Official Journal Issued by Faculty of Veterinary Medicine

Benha Veterinary Medical Journal

Journal homepage: https://bvmj.journals.ekb.eg/

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Original Paper

Hypoglycemic potential of chitosan Nano-selenium in experimentally induced diabetes mellitus in rats

Omayma A.R. Abo zaid[1], Sawsan M. El-Sonbaty[2], Neama M.A. Hamam[1,*]

[1]*Clinical Biochemistry, Faculty of Veterinary Medicine, Benha University, Egypt*

[2]*National Center for Radiation Research and Technology, Atomic Energy Authority, Cairo, Egypt*

ARTICLE INFO

Keywords

AFP
Diabetes Mellitus
Glibenclamide
Nanoparticles
Selenium
Streptozotocin

Received 05/02/2021
Accepted 05/03/2021
Available On-Line 01/04/2021

ABSTRACT

The present study investigated the effects of chitosan selenium nanoparticles (CTS-SeNPs) at a dose of 2 mg/kg body weight in streptozotocin (STZ) induced diabetes in rat model at a dose of 50 mg/kg body weight. Rats administered SeNPs orally in normal and experimentally induced diabetic rats for 35 days and glibenclamide (Glib) at a dose rate of 20 mg/kg was used as a reference drug. Blood samples were collected at the end of the experiment. Administration of SeNPs that was significantly decreased blood glucose levels and enhanced serum insulin concentration, Results showed a decrease in liver function enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST), all groups' showed non-significant changes in serum creatinine levels, decrease cardiac function enzymes creatine kinase-MB (CK-MB). Results also showed a significant decrease in the activity of α-amylase. Also, Glib administration exhibited a significant improvement in diabetic animals after 35 days of treatment. This study suggests that SeNPs capped with chitosan can alleviate hyperglycemia and showing synergistic effect in STZ-induced diabetic rats and this protection may be possibly by eliciting or SeNPs showed enhancement of the elimination of the diabetes-induced oxidative stress injuries.

1. INTRODUCTION

Diabetes mellitus (DM) is a common metabolic endocrine disorder that remains to be a concerning epidemic in the last century and is one of the main leading causes of worldwide death (Glovaci et al., 2019). Diabetes mellitus is a chronic, non-communicable disease (NCD) which has emerged as one of the leading global health problem associated with the pancreas in the production of insulin leading to hyperglycemia (WHO, 2014; Cho et al., 2018).

There are three types of DM. Type 1 Diabetes mellitus (T1DM) is an auto immune disease where pancreases produce little or no insulin at all. Type 2 Diabetes mellitus (T2DM) T2DM is a lifestyle disease because it is triggered by obesity, physical inactivity and sedentary lifestyle and type 3 is a condition specific to women when they are pregnant and it disappears after birth (Mayer-Davis et al., 2017; Zheng et al., 2018). It is said that T2DM is the end stages of diabetes where it mostly affects people later in their lives or it can be hereditary affect some in their younger years (Chen et al., 2012; Evans et al., 2000). Sedentary lifestyle with low/zero amounts of physical exercise is a huge contributing factor to fat build up in the body, This could be prevented through living a healthy lifestyle (Association, 2005).

Oxidative stress may play a role in the pathogenesis of human diseases. Many studies have investigated the relationship between oxidative stress parameters and various diseases such as some cancers, cardiovascular disease, Type II diabetes, cataracts and aging (Giugliano et al., 1996). Oxidative stress is believed to play a role both in the initial pathology of diabetes and in the development of vascular complications during the course of the disease (Craciun et al., 2016; Barseem and Elsamalehy, 2017). It can cause irreversible damage to the β-cells of the pancreatic islets (Xie et al., 2018). As a result, diabetic patients are susceptible to developing atherosclerotic cardiovascular diseases at early ages compared to healthy subjects (Craciun et al., 2016). Many antioxidants are produced in the body to prevent the harmful effects of these oxidants (Castro-Correia et al., 2017).

Individuals with diabetes were reported to have inflammatory cytokines. Trace element such as selenium can potentiate insulin activity and are essential for some activities in the body they affect the pathogenesis of diabetes via their role in peroxidation and inflammation (Donath and Shoelson, 2011) and the oral administration or intraperitoneal injection of daily doses of selenite for 3-8 weeks in streptozotocin-induced diabetic rat reduced glucose level (McNeill et al., 1991; Battell et al.,1998).

