ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Fruit extract nanoparticles increase the efficiency of *Balanites sp* against diabetes mellitus in albino male rats

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Manuscript Info

Manuscript History:

Received: 12 November 2015
Final Accepted: 28 December 2015
Published Online: January 2016

Key words:
Nanotechnology, Balanites sp, Diabetes, DNA Fragmentation, Gene expression.

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Abstract

Type II diabetes mellitus is increasing health problems that negatively affect health care systems worldwide. There is a constant urge to develop new therapies with better effects, lower side effects at lower prices to treat this disease. Because nano-structured systems could promote sustained release of active constituents, reduce the required dose, low toxicity, decrease side effects, and improve activity. Therefore, the present study carried out to investigate whether *Balanites sp* fruit extract nanoparticles could treat the hyperglycemic, liver toxicity and gene expression changes in pancreatic, and hepatic tissues of diabetic rats. The protective effect of *Balanites sp*-NPs was studied on type-II diabetes through determination of serum glucose and levels, enzymes activities, DNA fragmentation and expression alterations of insulin and gluconeogenic genes in diabetic rats induced by alloxan. The current study showed that oral administration of *Balanites sp*-NPs significantly decreased the blood glucose, alterations in the expression of insulin (I&II) and gluconeogenic genes, DNA Fragmenation. Also, *Balanites sp* -NPs restored the altered plasma enzyme (AST and ALT) levels to near normal. The results could be concluded that the nanoparticles of *Balanites sp* extract increased the potential to be developed as an antidiabetic agent**.**

Introduction:-

The importance of human diabetes mellitus as a world health problem is attributes to the fact that at least 150 million people are affected, thus the necessity to seek new drugs (Soto et al., 2004). Nature is an extraordinary source of antidiabetic medicines. Where, many herbal products have recommended for the treatment of diabetes mellitus since antiquity (Fornasini et al., 2012).

Alloxan causes severe necrosis of pancreatic β-cells (Shafrir, 2003) with the consequent lack of insulin secretion. For this reason, it has been widely used to induce experimental diabetes mellitus, and many studies have performed using this model to explore pancreatic damage (Soto et al., 2004).

Balanites sp. such as *Balanites aegyptiaca* (L) Del. is a perennial tropical plant used in food preparations and herbal medicine, especially in Africa, Asia and some developing countries (Nadro and Samson, 2014). It is also called desert date (English), adua (Hausa, Nigeria), tanni (Fulfulde, Nigeria) and heglig (Arabic). *Balanites sp* belongs to the family Balanitecea. The plant attains a height of more than 6 meters. It has a multiplicity of uses and almost every part of the plant is useful including, leaves, thorns, back of root and fruit (Nadro and Samson, 2014). *Balanites sp* is used to treat so many illnesses including, diarrhoea, hermorrhoid, stomach aches, jaundice, yellow fever, syphilis and epilepsy (Ojo et al., 2006). For instance, the fruit is used to treat liver disease and as a purgative and sucked by schools children as a confectionary in some countries. The bark is used in the treatment of syphilis, round worm infection and as fish poison [6] (Gajalakshmi et al., 2013).

Although nanotechnology contributions are advantageous for several medicinal areas, it is essential to highlight some of the disadvantages. Clinical researchers have mentioned some negative factors, such as toxicity of metal

nanoparticles through easy inhalability of nanoparticles which can result in dangerous lung diseases, and often lead to other diseases that can lead to changes in homeostasis, or even death (Yadav et al., 2011, Singh et al., 2013). However, the strategy of applying nanotechnology to plant extracts has been widely cited in the literature, because nano-structured systems could potentiate action of plant extracts, promote sustained release of active constituents, reduce the required dose, low toxicity, decrease side effects, and improve activity (Ghosh et al., 2013, Rajendran et al., 2013). Moreover, several studies have been used nano-encapsulated form of the plant extracts to increase the efficiency of its biological action. Kesarwani and Gupta published a review that mentioned several studies which employed nanostructured systems to optimize the properties of plant extracts (Kesarwani and Gupta, 2013). Bhattacharya and Ghosh (2009) used lipid-based systems incorporated green tea and ginseng (*Panax ginseng* CA Meyer) (Araliaceae) extracts, in various formulations, to increase the absorption of the active components.

Currently, the treatment of diabetes mainly involves a sustained reduction in hyperglycemia by the use of biguanides, thiazolidinediones, sulphonylureas, Diphenylalanine derivatives, meglitinides and α-glycosidase inhibitors in addition to insulin. However, due to unwanted side effects the efficacies of these compounds are debatable and there is a demand for new compounds for the treatment of diabetes (Thirunavukkarasu et al., 2003). Hence, plants have been suggested as a rich, as yet unexplored source of potentially useful anti diabetic drugs. However, only a few have been subjected to detailed scientific investigation (Saxena and Vikram, 2004). Therefore, the present investigation was designed to study the normo-glycemic and hypolipidemic effects of nanoparticles of *Balanites sp.* fruit extract in alloxan-induced diabetic rats. In addition, the DNA fragmentation and expression alterations of Insulin (I&II glycolytic and gluconeogenic genes in diabetic rats treated with *Balanites sp*. NPs were determined.

Material and Methods:-

Drugs and chemicals

Alloxan was purchased from Sigma–Aldrich (USA). Reagents for RT-PCR were purchased from Invitrogen (Paisley, UK) and Fermentas (Leon-Rot, Germany).

