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Assessment of Antigenotoxic Effect of Nanoselenium and Metformin on Diabetic Rats

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Abstract

The present study was conducted to investigate the protective role of selenium nanoparticles on type-2 diabetes mellitus (T2DM) in male rats treated with metformin drug compared to the control. Chromosomal aberrations, DNA fragmentation, micronucleus tests as well as comet assay were carried out. Rats were divided into four groups: group1, normal control in which the rats were received normal saline solution, group 2, control diabetic group in which the diabetic rats were induced using 45mg/Kg body weight Streptozotocin (STZ). Group 3, Metformin treated group in which the diabetic rats were treated with a standard oral hypoglycemic agent, metformin (100 mg/kg body weight (b.wt) while, Group 4, selenium nanoparticles (SeNPs) treated group, in which the rats were treated with nanoselenium stabilized in liposome (0.1 mg/kg b. wt) orally plus metformin. Rats were sacrificed after 21 days following the last injection. The results demonstrated that diabetes caused increase in chromosomal aberrations, micronucleus formation, DNA fragmentation as well as DNA damage using comet assay, but these parameters decrease in treated animals with metformin. In addition, metformin + nanoselenium combined treatment caused decreased in all studied parameters relative to metformin alone. In conclusion, it appeared that SeNPs combined with metformin have antidiabetic effect in experimental model of T2DM. This effect could be ascribed to their ability in decreasing frequencies of chromosomal aberrations, decreasing the number of micronucleus as well as decreasing the percentages of DNA damage. Thus, SeNPs may benefit in clinical purposes especially in enhancing the effect of T2DM.

Keywords: Selenium nanoparticles, Diabetes, Bone marrow, DNA damage, Rats.

1. Introduction

Diabetes mellitus is the most common endocrine disease in which there is an increase in the level of blood sugar over a prolonged period (hyperglycemia) (World Health Organization (WHO), 2014). There are many symptoms of this disease, such as increased ingestion of food, increased thirst, muscular proteolysis, postprandial sickness and damage of many organs, such as eyes, nerves, kidneys and heart, these symptoms affect the human life as a whole. If untreated, diabetes causes many problems, such as cardiovascular diseases, kidney failure and eye damage (WHO, 2013), diabetic ketoacidosis and nonketetotic hyperosmolar coma (Kitabchi *et al*., 2009).

There are three types of diabetes mellitus: type 1, which results from a failure in the pancreas (WHO, 2013). Type 2 (the most common type of diabetes) in which the cells do not respond to insulin property, and the number of diagnosed cases of this type has increased over the past few decades (Olansky *et al.*, 2003), and it forms about 90% of the diabetes cases (Shi and Frank, 2014) distributed equally between women and men (Vos *et al*., 2012) and the number is expected to reach 592 million in 2035 (IDF, 2014). Type 3 is gestational diabetes and this type occurs in pregnant women who have diabetes history in their family (WHO, 2013).

The treatment of type 2 diabetes depends on using many drugs, such as sulfonylureas, Insulin, peroxisome proliferator-activated receptor- γ agonists and metformin.

Metformin (dimethylbiguanide) is the most described oral drug used in the treatment of type 2 diabetes (The American Society of Health-System Pharmacists, 2016; Maruthur *et al.*, 2016) to control the blood sugar level with or without Insulin in conjunction with life modification, such as weight control, special diet and physical activity (Sardas *et al.*, 2001). Metformin decreases the resistance of insulin, hepatic glucose output and enhances the uptaking of glucose (Bailey and Turner, 1996; Nisbet *et al.*, 2004); these to decrease fasting and blood glucose by 20 to 40 percent, decreasing of hemoglobin and body weight and increase high density lipoprotein (HDL) (Howlett and Bailey, 1999).

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Selenium (Se) is an important element for health with important biological and biochemical functions due to its antioxidant properties (Chunying *et al.*, 2006). Se is a basic component of Selenoprotein which reduces oxidative stress (Rayman, 2012, Ahmed *et al.*, 2016).

High selenium level in blood plasma has been correlated with prevention of several diseases, such as cancers, cardiovascular diseases, muscle disorders as well as diabetes mellitus (Navarro-Alarco´n and Lo´pez-Martı´nez, 2000).

The relation between selenium and T2DM is controversial (Wang *et al.*, 2016). So untreated diabetes causes oxidative stress which leads to complications of the disease and the antioxidant intake is considered as being favorable for the therapy of diabetes (Mueller *et al.*, 2009), and results in decreasing insulin, homeostasis model of assessment-insulin resistance, serum high-sensitivity C-reactive protein, and increasing the quantitative insulin sensitivity check index score as well as the concentration of the total antioxidant capacity (Farrokhian *et al.*, 2016). It is clear that Selenium plays a protective role against T2DM (Steinbrenner and Sies, 2009).

Furthermore, it has been found that the half-life of some therapeutics can be improved by conjugating them to nanocarriers (Makhluf *et al*., 2008; Liu *et al.*, 2012). It is obvious that selenium nanoparticles (SeNPs) have been considered a promising tool in drug therapies of type 2 diabetes; this is due to its low toxicity and high therapeutic properties (Rao *et al.*, 2014).