Nanoparticles are gaining lots of attention and concerns in biomedical and industrial application (Shirkhanloo et al., 2017; Zarchi et al., 2018). Especially metal nanoparticles with extraordinary characteristics are capable of many diagnostic (Fatemi et al., 2017), therapeutic (Amini et al., 2017; Karimi et al., 2018), health (Darabpour et al., 2017; Shaabani et al., 2017) and nutrition application (Amini et al., 2014). Recently many reports about a different biomedical application of selenium nanoparticles were

* Corresponding author: Neama M.A. Hamam,, Clinical Biochemistry, Faculty of Veterinary Medicine, Benha University, Egypt

published with anti-diabetic, antioxidant and anticancer 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 and micro-particles have shown interesting promise for protein delivery (Delie et al., 2005). Nano-particulate hydrogels consisting of chitosan or synthetic polymers have been developed and tested over the past two decades (des Rieux et al., 2006; Galindo-Rodriguez et al., 2006). Nano-particulate delivery systems have the potential to improve protein stability, increase the duration of the therapeutic effect and permit administration through non-parental routes (Florence, 1997).
Glib is an important drug for the management of hyperglycemia in moderate diabetes mellitus (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).
In this study, we designed new therapeutic strategies for diabetes mellitus with Chitosan SeNPs in order to investigate whether SeNPs can show antidiabetic activity, or even improve its therapeutic effects 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 housed at the animal house at the National Center for Radiation Research and Technology (NCRRT) (Cairo, Egypt).The rats were housed in metal cages at a temperature of 25 ± 2ºC for one 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:
Streptozotocin (STZ), Sodium selenite (Na2SeO3), Acetic acid, Chitosan (CTS), were purchased from Sigma Chemical, St. Louis, USA, Glib tablets (Dianil® 5 mg) dietary supplement was purchased from Sanofi- Egypt

2.3. Preparation 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 nanoparticle size and took the red color as in fig (1).

2.4. Characterization of SeNPs:
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 intra-peritoneal injection (50 mg/kg b. w.) solved 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 (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 gavage needle.
Group II:(CTS-SeNPs group): Rates were administered with CTS-SeNPs at a dose of (2 mg Se/kg b. w., in 1 ml saline) daily, by oral intubations using gavage needle (Zeng et al., 2018).
Group III: (Glib group): Rates were administered with Glib at a dose of (20 mg/kg b. w., in 1 ml saline) daily, by oral intubations using gavage needle (Zeng et al., 2018).
Group IV: (STZ group): Rats received a single intra-peritoneal injection of STZ(50 mg/kg b. w.) once at the first day of experiment.
Group V:(STZ+CTS-SeNPs): Rats received a single intra-peritoneal injection of STZ as group IV and after 3 days were administered with CTS-SeNPs at dose of (As group II) daily, by oral intubations using gavage needle.
Group VI: (STZ+Glib): Rats received a single intra-peritoneal injection of STZ as group IV and after 3 days were administered with Glib at a dose of (As group III).
Animals were sacrificed after 35 days of treatment.

2.7. Sampling:
2.7.1. Blood samples:
Blood samples were 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, then stored at -20°C in a deep freezer until determination of the following biochemical parameters.
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).ALT & AST 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), and the activity of α-amylase was determined in the serum spectrophotometrically using a kit of Bio-diagnostic co., Egypt
2.8.2. 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 $p < 0.05$.

Fig. 1 Photo of CTS-SeNPs samples; A: before nanoparticle formation B: after nanoparticle formation showing red color

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 were 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-SeNPs formation was approved by the appearance of a peak in the UV visible region at 270.5 nm characterize for CTS-SeNPs formation as seen.

3.2. Biochemical analysis:

Data were presented in table (1) showed that glucose and insulin levels not 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 ($p \leq 0.05$). Simultaneously, insulin levels drastically declined in STZ groups compared to the control group, while treatment with CTS-SeNPs and Glib restored the level of serum insulin in STZ+CTS-SeNPs and STZ+Glib group.

Analysis of the data of α-amylase activity (Table 2) of diabetic group (STZ) showed significant ($p \leq 0.05$) elevation in the enzyme activity compared to normal control group. Diabetic rats treated with CTS-SeNPs or Glib revealed significant decrease in enzyme activity ($p \leq 0.05$) in relevant to STZ group.

Table (3) showed that, ALT, AST, and Creatinine levels nearly to control group in CTS-SeNPs and Glib group (P>0.05). STZ group showed elevated activities of liver enzymes (ALT, AST). As shown after administering CTS-SeNPs and Glib to STZ group, a marked decrease in ALT, AST level was noted in comparison with the STZ group ($p \leq 0.05$). Non-significant changes in creatinine levels compared to STZ group.

Table 1 Effect of CTS-SeNPs on glucose and insulin levels in serum

Group	Glucose (mg/dl)	Insulin (ng/mL)
Control	86. 2± 2.7^{b}	2.3 ± 0.08^{b}
CTS-SeNPs	80.3± 5.2^{b}	2.4 ± 0.16^{b}
Glib	70.0 ± 4.2^{b}	2.5 ± 0.12^{b}
STZ	589.0 ± 38^{a}	1.3 ± 0.18^{a}
STZ+CTS-SeNPs	88.0± 7.4^{b}	1.9 ± 0.23^{a,b}
STZ+Glib	169.8 ± 30.5^{a,b}	1.89 ± 0.11^{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).