Animals:-

Swiss albino male rats strain with body weight ranging between 120-140 gm, purchased from the Animal House Colony, Jeddah, Saudi Arabia., were maintained on standard laboratory diet (protein, 16.04%; fat, 3.63%; fiber, 4.1%; and metabolic energy, 0.012 MJ) and water *ad libitum* at the were obtained from the Animal House Colony of the Department of Biology, King Abdulaziz University, Jeddah, Saudi Arabia. After an acclimation period of 1 week and at 50 days of age, animals were divided into seven groups (10 rats/ group) and housed individually in filter-top polycarbonate cages housed in a temperature-controlled (23 ± 1°C) and artificially illuminated (12 h dark/light cycle) room free from any source of chemical contamination

Induction of experimental diabetes:-

Induction of diabetes mellitus by giving freshly prepared alloxan solution 120 mg/ kg b.wt dissolved in 0.5 ml acetate buffer (pH 5.5) to overnight fasted male albino rats (Helal et al., 2012). The control animals were administered with citrate buffer. After 3 days, the blood was collected by sinocular puncture and the plasma glucose level of each rat was determined. Rats with a fasting plasma glucose range of 250–300 mg/dl (Thirunavukkarasu et al., 2003) were considered diabetic and included in the study.

Experimental design:-

Seventy adult male albino rats of local strain with body weight ranging between (120-140 gm.) were divided into two groups as following: group I= control non diabetic rats; Group II = alloxan-induced diabetic rats which divided also into two subgroups; subgroup1: diabetic untreated rats, subgroups 2 and 3: diabetic treated orally with aqueous extract of Balanites sp. fruit (20 and 42mg/ kg b.wt. dally, respectively) for 30 days, subgroup 4 and 5: diabetic treated with aqueous extract nanoparticles of Balanites sp. fruit (20 and 42mg/ kg b.wt. dally) by gastric intubation for 30 days, subgroup6: diabetus mellitus induced-rats were administered 10 units insulin subcutaneously (Principato et al., 1985).

At the termination of the experiment blood samples from fasting rats were withdrawn from retro-orbital venous plexus under diethylether anaesthesia in dry clean centrifuge tubes and left to clot. The animals were anesthetized with ether, and blood was collected from retro-orbital puncture. Serum was then separated for the estimation of

glucose. Afterwards, the animals were sacrificed and pancreas, liver and muscle tissues were used for biochemical analyses and DNA fragmentation as well as gene expression assessment.

Plant sampling:-

Balanites sp. fruits collected from private farm in Jeddah, Saudi Arabia were dried by oven at 50°C. Dry fruits material was grinded and boiled in water for 30 min, filtered and evaporated by evaporator. The extract was dried by freeze dry as water extract. The samples have been preserved in the refrigerator (−20°C). Authentication of plant materials was identified by comparing against the specimens deposited King Abdulaziz University, where herbarium vouchers have been kept.

Preparation of the extract:-

The extract of *Balanites sp.* was collected, washed three times with water, dried over anhydrous sodium sulfate and evaporated to dryness according to Tasanarong et al., (2014).

Formation of *Balanites sp.*loaded nanoparticles:-

Solvent displacement technique of Samadder et al. (2012) was deployed under optimal conditions to prepare the poly-lactic-co-glycolic acid (PLGA) encapsulation of *Balanites sp.* extract. Briefly, to prepare the poly-lactic-co-glycolic acid (PLGA) encapsulation of *Costus speciosus*, solvent displacement technique of Samadder *et al*. (2012) deployed under optimal conditions. To 20 mL of an aqueous solution of F68; w/v stabilizer (1% polyoxyethylene-polyoxypropylene), an organic phase mixture containing 10 mg of dried *Balanites sp.* fruit dissolved in 3 mL acetone along with 50 mg PLGA in a dropwise manner (0.5 mL/min) was added. Stirring the mixture continuously was performed at room temperature until complete evaporation of the organic solvent; the redundant stabilizer was removed by centrifugation at 2500 g at 4ºC for 30 minutes. The pellet was re-suspended in Milli-Q water and washed three times and the nanoparticles obtained were stored in suspensions at 4ºC until further use.

Transmission electron microscopy:-

The particle size and shape were of *Balanites sp.* nanoparticles were characterized using high resolution transmission electron microscopy (HR-TEM) JEM 2100 LB_6 under operating voltage of 200 kV to investigate the micrograph of prepared PLGA encapsulation of *Balanites sp.* extract nanoparticles under operating voltage of 200 kV for different samples (Fig. 1).

Plasma enzyme assessments:-

Alanine aminotransferase (ALT; EC 2.6.1.2) and aspartate aminotransferase (AST; EC 2.6.1.1) activities were assayed by the method of Reitman and Frankel (Reitman and Frankel, 1956).