SeNPs have been widely studied because they have advanced biological activity and high photoelectric performance, antioxidant activity, anticancer, and immune-system enhancements (Gao *et al*., 2002; Gates *et al*., 2002). By comparing Se with SeNPs, it was found that SeNPs can serve as a potential chemopreventive agent with reduced risk of Se toxicity (Zhang *et al*., 2005; Wang *et al*., 2007).

The purpose of the present study is to investigate the action of nanoselenium, combined with metformin, on the induction of chromosomal aberrations, formation of micronuclei and DNA fragmentation in streptozotocin induced T2DM rats compared to those healthy controls.

2. Materials and Methods

2.1. Experimental Animals

For the present study, adult female albino rats of Westar Strain weighing 180- 200 g were obtained from the Holding Company for Biological Products and Vaccines (VACSERA, Cairo, Egypt). After an acclimatization period of one week, the animals were classified into four groups of equal average body weight and housed in wire-bottomed cages in room under standard condition of illumination with 12 hours light-dark cycle at 25+1℃. They were provided with water and rodent chow *ad libitum*. All animals received care in compliance with the Egyptian rules of animal experiments which were approved by the ethical committee of medical research of the National Research Centre, Cairo, Egypt.

2.2. Induction of Diabetes

Rats were fasted for 18 – 24 h before induction diabetes by using streptozotocin (STZ) from Sigma. St. Louis, MO, USA. (CAS no 18883-66-4). The rats received a single intra-peritoneal (i. p) injection of 45 mg/Kg/wt of STZ, which was freshly prepared and dissolved in 0.05 M citrate buffer, pH 4.5 as described previously (Wei *et al*., 2003). Blood glucose was contoured every 2 days using an Accu-chek blood glucose meter (Roche Diagnostics, Basel, Switzerland). Rats with blood glucose levels > 15 Mm (200mg/dl) for 7 consecutive days were considered diabetic.

2.3. Experimental Design

Rats were divided into four groups: group1, a normal control group in which the rats received normal saline solution; group 2, a diabetic control group in which the rats were diabetic using Streptozotocin (45mg/Kg b.wt) and left untreated; group 3, a group treated with Metformin in which the diabetic rats were treated with a standard oral hypoglycemic agent, metformin from Merck, CAS no 657-24-9, USA (100 mg/kg b.wt) (Kosegawa *et al*., 1999); and group 4, selenium nanoparticles (SeNPs)-treated group, in which the rats were treated with nanoselenium stabilized in liposome (0.1 mg/kg b. wt) orally (Loeschner *et al*., 2014) in addition to metformin treatment. After 14 days of daily treatment, overnight fasting animals euthanized under mild ether anesthesia.

2.4. Biosynthesis of Selenium Nanoparticles

Nanoselenium was prepared according to the method of Dwivedi *et al.*(2011) by a simple wet chemical method. Sodium selenosulphate (Na2SeSO3) (Sigma. St. Louis, MO, USA) precursor reacted with different organic carboxylic acids in aqueous medium by using polyvinyl alcohol to stabilize selenium nanoparticles. Then, the synthesized nanoparticles were separated from their solution by centrifugation at 15,000 rpm and redispersed in aqueous medium with a sonicator.

2.5. Characterization of Selenium Nanoparticles

Characterization of selenium nanoparticles was carried out by examination of a sample under Transmission Electron Microscope (TEM) (JEOL-100 CX).

2.6. Liposome Preparation

Elemental selenium nanopartiacles in molar ratio7:2 were used to prepare neutral mutilamellar vesicles using the method of Kim *et al.* (1985). Briefly, 10 mg of high purity L-alpha-diapalmitoyl phosphatidyl choline (DPPC) from (Lipoid KG-Germany) and 1 mg of selenium were transferred to 50 ml round bottom flask. Then, 15 ml of Chloroform was added, and the flask was shaken until all lipids dissolved in Chloroform. The solvent evaporated under vacuum using rotary evaporator until a thin dry film of lipids was formed. The flask left under vacuum for 12 h to ensure the evaporation of all traces of chloroform. 10 ml of buffer (10 mM Trizma, pH 7) added to the flask in mechanically shaken for 1 h at temperature of 45℃. The suspension centrifuged at 8000 rpm for 20 min and the supernatant discarded. The liposome re-suspended in 10 ml buffer solution. Control liposome was prepared following the same classical method as before using only

aliquots of 10 mg of DPPC. The concentration of free selenium/ml of buffer was adjusted to be 1/mg/ml.

2.7. DNA Fragmentation Assay

DNA fragmentation was measured by spectrophotometer using diphenylamine (DPA) method, according to the method of (Perandones *et al.*, 1993) with some modifications. Samples of liver were homogenized in lyses buffer containing 5mM Tris–HCl, pH 8.0, 20mM EDTA and 0.5% Triton X-100. Then, centrifuged at 1500×*g* for 20 min. Pellets were resuspended in 0.5N perchloric acid and 5.5N perchloric acid was added to supernatant, centrifuged again at 1500×*g* for 10 min to remove proteins. Samples were heated at 90 ◦C and after cool reacted with diphenylamine (DPA) for 16–20h at room temperature. Absorbance was measured at 600 nm using a UV-double beam spectrophotometer (Shimdazu 160 A). DNA fragmentation in samples = (fragmented DNA in supernatant.)/[(fragmented DNA in supernatant intact + DNA in pellet)] were expressed as percentage of total DNA appearing in the supernatant fraction.