Table 2 Effect of CTS-SeNPs on serum α- amylase

Group	α-amylase (U/ml)
Control	316.5 ± 7.2^{b,d}
CTS-SeNPs	323 ± 5.3^{b,d}
Glib	308.5 ± 3.8^{b,c,d}
STZ	482.5 ± 17.4^{a,c,d}
STZ+CTS-SeNPs	326.8 ± 6.2^{b,d}
STZ+Glib	340.5 ± 12.9^{a,b,c}

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 3 Effect of CTS-SeNPs on serum ALT, AST and creatinine levels

Group	ALT (U/L)	AST (U/L)	Creatinine (mg/dl)
Control	32.7 ± 4.0^{b}	60.6 ± 2.7^{b}	0.7 ± 0.13
CTS-SeNPs	34.5 ± 5.0^{b}	59.3 ± 3.4^{b}	0.6 ± 0.08^{b}
Glib	38.2 ± 7.3^{b}	58.2 ± 5.7^{b}	0.7 ± 0.14
STZ	86.8 ± 3.7^{a}	94.8 ± 7.0^{a}	0.8 ± 0.09^{c,d}
STZ+CTS-SeNPs	39.2 ± 4.4^{b}	60.5 ± 6.0^{b}	0.6 ± 0.15^{b}
STZ+Glib	37.0 ± 4.5^{b}	69.7 ± 4.8^{a,b}	0.6 ± 0.15^{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).

4. DISCUSSION

The obtained results revealed selenium (Se), an essential trace element that shows antioxidant active oxygen free radical scavenging, defends the organs and tissues against oxidative damage and improves the body's immune system (Wang et al., 2013). Several studies have revealed Se to be an insulin-mimetic because it plays roles in the regulation of enzymes in the insulin signaling cascade, the expression of lipogenic enzymes, and in carbohydrate metabolism in the liver (Chen et al., 2015; Iizuka et al., 2010; Mao, Teng, 2013). Elevated Se blood levels could result in toxicity. On the other hand, SeNPs are more biocompatible with no or low toxicity when compared to seleno-methionine or selenite (Deng et al., 2019). SeNPs have received great attention because of their unique biological activities and low toxicity (Srivastava et al., 2014). These SeNPs also exhibit high biological activity and good absorptive ability due to the interaction between –NH2, C=O, −COO, and –C–N– groups of proteins and the nanoparticles of selenium (Hassanin et al., 2013). Insulin levels in diabetic rats once they are treated with SeNPs and/or insulin results in improved glycemic control (Becker et al., 1996). Selenium is capable of eliciting insulin-mimetic effects by activation of Akt and other kinases of the insulin signaling cascade such as p70 S6 kinase (Steinbrenner et al., 2011).

Diabetes mellitus is a metabolic disorder, Insulin is key player to regulate carbohydrate, fat, and protein metabolism. Insulin deficiency may affect the above important metabolisms; alpha amylase enzyme may be responsible for the breakdown of carbohydrates into glucose. The main enzyme involved in the digestion of carbohydrates is alpha-amylase. The alpha-linked polysaccharides are hydrolyzed by alpha-amylase to oligosaccharides, membrane-bound enzymes located in the brush border of the small intestine; facilitate the final stage of the carbohydrate digestive process to release absorbable monosaccharides, like glucose. (Abhijit et al., 2014). Reducing of alpha amylase will therefore slow the release of absorbable monosaccharides from dietary complex carbohydrates, postpone the absorption of glucose into blood and thus avoid any sudden increase in the amount of blood glucose caused in meals (Raman et al., 2012). Reducing of A amylase via certain inhibitors is used to control hyperglycemia, CTs-SeNPs also reduced glucose concentration in the serum through reducing the activity of α-amylase which reduce the viability of glucose and maltose in the blood as products of carbohydrates hydrolysis and delayed glucose absorption through delayed carbohydrate digestion and extended digestion time (Chiba, 1997; Perry et al., 2007).

ALT and AST are considered markers of liver toxicity (Mori et al., 2003). Our results showed that 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 enzymes of liver to normal values. Radical scavenging activity besides its integrity and functions of liver tissues results from the role of SeNPs in protection, 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, a non-significant change in serum creatinine levels was observed in diabetic rats this proves that the kidney injury is long-term. These results were in agreement with Gavin et al., (2003), who explain Long-term damage, dysfunction, and kidney failure are major complications of diabetes. The hypoglycemic effect of SeNPs explains 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 seleno-protein and modulating the endogenous antioxidants (Douillet et al., 1999).

5. CONCULOSIONS

This study will serve to manage the nanoparticle synthesis and restore damaged pancreatic tissue of diabetic rats. With more extensive research, SeNPs could be used in the future as an agent that can manage diabetes.

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