DNA Fragmentation Analysis:-

a- Diphenylamine reaction procedure:-

Rats liver tissues were used to determine the quantitative profile of the DNA fragmentation. Liver samples were collected immediately after sacrificing the animals. The tissues were lysed in 0.5 ml of lysis buffer containing, 10 mM tris-HCl (pH 8), 1 mM EDTA, 0.2% triton X-100, centrifuged at 10 000 rpm (Eppendorf) for 20 min at 4°C. The pellets were resuspended in 0.5 ml of lysis buffer. To the pellets (P) and the supernatants (S), 0.5 ml of 25% tri-chloroacetic acid (TCA) was added and incubated at 4°C for 24 h. The samples were then centrifuged for 20 min at 10 000 rpm (Eppendorf) at 4°C and the pellets were suspended in 80 ml of 5% TCA, followed by incubation at 83°C for 20 min. Subsequently, to each sample 160 ml of DPA solution [150 mg DPA in 10 ml glacial acetic acid, 150 ml of sulfuric acid and 50 ml acetaldehyde (16 mg:ml)] was added and incubated at room temperature for 24 h(Burton, 1956). The proportion of fragmented DNA was calculated from absorbance reading at 600 nm wavelength using the formula:

$$\%\text{Fragmented DNA} = \frac{OD(S)}{OD(S) + OD(P)} \quad X\ 100$$

b- DNA gel Electrophoresis Laddering Assay:-

Apoptotic DNA fragmentation was qualitatively analyzed by detecting the laddering pattern of nuclear DNA as described by Lu et al. (2002). Briefly, liver tissues were *homogenized,* washed in PBS, and lysed in 0.5 ml of DNA extraction buffer (50 mM Tris–HCl, 10 mM EDTA. 0.5% Triton, and 100 µg/ml proteinase K, pH 8.0) for overnight at 37 °C. The lysate was then incubated with 100 µg/ml DNase-free RNase for 2h at 37 °C, followed by three extractions of an equal volume of phenol/chloroform (1:1 v/v) and a subsequent re-extraction with chloroform by centrifuging at 15,000 rpm for 5 min at 4 °C. The extracted DNA was precipitated in 2 volume of ice-cold 100% ethanol with 1/10 volume of 3 M sodium acetate, pH 5.2 at −20 °C for 1h, followed by centrifuging at 15,000 rpm for 15 min at 4 °C. After washing with 70% ethanol, the DNA pellet was air-dried and dissolved in 10 mM Tris–HCl/1 mM EDTA, pH 8.0. The DNA was then electrophoresed on 1.5% agarose gel and stained with ethidium bromide in Tris/acetate/EDTA (TAE) buffer (pH 8.5, 2 mM EDTA, and 40 mM Tris–acetate). A 100-bp DNA ladder (Invitrogen, USA) was included as a molecular size marker and DNA fragments were visualized and photographed by exposing the gels to ultraviolet transillumination.

Gene expression analysis using *Quantitative RT-PCR:-*

Total RNA (Poly(A)$^+$ RNA) was extracted from 50 mg of pancreas and muscles tissues using the standard TRIzol extraction method (Invitrogen, Paisley, UK) and recovered in 100 µL diethylpyrocarbonate (DEPC)-treated water by passing the solution a few times through a pipette tip.

Total RNA was treated with one unit of RQ1 RNAse-free DNAse (Invitrogen, Karlsruhe, Germany) to digest DNA residues, re-suspended in DEPC-treated water, and quantified photospectrometrically at 260 nm. Total RNA was assessed for purity from the ratio between quantifications at 260 nm and 280 nm, and was between 1.8 and 2.1. Integrity was verified with the ethidium bromide-stain analysis of 28S and 18S bands using formaldehyde-containing agarose gel electrophoresis. Aliquots were either used immediately for reverse transcription (RT) or stored at -80 °C.

To synthesise first-strand cDNA, 5 µg of complete Poly(A)$^+$ RNA was reverse transcribed into cDNA in a total volume of 20 µL using 1 µL oligo (poly(deoxythymidine)18) primer (Alakaili and Mahrous, 2015). The composition of the reaction mixture was 50 mmol L-1 $MgCl_2$, 10x RT buffer, 200 U μL^{-1} reverse transcriptase (RNase H free, Fermentas, Leon-Rot, Germany), 10 mmol L^{-1} of each dNTP, and 50 µmol L^{-1} of oligo(dT) primer. RT reaction was carried out at 25 °C for 10 min, followed by 1 h at 42 °C, and completed with denaturation at 99 °C for 5 min. Reaction tubes containing RT preparations were then flash-cooled in an ice chamber until used for DNA amplification through polymerase chain reaction (PCR) (Farsi et al., 2014]..

PCR reactions were set up in 25 µL reaction mixtures containing 12.5 µL 1× SYBR® Premix Ex TaqTM (TaKaRa, Biotech. Co. Ltd., Germany), 0.5 µL 0.2 µM sense primers, 0.5 µL 0.2 µM antisense primer, 6.5 µL distilled water, and 5 µL of cDNA template. The reaction program was allocated to 3 steps. First step was at 95.0°C for 3 min. Second step consisted of 40 cycles in which each cycle divided to 3 steps: (a) at 95.0°C for 15 sec; (b) at 55.0°C for 30 sec; and (c) at 72.0°C for 30 sec. The third step consisted of 71 cycles which started at 60.0°C and then increased about 0.5°C every 10 sec up to 95.0°C. At the end of each qRT-PCR a melting curve analysis was performed at 95.0°C to check the quality of the used primers**.** Each experiment included a distilled water control. Table 1 lists the specific gene primer sequences and PCR cycling conditions (Farsi et al., 2014).

The quantitative values of RT-PCR (qRT-PCR) of insulin and gluconeogenic genes (Insulin-I, Insulin-II, GLUT2 and GLUT4) were normalized on the bases of ß-actin expression. The primer sequences of liver cancer related genes are listed in Table 1.