2.8. Detection of Oxidative DNA Damage (Comet Assay)

According to the method of Singh *et al.* (1988), 0.5 g of crushed samples were transferred to 1 ml ice-cold PBS. This suspension was stirred for 5 min and filtered. Cell suspension (100 µl) was mixed with 600 µl of low-melting agarose (0.8% in PBS). 100 µl of this mixture was spread on pre-coated slides. The coated slides were immersed in lyses buffer (0.045 M TBE, pH 8.4, containing 2.5% SDS) for 15 min. The slides were placed in electrophoresis chamber containing the same TBE buffer, but devoid of SDS. The electrophoresis conditions were 2 V/cm for 2 min and 100 mA. Staining was done using 20µg/ml ethidium bromide at 4°C. A total of 100 randomly captured comets from each slide was examined at 400 x magnification using a fluorescence microscope connected to CCD camera to an image analysis system [komet 5 image analysis software developed by Kinetic Imaging, Ltd. (Liverpool, UK)]. A computerized image analysis system acquires images, computes the integrated intensity profiles for each cell, estimates the comet cell components and, then, evaluates the range of derived parameters. To quantify the DNA damage, Tail Length (TL), the percentage of migrated DNA (Tail DNA %) and Tail Moment (TM) were evaluated. Tail length (length of DNA migration) is related directly to the DNA fragment size and presented in micrometers. It was calculated from the centre of the cell. Finally, the program calculates tail moment.

2.9. Bone Marrow Chromosomal Aberration Assay

At first, the mice were injected with 4 mg/kg b.wt. colchicine two hours before sacrifice. Metaphase cells were prepared according to the standard technique of Preston *et al.* (1987). Bone marrow cells were aspirated from both femurs of each animal; then, the cells were centrifuged at 1000 rpm for 10 min. and resuspended in pre-warmed (37°C), hypotonic solution (0.075M potassium chloride) for 20 min at 37°C. The samples were centrifuged and fixed in cold 3:1 methanol: glacial acetic acid. Each sample was washed five times fixative. The slides were stained in 10% buffered Giemsa (pH 7.0), air-dried and mounted in DPX. Chromosome aberrations were identified according to criteria described by Savage (1975).

2.10. The Micronucleus Test

Bone marrow slides were prepared according to the method described by Hayashi *et al.* (1983). The bone marrow was washed with 1 ml of fetal calf serum and then smeared on clean slides. The slides were left to air-dry and then fixed in methanol for 5 minutes followed by staining in May-Grunwald- Gemisa for 5 minutes then washed in distilled water and mounted. For each animal, at least 2000 polychromatic erythrocytes (PCEs) per animal were examined for the presence of micronuclei.

2.11. Statistical Analysis

Statistical analysis was carried out with SPSS software. Data were analyzed using one-way analysis of variance (ANOVA) followed by Duncan's post hoc test for comparison between different treatments. The values were expressed as mean ± S.E and differences were considered as significant when $< P$ 0.05.

3. Results

3.1. Chromosomes Examination

The analysis of bone marrow chromosomes in diabetic animals depicted various types of chromosomal aberrations, which included gap, break, deletions, fragments, centromeric attenuations and endomitosis as structural aberrations as well as polyploidy and prediploidy as numerical aberrations.

The results in table 1 represent the chromosomal aberrations analysis and showed that the frequencies of structural and numerical chromosomal aberrations were significantly increased ($P< 0.05$) in diabetic animals (STZ-challenged group) comparing to control group. In contrast, the diabetic animals treated with Metformin had significant ($P< 0.05$) decreases in the frequencies of all structural and numerical chromosomal aberrations than diabetic group. In addition, metformin/nanoselenium group had the lowest frequencies of most structural aberrations (gap, fragment, endomitosis, C.A, total structural aberrations) and total numerical chromosome aberrations relative to Metformin group.

Table 1. Effect of metformin and nanoselenium treatments on the frequency of chromosome aberrations in STZ- induced diabetic rats

Treatment	Structural aberrations							Numerical aberrations			Total aberrations
	Gap	Break	Deletion	Fragment	Centromeric ttenuation	Endomitosis	Total	Preidiploidy	Polyploidy	Total	
Control	0.67±0.21^{c}	0.33±0.21^{c}	0.33±0.21^{c}	0.17±0.16^{c}	0.50±0.22^{c}	0.67±0.21^{c}	2.17±0.40^{d}	1.33±0.21^{b}	0.33±0.21^{b}	1.67±0.21b^{c}	3.84±0.30^{d}
D	3.67±0.21^{a}	2.67±0.21^{a}	2.67±0.33^{a}	2.50±0.22^{a}	4.50±0.22^{a}	3.67±0.21^{a}	18.33±0.42^{a}	4.67±0.21^{a}	2.50±0.22^{a}	7.17±0.30^{a}	25.50±0.56^{a}
D+M	2.00±0.26^{b}	1.50±0.22^{b}	1.33±0.21^{b}	1.50±0.22^{b}	2.33±0.21^{b}	2.00±0.26^{b}	9.17±0.47^{b}	1.50±0.22^{b}	0.67±0.21^{b}	2.17±0.16^{b}	11.34±0.42^{b}
D+M+Se	1.17±0.30^{c}	1.46±0.19^{b}	1.17±0.23^{b}	0.50±0.22^{c}	0.83±0.16^{c}	1.17±0.30^{c}	4.50±0.34^{c}	1.17±0.16^{b}	0.33±0.21^{b}	1.33±0.21^{c}	5.83±4.77^{c}

D: Diabetic rats by using streptozotocin (STZ); D+M: Diabetic group treated with metformin drug; D+M+Se: Diabetic group treated with metformin and nanoselenium particles. All data are expressed as mean ± SEM. Means bearing different letters superscripts are significantly different at ($P < 0.05$).

