow), and average interstitial blood vessels (yellow arrow) (H&E X 400).The pancreas of the control (Fig. 3:A), CTS-SeNPs (Fig. 3:B) and Glib (Fig. 3:C) groups were showed a normal texture pattern with ordinary thickness. In STZ group atrophy and shrinking of Langerhans islets with lymphocytic cellular infiltration were detected (Fig. 3:D). As shown in STZ+CTS-SeNPs group (Fig. 3:E) and STZ+ Glib (Fig. 3:F), Langerhans islets were repair/regeneration of β –cells in islets of Langerhans in the diabetic rats treated with SeNPs and Glib.

4. DISCUSSION

Selenium (Se) is an antioxidant; which is vital micronutrient in our diet and it could minimize diabetic symptoms (Pillai et al., 2012). According to Hwang et al. (2007), Se can facilitate the transportation of glucose to cells which acts like insulin in streptozotocin (STZ)-induced diabetic rats and coordinate the action of several enzymes that are involved in glycolysis, gluconeogenesis and regarded essential factor of antioxidant enzyme production. On the other hand,Deng et al., (2019)investigated that comparison between seleno methionine or selenite and SeNPs, SeNPs are more biocompatible with no toxicityand biologically active (AlBasher et al., 2019). While, chemical form of Se as selenite was not efficient for glucose recovery (Ayaz et al., 2004; Battell et al., 1998; Becker et al 1996). Therefore, the recorded finings in the present investigation were reinforced by those of Vural et al. (2017), who demonstrated that diabetic rats were orally treated with the SeNPs for a 7 week causing noticeable decrease in glucose

levels. Se nano-particles has been proven able to output the hypoglycemic effect comparable to that of insulin (Abdulmalek and Balbaa, 2019). According to Hwang et al. (2007), Se markedly stimulated glucose transport and insulin-sensitive cyclic adenosine mono-phosphate phosphodiesterase because it has insulin-like effect both in vitro and in vivo. Moreover, the glucose lowering effect of SeNPs might be corroborative by other mechanisms, such as stimulation of adipogenesis in adipocytes via stimulating serine/threonine kinases, including the p70 S6 kinase or acceleration of kidney glucose excretion in rats (Al-Quraishy et al., 2015). While, Kamel, (2014) found that the best anti diabetic and anti insulinemic effect was noticed in rats treated with Glib, it has the ability to increase pancreatic beta cells production of insulin and the long duration of Glib action and its metabolites could increase its prolonged hypoglycemic risk.

ALT and AST are considered markers of liver toxicity (Mori et al., 2003). STZ can produce a change in these enzymes in the serum of diabetic rodents. It has been reported that the transaminases are increased in insulin deficiency; these changes can be associated with the increase of gluconeogenesis and ketogenesis during diabetes (Fleig et al., 1970). This study has shown that treatment with SeNPs repair the activities of liver enzymes to normal values. Radical scavenging activity besides its integrity and functions of liver tissues results from the role of SeNPs in protecting, this results were in agreement with Al-Quraishy et al., (2015). Glib controlled production some enzymes in liver in diabetic rats through regulation of metabolic enactment and restraint of glycolysis and gluconeogenesis further, Our outcomes showed that the Glib treatment makes more insulin from pancreatic β-cells and augmentation of glycogen content in the liver among diabetic rats by extending the activity of glycogen synthase and hinders glycogen phosphorylase (Golden et al., 1979; Pederson et al., 2005).

In the present study, Non-significant changes in serum creatinine levels were observed in diabetic rats this proves that the kidney injury is long-term. These results were in agreement with Gavin et al., (2003) who explained Long-term damage, dysfunction, and kidney failure are the major complications of diabetes. It was noticed that, the hypoglycemic effect of SeNPs was associated with the improvement renal function in chronic cases in the SeNPs treated diabetic rats. Moreover, diet rich with Se help in delaying diabetic nephropathy by activating several selenoprotein and modulating the endogenous antioxidants (Douillet et al., 1999).

Cardiac injury was associated with induction of diabetes in rat model in the current study and was evidenced by the increased circulating values of CK-MB these findings were supported by those of Hall, (1991). Serum CK-MB activities were elevatedin STZ diabetic rats and it was reported to be increased in diabetic patients (Huang et al., 2006). In the present study, there was a significant decrease in serum CK-MB levels observed with treatment of SeNPs, indicating good cardiac protective effect of SeNPs. SeNPs raised considerable expectations for the prevention of cardiovascular diseases including diabetic cardiomyopathy. Also, SeNPs has useful effects on pathologic changes in heart, liver and kidney in induced diabetes in rats (Battellet al. 1998).Moreover, it was demonstrated that Glib was associated with a reduced risk of developing cardiovascular disease (including congestive heart failure) (Kahn et al 2006).

Oxidative stress secondary to persistent hyperglycemia is the main reason for hyperlipidemia observed in STZ-induced diabetic rats (Kumar et al., 2013), or due to insulin resistance as insulin resistance correlates with hyperglycemia, and alteration in lipid metabolism (Stahlman et al., 2013). In the present work, STZ-diabetic rats showed a marked reduction in serum HDL while there was a markedincrease in lipid profile parameters, cholesterol, triglyceride, LDL and VLDL. Demonstrated that the oral treatment of SeNPs and Glib a remarkable results a decrease in serum levels of cholesterol, triglyceride, HDL were observed linked with a significant decrease in LDL levels compared to the untreated diabetic rats also, the action of increases of HDL levels that could raise the efflux of cholesterol, triglyceride to liver tissue for catabolism this make reduction in cholesterol, tri-glyceride results (Jiang et al., 2015).

5. CONCULOSIONS

This study will serve to manage the nano-particle synthesis and restore damaged pancreatic tissue of diabetic rats. More extensive research is required; SeNPs could be used in the future as an agent that can manage diabetes. Moreover, administering Glib to diabetic rats reverses the changes caused by diabetes and has an effective drug to facilitate insulin secretion from beta cells, as it is used in present work as a positive drug control in diabetic rats.

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