At the end of each qRT-PCR a melting curve analysis was performed at 95.0 °C to check the quality of the used primers.

First the amplification efficiency (Ef) was calculated from the slope of the standard curve using the following formulae (Bio-Rad, 2006):

$Ef = 10^{-1/slope}$

Efficiency (%) = (Ef – 1) x 100

The relative quantification of the target to the reference was determined by using the

ΔCT method if E for the target (Insulin I&II, GLUT2 and GLUT4) and the reference primers (β-Actin) are the same (Bio-Rad, 2006).

$Ratio_{(reference/\ target\ gene)} = Ef^{CT(reference) - CT(target)}$

Statistical Analysis:-

All results were expressed as Mean±S.E of the mean. Data were analyzed by one way analysis of variance (ANOVA) using the Statistical Package for the Social Sciences (SPSS) program, version 11 followed by least significant difference (LSD) to compare significance between groups. Difference was considered significant when $P < 0.05$.

Results:-

Serum glucose levels:-

The anti-diabetic effect of the *Balanites sp* or *Balanites sp*-NPs on the fasting serum glucose levels in diabetic rats was determined. Diabetic rats revealed extremely high levels of glucose compared with control rats. However, daily treatment of *Balanites sp*-NPs led to a dose dependent fall in serum glucose levels. Administration of DM-rats with low and high doses of *Balanites sp*-NPs revealed highly antihyperglycemic effect on serum glucose levels compared with the DM-rats.

Effect of Balanites sp. & Balanites sp.-NPs on plasma enzymes in diabetic rats:-

The activities of plasma enzymes AST and ALT significantly increased in diabetic rats when compared to normal controls. However, oral administration of *Balanites sp. or Balanites sp*-NPs for 30 days significantly restored the enzyme levels to near normal in diabetic rats (Table 2). Moreover, the improvement impact of *Balanites sp*-NPs on plasma enzymes was more effectively compared to *Balanites sp* alone especially with the high dose.

Effect of Balanites sp and Balanites sp-NPs on the DNA fragmentation:-

DNA fragmentation assay revealed that treatment of diabetic rats with different doses of *Balanites sp* and *Balanites sp--NPs* induced different rats of DNA fragmentation (Fig. 2 and Table 3).

The rate of DNA fragmentation in DM-rats revealed high rate of DNA damage compared with control rats (Fig. 2 and Table 4). While, treatment of DM-rats with different doses of *Balanites sp* and *Balanites sp--NPs revealed significantly low rats compared with DM-rats.* Moreover, the protective action of *Balanites sp*-NPs on the DNA fragmentation was more effectively compared to *Balanites sp* alone especially with the high dose. Treatment of DM-rats with low and high doses of *Balanites sp--NPs revealed lower rates of fragmentation* compared with those in DM-rats treated with *Balanites sp* alone (Fig. 2 and Table 3). On the other hand, treatment of DM-rats with insulin induced low rats of DNA fragmentation compared with DM-rats.

Expression of insulin and gluconeogenic genes in diabetic rats treated with *Balanites sp and Balanites sp*-NPs:-

The expression of diabetic-associated insulin (I & II) and gluconeogenic genes (GLUT2 and 4) in diabetic rats treated with *Balanites sp* and *Balanites sp--NPs* was determined using RT-PCR (Figures 3-6).

The results revealed that DM-rats showed significantly lower expression values of pancreatic insulin 1 and II and muscle Glucose transporter type 4 (GLUT4) genes in comparison with the control rats (Figures 3, 4 & 6). While, DM-rats treated with Low and high doses of *Balanites sp* and *Balanites sp--NPs* caused significant increase in insulin I and II and GLUT4 expression as compared with the DM-rats. Furthermore, the highest expression levels of insulin I and II as well as GLUT4 genes were showed in DM-rats treated with the low and high doses of *Balanites sp--NPs* (Figures 3, 4 & 6). In addition, treatment of DM-rats with insulin increased significantly the expression of insulin I and II and GLUT4 genes, however with low efficiency compared with *Balanites sp--NPs*.

Concerning the Glucose transporter type 2 (GLUT2) gene, the present results revealed that DM-rats showed significantly higher expression values of GLUT2- mRNA in comparison with the control rats (Fig. 5). However, DM-rats treated with Low and high doses of *Balanites sp* and *Balanites sp--NPs* caused significant decrease in GLUT2- mRNA expression as compared with the DM-rats. Moreover, lowest expression levels of GLUT2- mRNA genes were showed in DM-rats treated with the low and high doses of *Balanites sp--NPs* (Fig. 5). Moreover, DM-rats treated with insulin showed significantly lower expression values of GLUT2- mRNA in comparison with the DM-rats.

Discussion:-

The rapidly increasing incidence of diabetes millets is becoming a serious threat to humankind's health in all parts of the world [Harlev et al., 2013). There is a constant urge to develop new therapies with better effects, lower side effects at lower prices to treat this disease (Fornasini et al., 2012). Therefore, the current study aimed to use a novel approach to treat diabetes with natural product nanoparticulate system to enhance the antidiabetic activity on animal models.

The currently available drug regimens for management of diabetes mellitus have certain drawbacks and therefore there is a need to find safer and more effective antidiabetic drugs (Grover et al., 2000). Traditional antidiabetic plants might provide a useful source of new oral hypoglycemic compounds for development as pharmaceutical entities, or as simple dietary adjuncts to existing therapies (Helal, 2000). Where, plants are rich sources of antidiabetic, antihyperli pidemic and antioxidant agents such as flavonoids, gallotannins, amino acids, and other related polyphenols (Ashok-Kumar et al., 2012).