3.2. Influence of Diabetes on the DNA Damage (Comet Assay)

The DNA damage resulted from diabetes was investigated by comet assy. the lengths of the comets (DNA tails) depended on the effect of diabetic in rats, longer tails indicating more DNA damage.

Figure 1 shows representative examples of diabetic rats. Control liver rat cells showed no tails (Figure A). Tails appeared in diabetic rats (Figure B) and were substantially less in diabetic treatment one (Figures C and D). The mean value of tail length in Diabetic group was increased rapidly and significantly more than that of their controls, as shown in table 2, and more DNA damage was observed in the tail when compared with control group (Figure 1).

As shown in table 2, the extent of DNA damage, measured in TM, increased rapidly in diabetic group when compared to control. Treated groups with metformin or metformin+ nanoselenium showed a significant decrease in comet TM values when compared to diabetic group ($P<0.05$) but is still a significant increase compared with the control.

The maximum extent of DNA damage, increased rapidly in diabetic group when compared to control. Then, the extent of damage decreased significantly ($P<0.05$) in both treated groups with metformin or metformin+nanoslenium but it did not reach the control level.

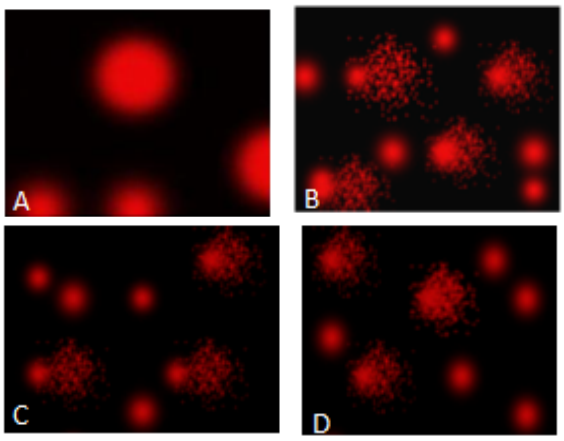

Figure 1. Effect of metformin, nanoselenium treatments in STZ-induced diabetic rats on bone marrow DNA as detected by comet assay. (A) Normal untreated cell; (B) diabetic cell, (c) diabetic rat treated with metformin and (D) diabetic rat treated with nanoselenium and metformin.

Table 2. Comet parameters in liver cells of metformin, nanoselenium treatments in STZ- induced diabetic rats as measured by comet assay.

Treatment	Tailed %	Untailed %	Tail length µm	DNA %	Tail moment (unit)
Control	2.33± 0.33^{d}	97.67± 0.33^{a}	2.16± 0.09^{c}	1.33± 0.22^{d}	2.84± 0.41^{d}
D	21.0± 0.58^{a}	79.0± 0.58^{d}	5.27± 0.18^{a}	6.57± 0.28^{a}	34.65± 2.41^{a}
D+M	17.67± 0.33^{b}	82.33± 0.33^{c}	4.5± 0.29^{b}	4.27± 0.15^{b}	19.25± 1.66^{b}
D+M+Se	15.0± 0.58^{c}	85.0± 0.58^{b}	3.77± 0.28^{b}	3.47± 0.26^{c}	13.19± 1.88^{c}

D: Diabetic rats by using streptozotocin (STZ); D+M: Diabetic group treated with metformin drug; D+M+Se: Diabetic group treated with metformin and nanoselenium particles. All data are expressed as mean ± SEM. Means bearing different letters superscripts are significantly different at ($P < 0.05$).

3.3. Micronucleus Test

The effect of diabetes and treatments with metformin and nanoselenium on micronucleated polychromatic erythrocytes (MnPCEs) formation in the bone marrow cells of male rats is shown in Figure 2. In this Figure, it is demonstrated that there were few formations of MN in rats belong to control group (3.33±0.21), but MN formation significantly increased ($P<0.05$) in diabetic animals (26.00±0.25) comparing to control group. Meanwhile, treated groups with metformin significantly reduced the MNPCE when compared with diabetic group. Additionally, MNPCE in the combined metformin and nanoselenium (14.00±0.25) significantly reduced more than the group treated with metformin alone (20.67±0.33).