Balanites sp is a plant commonly used in African and Asian folk medicine as a hypoglycemic agent (Gad et al., 2006). However, there are very few studies concerning the effect of the fruit extract as antidiabetic agent. Additionally, this study is the first one used nanoparticles from *Balanites sp* to increase the efficiency of this plant against diabetes. Therefore, the aim of the present study was to evaluate the normo-glycemic, gene expression alteration and anti-genotoxicity effects of *Balanites sp*-NPs in alloxan-induced diabetic rats

The experimental diabetic model used in this study was type II since alloxan dose destroyed half a population of pancreatic beta cells (Fischer and Homburger, 1980). Where, alloxan has direct effect on β-cells membrane permeability by causing failure of ionic pumps and increasing cell size which inhibits intra cellular energy generation by inhibiting enzymes of tricarboxylic acid cycle and Ca+2 dependants dehydrogenises in their mitochondrion, causing ATP deficiency, cessation of insulin production and cell necrosis [3, Harvey and Ferrier, 2011]. In addition, may be due to its sudden activation of quiescent cell for a high level of protein synthesis and produced rapid and massive β-cell death which, leading to a decrement in β-cells number (Fischer and Homburger, 1980). Moreover, showed β-cells' cytoplasmic vacuolation which may be attributed to the diabetogenic action of alloxan where, it induced highly reactive oxygen radicals whom have cytotoxic effect on β-cells as explained by Fischer and Homburger (1980). These results are in agreement with finding of Kessler et al. (1999) who reported that vacuolation of the islet in the most prominent with lesion associated with functional islet abnormality and development of hyperglycemia.

The improvement of general diabetic conditions in rats treated by the extract of *Balanites sp* (fruits) is possibly due to recovered endocrine pancreatic tissue at both structural and functional levels. This can lead to elevated insulin level and improved insulin sensitivity that lowers the concentration of glucose in blood. Where, insulin inhibits hepatic glucose production, stimulates both of glucose uptake and of metabolism by muscle and adipose tissues and increases liver glycogen content. In addition, *Balanites sp* containing diosgenin (Chapagain and Wiesman, 2005) which may be useful for ameliorating the glucose metabolic disorder, associated with diabetes and obesity. Where, diosgenin can be absorbed through the gut and plays an important role in the control of metabolic diseases such as diabetes and obesity as reported by Ulbricht et al. (2007). Furthermore, *in vitro* experiment showed that diosgenin promoted 3T3-L1 adipocyte differentiation to enhance insulin-dependent glucose uptake (Uemura et al., 2010). While, Abdel Motaal et al. (2012) attributed the antihyperglycemic activity of *Balanites sp* fruits to increase muscle basal glucose uptake significant insulin-like and partly glitazone-like activities in peripheral tissues.

Moreover, the current results revealed that the activities of plasma AST and ALT were increased which indicated that diabetes might be induced due to liver dysfunction. Ohaeri (2001) also found that liver was necrotized in diabetic rats. Therefore, an increase in the activities of AST and ALT in plasma might be mainly due to the leakage of these enzymes from the liver cytosol into the blood stream (Navarro et al., 1993) which gives an indication on the hepatotoxic effect of alloxan. On the other hand, we found that treatment of the diabetic rats with *Balanites sp*-NPs caused reduction in the activity of these enzymes in plasma when compared to the diabetic group and consequently alleviated liver damage caused by alloxan-induced diabetes. These results are in agreement with those obtained by El-Demerdash et al. (2005) in rats.

The current study observed that the rate of DNA damage in liver tissues of DM- male rats induced a high rate of DNA fragmentation with control rats. While, treatment of DM-rats with *Balanites sp*-NPs *revealed significantly low rates compared with DM-rats.* Also, in agreement with our findings Harvey and Ferrier [33] reported that, the metabolic abnormalities of type II diabetes area result of insulin resistance which lead to dislipidemia in the liver where, fatty acids are converted to triacylglycerol which are packaged and secreted in VLDL. Both lipid accumulations particularly triglycerides and reduction in antioxidant activity are contributed to the development of oxidative stress induced DNA damage in diabetic rats (Budin Balkis et al., 2009)

The current study revealed that DM-rats treated with Low and high doses of *Balanites sp* and *Balanites sp--NPs* caused significant increase in the expression of insulin I and II and GLUT4 genes as compared with the DM-rats. In same line with our findings, it has been reported that reduction in protein synthesis which in turn may be due to a decrease in the amount and availability of mRNA (Qari, 2010) and also, a reduction in ribosomal protein synthesis as a result of insulin deficiency(Rose et al., 1982). While, Shafrir [3] explained the decrease of total proteins and albumin in alloxan diabetic rats to enhanced proteolysis in tissues, which lead to reduce production of growth factors and increase growth factor binding protein by a rapid mechanism and slow, long-losing activations of a myofbrillar protease.

The protective effect of the *Balanites sp*-NPs on the **molecular mechanism inhibiting changes in the gene expression is not clear understood. However, several studies suggested the protective effects of** *Balanites sp*-NPs **may be attributed to its antioxidant activity.** Free radicals have aroused significant interest among scientists in the past decade (Mahrous et al., 2006). Their broad range of effects in biological systems has drawn the attention of many experimental works. It has been proved that these mechanisms may be important in the pathogenesis of certain diseases and ageing. There are many reports that support the use of antioxidant supplementation in reducing the level of oxidative stress and in slowing or preventing the progress of complications associated with diseases [45]. Many synthetic antioxidant components have shown toxic and/or mutagenic effects, which have shifted the attention towards the naturally occurring antioxidants. Numerous plant constituents have proven to show free radical scavenging or antioxidant activity (Aruoma and Cuppett, 1997). Flavonoids and other phenolic compounds (hydroxyl cinnamic derivatives, catechines, etc.) of plant origin have been reported as scavengers and inhibitors of lipid peroxidation (Formica and Regelson, 1995).

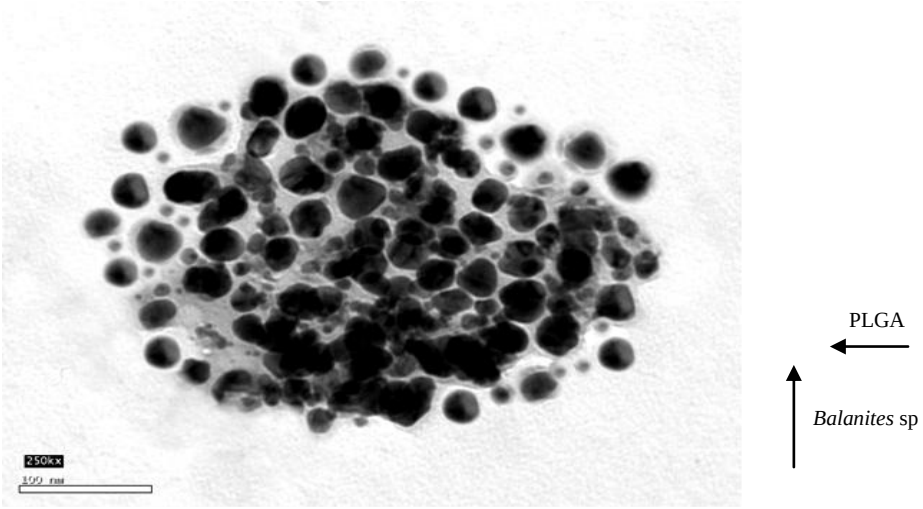

Figure 1: Cross-sectional transmission electron microscopy image of the of poly-lactic-co-glycolic acid (PLGA) encapsulation of *Balanites sp* nanoparticles.

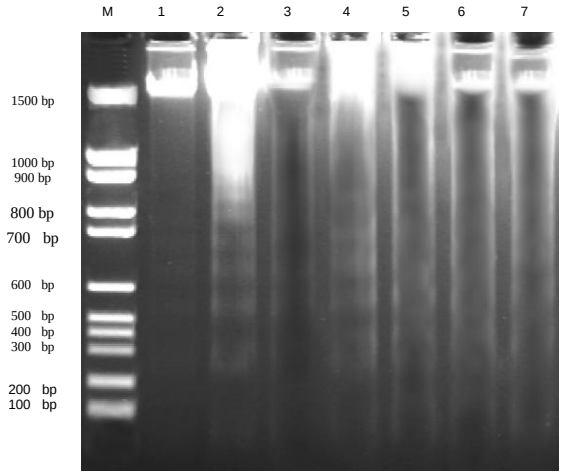

Figure 2: DNA fragmentation in liver tissues of male DM-rats treated with different doses of *Balanites sp* or *Balanites sp* nanoparticles. M: DNA marker. Lane 1 represents PCR products of untreated control rats; lane 2 represents DM-rats; lane 3 represents DM-rats treated with insulin; Lanes 4 and 5 represent DM-rats treated with 20 and 42 mg/kg *Balanites sp* extract. Lanes 6 and 7 represent DM-rats treated with 20 and 42 mg/kg *Balanites sp* nanoparticles.