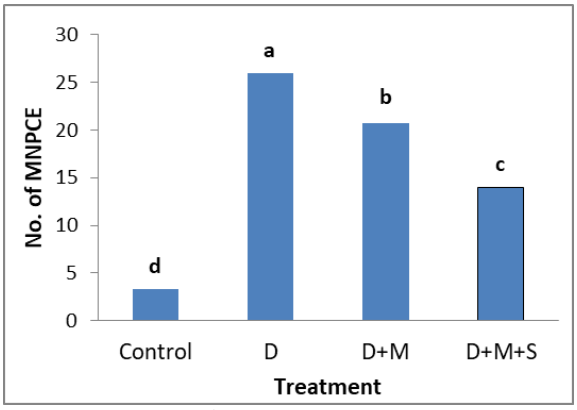

Figure 2. Frequencies of micronucleated polychromatic erythrocytes (MPCEs) in rat bone marrow cells in all experimental groups.

3.4. DNA Fragmentation

The effects of nanoselenium and metformin drug on diabetic rats were investigated by DNA fragmentation assay (Figure 3). The result demonstrated that fragmented DNA, in the control group (8.33±0.33), was lower than that in the other treated groups. Also, fragmented DNA in diabetic animals (24.33±0.33) was significantly higher than that of the control group ($P< 0.05$). On the other hand, treated animals with metformin decreased the DNA fragmentation comparing with diabetic animals ($P<0.05$). In addition, the combined treatment with metformin /nanoselenium (12.17±0.47) decreased the fragmentation of DNA compared to the treatment with metformin alone (18.33±0.33) ($P<0.05$).

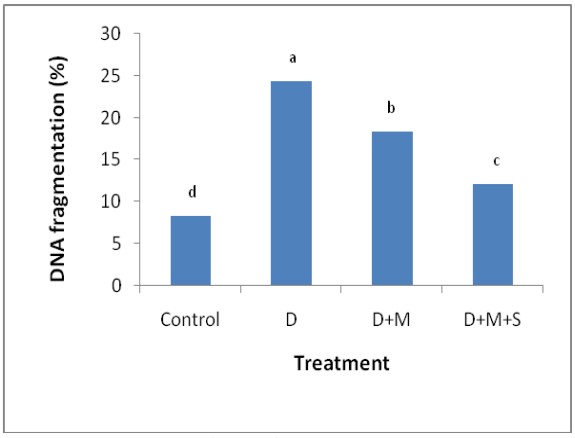

Figure 3. Percentages of DNA fragmentation in rat liver cells in all experimental groups.

4. Discussion

The present study indicated that the administration of STZ increased genetic alterations (micronuclei frequency, fragmented DNA, as well as chromosomal aberrations). The genotoxic effects of STZ, observed in the present study, are in agreement with the results obtained from previous studies (Vikram *et al.*, 2007; Attia *et al*., 2009). Also, Martínez-Pérez *et al.* (2007) found that there were high levels of micronucleus frequency in type 2 diabetes with no microvascular or macrovascular complications. In addition, Corbi *et al*. (2014) indicated that there is an association between T2DM and DNA Damage by studying the frequency of micronucleated polychromatic erythrocytes. This genotoxicity may be due to the presence of hyperglycemia condition in T2DM that caused production of reactive oxygen species (ROS) (Pham-Huy *et al.*, 2008; Robertson, 2004). Accumulation of ROS caused an oxidative stress, which plays a crucial role in cellular mechanism of tissue injury in a wide spectrum of disease states. It may lead to a constant threat to all living organisms and antioxidant defense system is employed by the body to eliminate it (Ceconi *et al.*, 2003). In addition, stress causes increase resistance of insulin and disturbance in pancreatic β-cell function (Houstis *et al.*, 2006).

Oxidative stress can attack all types of macromolecules including DNA by attacking the cell membrane, nucleus and then genetic materials, which leads to chromosomal aberrations (Attia, 2010; Otton *et al.*, 2004). DNA oxidative damaging occurs due to modifications in nucleotide bases or sugars, these modifications lead to mutations which may cause formation of tumors if they occur in somatic cells (Selvakumar *et al.*, 2006), then early aging may occur which leads to death (Rehman *et al.*, 1999).

Moreover, ROS damage the cell by different pathways, such as advanced formation of advanced glycation end production, hexosamine pathway, protein kinase and lipid peroxidation (Piconi *et al*., 2003), which is a result of attacking ROS to the residues of polyunsaturated fatty acid of cell membrane. The imbalance between production of ROS and the ability of antioxidant to detoxify their effect cause oxidative stress (Echtay, 2007; Roberts and Sindhu, 2009).

By using comet assay, the present study revealed that diabetes cause DNA damage. This result is similar to that obtained from the study of Sardas *et al.* (2001) who reported increase in oxidative DNA damage in type 1 and type 2 diabetes, and these damage was higher in type 2 diabetes compared to type 1 diabetes. Also, Marra *et al.* (2002) found that oxidative stress is increased in diabetes and this leads to cardiovascular disease.