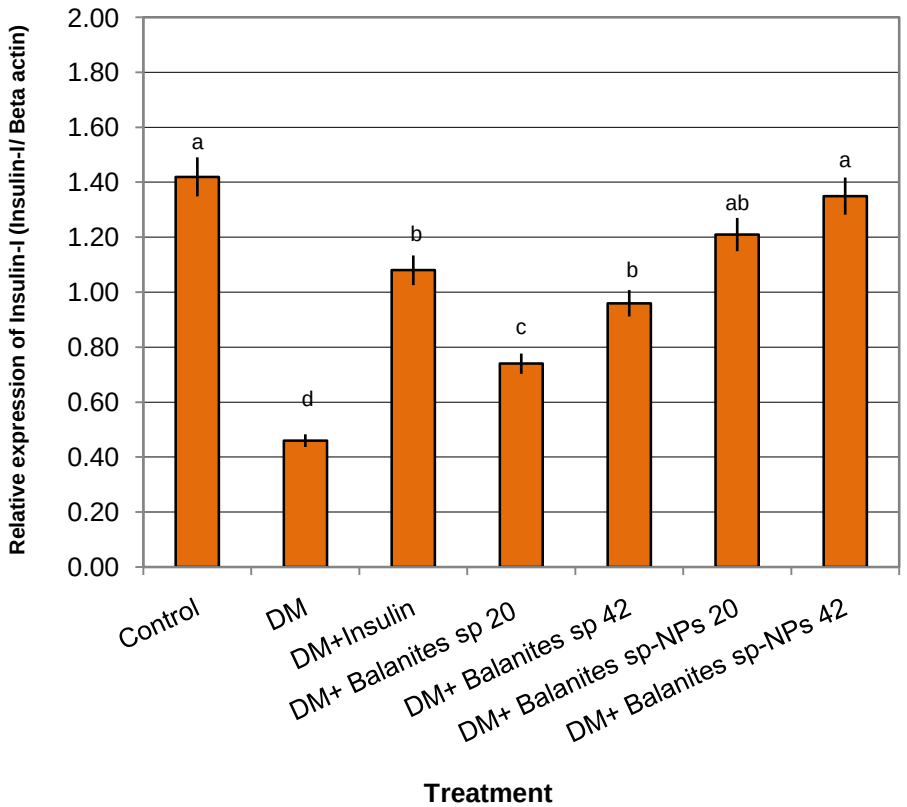

Figure 3: The alterations of Insulin-I mRNA in pancreas tissues isolated of male DM-rats treated with different doses of *Balanites sp* or *Balanites sp* nanoparticles. [a,b,c,d] Mean values within tissue with unlike superscript letters were significantly different (P<0.05).

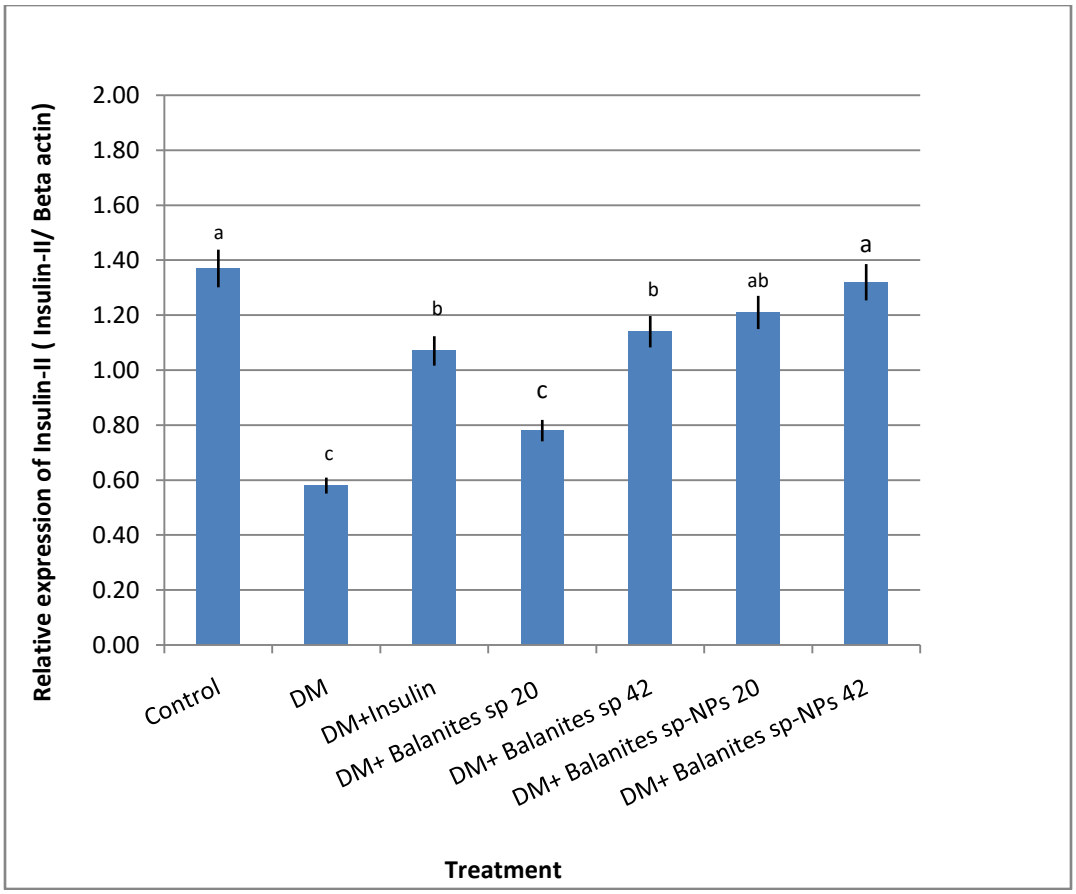

Figure 4: The alterations of Insulin-II mRNA in pancreas tissues isolated of male DM-rats treated with different doses of *Balanites sp* or *Balanites sp* nanoparticles. [a,b,c] Mean values within tissue with unlike superscript letters were significantly different (P<0.05).