Diabetic animals treated with Metformin showed decrease of most frequencies of structural and numerical chromosome aberrations and reduced the incidence of MN formation in PCEs in the diabetic animals. These may be due to the lowering of blood glucose by Metformin as a result of stimulating insulin releasing from functioning pancreatic beta cells (Chunying *et al*., 2006; Kirpichnikov *et al*., 2002) and hepatic glucose output as well as enhancing peripheral glucose uptake (Bailey and Turner, 1996), these to decrease fasting blood glucose by 20 to 4%, decrease body weight, decrease low density lipoprotein and increase high density lipoprotein (Howlett and Bailey, 1999). Therefore, a diabetes patient has three times normal rate of gluconeogenesis but metformin reduces this by one-third (Hundal *et al*., 2000). Metformin increased the insulin sensitivity and reduced the oxidative stress levels and the activity of catalase and superoxide dismutase when compared with diabetes rats (Vilela *et al.*, 2016). So, the dual therapy with metformin drug promotes more benefits to oxidative stress control in rats.

The current data revealed that the treatment with SeNPs in addition to metformin drug resulted in a significant reduction in the genetic alterations (micronucleus formation, chromosomal aberrations) as well a comet assay. This may be due to the antioxidant property of a compound which could play a significant role in decreasing the nuclear injury caused by diabetes (Adler *et al*., 2009). These observations are consistent with those of Abdelaleem *et al.* (2016) who found that selenium nanoparticles possesses antioxidant and anti-diabetic activities by decreasing oxidative stress biomarkers as well as blood glucose level. Similarly, Ahmed *et al.* (2016) found that selenium nanoparticles have antidiabetic potency, repression of oxidative stress, potentiating of the antioxidant defense system, and inhibition of pancreatic inflammation. So, selenium plays a protective role against type 2 diabetes (Steinbrenner and Sies, 2009). The protective roles of selenium in mammalian cell are due to its function in the active site of many antioxidant enzymes, such as thioredoxin reductase, glutathione and GR (Flora

et al., 2002). Dietary selenium may cause an increase in glutathione peroxidase, which has the ability to detoxify ROS (Köhrle and Gärtner, 2009) that can interfere with insulin signaling and result in regulation of glucose levels and prevention of diabetes (Goldstein *et al.*, 2005). Selenium is also a basic component of selenoprotein, an important enzyme in the body which protects from oxidative stress and inflammation (Rayman, 2012).

The nutritional deficiency of selenium causes muscular dystrophy, endemic fatal cardiomyopathy, and chronic degenerative diseases when used alone or in combination (Rayman, 2002).

5. Conclusion

In conclusion, the present study demonstrated the antidiabetic potential of SeNPs delivered in liposomes in the experimental model of T2DM. This effect appeared in decreasing the frequencies of chromosomal aberrations, in addition to decreasing the number of MNPCEs as well as repairing damaged DNA which is represent in DNA fragmentation assay and comet assay. Thus, SeNPs may benefit in clinical purposes, especially in enhancing the effect of T2DM.

References

Abdelaleem RMA, abdelhameed HF, Askar ME, Hassan SHM and Elbatal AI. 2016. Modulatory role of selenium nanoparticles and grape seed extract mixture on oxidative stress biomarkers in diabetic irradiated rats. *Indian J Pharmaceutical Edu Res*., **50 (1):**170-178.

Adler AI, Shaw EJ, Stokes T and Ruiz F. 2009. Newer agents for blood glucose control in type 2 diabetes: summary of NICE guidance. *BM J.* **338**: 1668

Ahmed HH, Abd El-Maksoud MD, Abdel Moneim A.E and Aglan HA. 2016. Pre-Clinical study for the antidiabetic potential of selenium nanoparticles. *Biol Trace Elem Res*., **177(2):**267-280.

Attia SM. 2010. Deleterious effects of reactive metabolites. *Oxidative Med Cellular Longevity*, **3 (4)**: 238–253,

Attia SM, Helal GK and Alhaider A A. 2009. Assessment of genomic instability in normal and diabetic rats treated with metformin. *Chemico-Biological Interactions,* **180 (2):** 296–304.

Bailey CJ and Turner RC. 1996. Metformin. *N Engl J Med*., **334**:579.

Ceconi C, Boraso A, Cargoni A and Ferrari R. 2003. Oxidative stress in cardiovascular disease. *Arch. Biochem. Biophys*., **420**: 217-221.

Chunying C, Hongwei Y, Jiujiang Z, Baili LQ, Shuiping L, Peiqun Z and Zhifang C. 2006. The roles of serum Se and selenoproteins on Hg toxicity in environmental and occupational exposure. *Environ. Health Perspect* .,**114 (2)**: 297-301.

Corbi SCT, Bastos AS, Orrico SRP, Secolin R, Raquel A. Santos D, Takahashi CS and Scarel-Caminaga RM. 2014. Elevated micronucleus frequency in patients with type 2 diabetes, dyslipidemia and Periodontitis. *Mutagenesis,* **29 (6):** 433–439.

Dwivedi C, Shah CP, Singh K, Kumar M and Bajaj PN. 2011. An organic acid-induced synthesis and characterization of selenium nanoparticles. *J Nanotechnol*., **2011**: 1–6.

Echtay KS. 2007. Mitochondrial uncoupling proteins-what is their physiological role? *Free Radical Biol Med.,* **43 (10)**: 1351–1371.

Farrokhian A , Bahmani F , Taghizadeh M , Mirhashemi SM , Aarabi MH , Raygan F , Aghadavod E and Asemi Z. 2016. Selenium supplementation affects insulin resistance and serum hs-crp in patients with type 2 diabetes and coronary heart disease. *Horm Metab Res.,* **48 (4):** 263-8.

Flora SJS, Kannan GM, Pant BP and Jaiswal DK. 2002. Combined administration of oxalic acid, succimer and its analogue for the reversal of gallium arsenide induced oxidative stress in rats. *Arch Toxicol*., **76**: 269-76.

Gao X, Zhang J and Zhang L. 2002. Hollow sphere selenium nanoparticles: their in-vitro anti hydroxyl radical effect. *Adv Mater*., **14**: 290–293.

Gates B, Mayers B, Cattle B and Xia Y. 2002. Synthesis and characterization of uniform nanowires of trigonal selenium. *Adv Funct Mater*., **12**: 219–227.

Goldstein BJ, Mahadev K and Wu X. 2005. Redox paradox: insulin action is facilitated by insulin-stimulated reactive oxygen species with multiple potential signaling targets. *Diabetes* **54:** 311–21.

Hayashi M, Sofuni T and Ishidate M Jr. 1983. An application of acridine orange fluorescent staining to the micronucleus test. *Mutat Res.,* **120**: 241–247.

Houstis N, Rosen ED and Lander ES. 2006. Reactive oxygen species have a causal role in multiple forms of insulin resistance. *Nature*, **440**: 944–8.

Howlett HC and Bailey CJ. 1999. A risk-benefit assessment of metformin in type 2 diabetes mellitus. *Drug Saf*., **20 (6):** 489.

Hundal R, Krssak M, Dufour S, Laurent D, Lebon V, Chandramouli V, Inzucchi S, Schumann W, Petersen K, Landau B and Shulman G. 2000. Mechanism by which metformin reduces glucose production in type 2 diabetes. *Diabetes*, **49 (12):** 2063–9.

IDF. International Diabetes Federation. Retrieved 29 November 2014.

Kim S, Jacobs RE and White SH. 1985. Preparation of multilamellar vesicles of defined size-distribution by solvent-spherule evaporation. *Biochim Biophys Acta*., **812 (3):** 793–801.

Kirpichnikov D, McFarlane SI and Sowers JR. 2002. Metformin: an update. *Ann Intern Med.,* **137 (1):** 25–33.

Kitabchi AE, Umpierrez GE and Miles JM. 2009. Hyperglycemic crises in adult patients with diabetes. *Diabetes Care,* **32 (7):** 1335–1343.

Köhrle J and Gärtner R. 2009. Selenium and thyroid. *Best Pract Res Clin Endocrinol Metab*., **23 (6):** 815–827.

Kosegawa I, Chen S, Awata T, Negishi K and Katayama S. 1999. Troglitazone and metformin, but not glibenclamide, decrease blood pressure in Otsuka Long Evans Tokushima fatty rats. *Clin Exp Hypertens.,* **21(3)**:199–211.

Liu W, Li X, Wong YS, *et al*. 2012. Selenium nanoparticles as a carrier of 5-fluorouracil to achieve anticancer synergism. *ACS Nano*., **6**: 6578–6591.

Loeschner K, Hadrup N, Hansen M, Pereira SA, Gammelgaard B, Møller LH, Mortensen A, Lam HR and Larsen EH. 2014. Absorption, distribution, metabolism and excretion of selenium following oral administration of elemental selenium nanoparticles or selenite in rats. *Metallomics*, **6(2)**:330–337.

Makhluf SBD, Abu-Mukh R, Rubinstein S, Breitbart H and Gedanken A. 2008. Modified PVA-Fe3O4 nanoparticles as protein carriers into sperm cells. *Small* , **4**: 1453–1458.

Marra G, Cotroneo P, Pitocco D, Manto A, Di Leo MA, Ruotolo V, Caputo S, Giardina B, Ghirlanda G and Santini SA. 2002. Early increase of oxidative stress and reduced antioxidant defenses in patients with uncomplicated type 1 diabetes: a case for gender difference. *Diabetes Care*, **25**: 370-375.

Martínez-Pérez LM, Cerda-Flores RM, Gallegos-Cabriales EC, Dávila-Rodríguez MI, Ibarra-Costilla E and Cortés-Gutiérrez EI. 2007. Frequency of micronuclei in Mexicans with type 2 diabetes mellitus. *Prague Med. Rep*., **108**: 248-255.

Maruthur NM, Tseng E, Hutfless S, Wilson LM, Suarez-Cuervo C, Berger Z, Chu Y, Iyoha E, Segal JB and Bolen S. 2016. Diabetes medications as monotherapy or metformin-based combination therapy for type 2 diabetes: A Systematic Review and Meta-analysis. Annals of Internal Medicine 7 june.

Mueller AS, Mueller K, Wolf NM and Pallauf J. 2009. Selenium and diabetes: an enigma? *Free Radic Res.,* **43(11):** 1029-59.

Navarro-Alarco´n M and Lo´pez-Martı´nez MC. 2000. Essentiality of selenium in the human body: relationship with different diseases. *Sci Total Environ*., **249**: 347–371.

Nisbet JC, Sturtevant JM and Prins JB. 2004. Metformin and serious adverse effects. *Med J Aust.*, **180**:53-54.

Olansky L, Marchetti A and Lau H. 2003. Multicenter retrospective assessment of thiazolidinedione monotherapy and combination therapy in patients with type 2 diabetes: comparative subgroup analyses of glycemic control and blood lipid levels. *Clin Ther*. **25**: 64–80.

Otton R, Soriano F.G, Verlengia R and Curi R. 2004. Diabetes induces apoptosis in lymphocytes. *J. Endocrinol*., **182**: 145-156.

Perandones CE, Illera VA, Peckham D, Stunz LL and Ashman R F. 1993. Regulation of apoptosis *in vitro* in mature murine spleen T cells. *J Immunol.,***151**: 3521-3529.

Pham-Huy LA, He H and Pham-Huy C. 2008. Free radicals, antioxidantsin disease and health. *Inter J Biomedical Sci*., **4 (2):** 89-96.

Piconi, L, Quagliaro L and Ceriello A. 2003. Oxidative stress in diabetes. *Clin Chem Lab Med*., **41**: 1144-1149.

Preston RJ, Dean BD, Galloway S, Holden H., McFee AF and Shelly M. 1987. Mammalian *in vivo* cytogenetic assays: Analysis of chromosome aberrations in bone marrow cells. *Mutat. Res*., **189**: 157-165.

Rao L, Ma Y, Zhuang MJ, Luo TJ, Wang YY and Hong A. 2014. Chitosan-decorated selenium nanoparticles as protein carriers to improve the *in vivo* half-life of the peptide therapeutic BAY 55-9837 for type 2 diabetes mellitus. *Inter J Nanomedicine* , **9**: 4819-4828.

Rayman M. 2002. The argument for increasing selenium intake. *Proc Nutr Soc*., **61**:203-315.

Rayman MP. 2012. Selenium and human health. *Lancet*, **379**: 1256–68.

Rehman A, Nourooz-zadeh J, Moller W, Tritschler H, Pereira P and Halliwell B. 1999. Increased oxidative damage to all DNA bases in patients with type 2 diabetes mellitus. *FEBS Lett.,* **448**:120-122.

Roberts CK and Sindhu KK. 2009. Oxidative stress and metabolic syndrome. *Life Sci*., **84 (21):** 705–712.

Robertson R. 2004. Chronic oxidative stress as a central mechanism for glucose toxicity in pancreatic islet beta cells in diabetes. *J Biol Chem.*, **279**: 42351-42354.

Sardas S, Yilmaz M, Oztok U, Caki N and Karakaya AE. 2001. Assessment of DNA strands breakage by comet assay in diabetic patients and the role of antioxidant supplementation. *Mutat Res*., **490:**123–129.

Savage JRK. 1975. Classification and relationship of induced chromosomal structural changes. *J Med Genet.,* **12:** 103–122.

Selvakumar E, Prahalathan C, Varalakshmi P *et al*., 2006. Modification of cyclophosphamide-induced clastogenesis and apoptosis in rats by alpha-lipoic acid. *Mutat Res*., **606**:85–91.

Shi Y and Frank BHU. 2014. The global implications of diabetes and cancer. *The Lancet*, **383(**9933): 1947–1948.

Singh NP, McCoy MT, Tice RR and Schneider EL. 1988. A simple technique for quantitation of low levels of DNA damage in individual cells. *Exp Cell Res.,* **175**: 184-191.

Steinbrenner H and Sies H. 2009. Protection against reactive oxygen species by selenoproteins. *Biochim Biophys Acta*., **1790**: 1478–1485.

The American Society of Health- System Pharmacists. "Metformin Hydrochloride. Retrieved Jan 2016.

Vikram DN, Tripathi P and Jena Ramarao G B. 2007. Evaluation of streptozotocin genotoxicity in rats from different ages using the micronucleus assay. *Regulatory Toxicol Pharmacol*., **49**: 238–244.

Vilela DD, Peixoto LG, Teixeira RR, Baptista NB, Caixeta DC, de Souza AV, Machado HL, Pereira MN, Silva RS and Espindola FS. 2016. The role of metformin in controlling oxidative stress in muscle of diabetic rats. *Oxidative Med Cellular Longevity*, Article ID 6978625, 9 pages.

Vos T, Flaxman AD, Naghavi M, Lozano R, Michaud C, Ezzati M, Shibuya K,

Wang H, Zhang J and Yu H. 2007. Elemental selenium at nano size possesses lower toxicity without compromising the fundamental effect on selenoenzymes: comparison with selenomethionine in mice. *Free Radic Biol Med*., **42**:1524–1533.

Wang XL, Yang TB, Wei J, Lei GH and Zeng C. 2016. Association between serum selenium level and type 2 diabetes mellitus: a non-linear dose-response meta-analysis of observational studies. *Nutr J*., **15 (1):** 48.

Wei M, Ong L, Smith MT, Ross FB, Schmid K, Hoey AJ, Burstow D and Brown L. 2003. The streptozotocin-diabetic rat as amodel of the chronic complications of human diabetes. *Heart Lung Circ*., **12 (1):** 44–50.

World Health Organization. "About diabetes. Retrieved 4 April 2014.

World Health Organization. "Diabetes Fact sheet N°312". October 2013.

Zhang J, Wang H, Yan X and Zhang L. 2005. Comparison of short-term toxicity between Nano-Se and selenite in mice. *Life Sci*., **76**: 1099–1109.