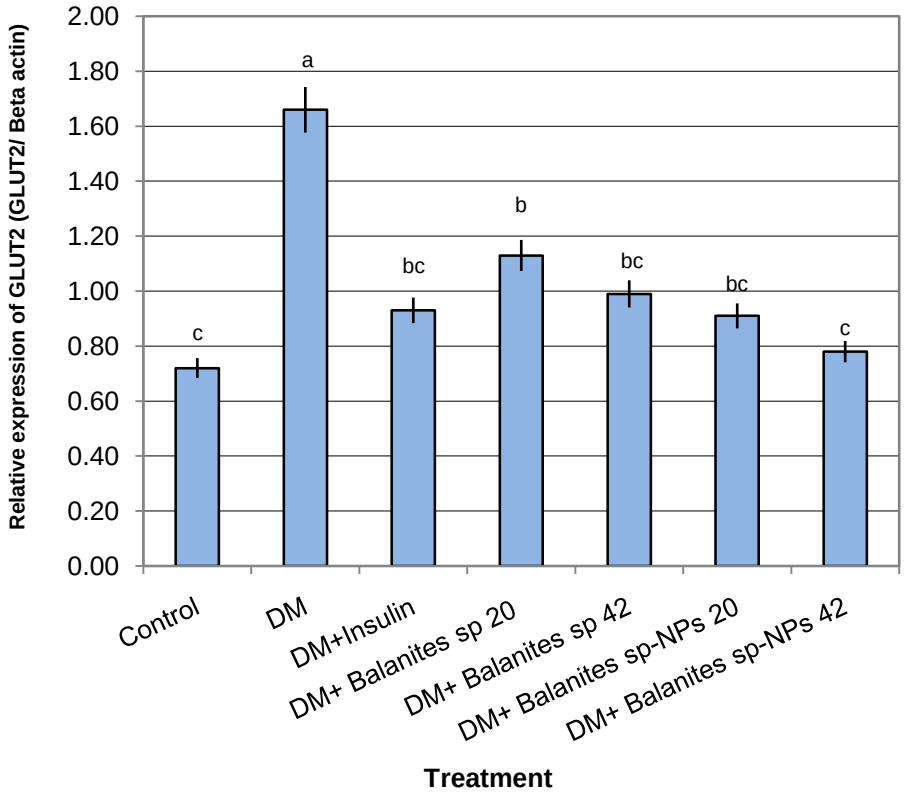

Figure 5: The alterations of GLUT2-mRNA in liver tissues isolated of male DM-rats treated with different doses of *Balanites sp* or *Balanites sp* nanoparticles. [a,b,c] Mean values within tissue with unlike superscript letters were significantly different (P<0.05).

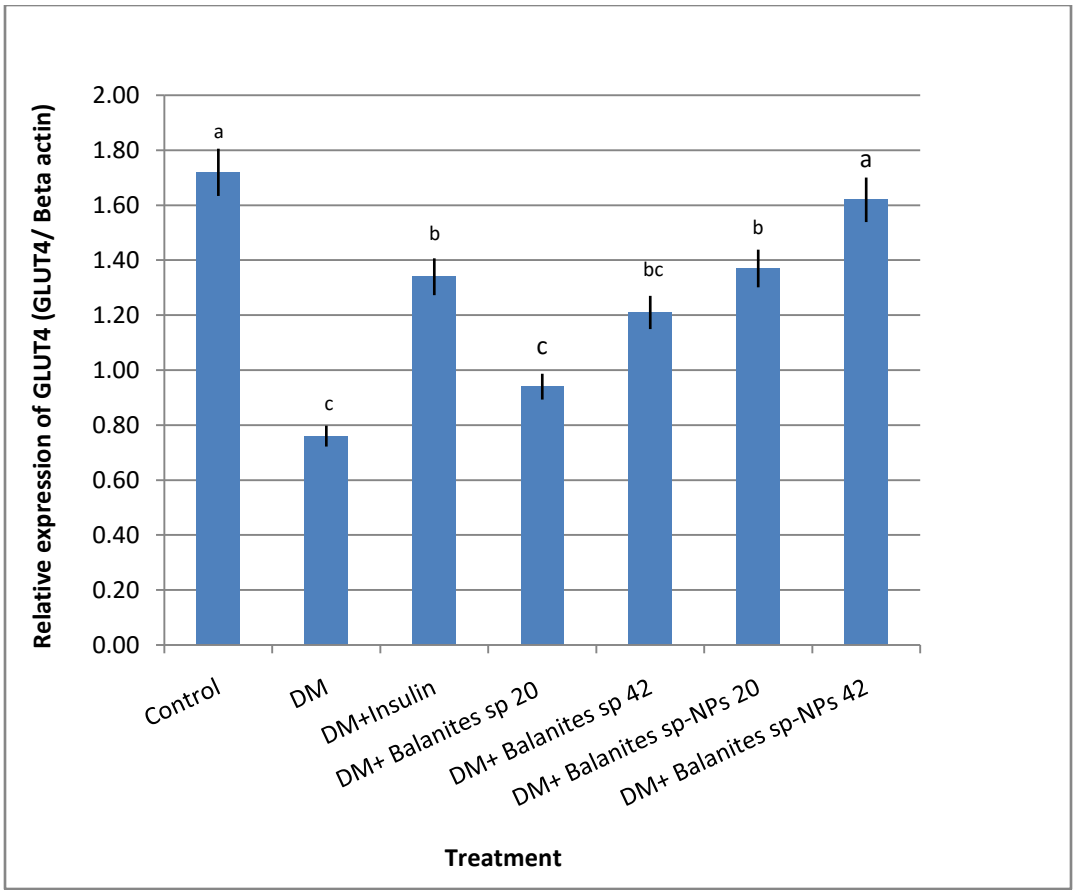

Figure 6: The alterations of GLUT4-mRNA in muscles tissues isolated of male DM-rats treated with different doses of *Balanites sp* or *Balanites sp* nanoparticles. [a,b,c] Mean values within tissue with unlike superscript letters were significantly different (P<0.05).

Table 1: List of primers, the primer sequences and the primer melting temperature (Tm)

Gene	Sequence (5'-3')	Annealing Tm°C
Insulin I	CCT GTT GGT GCA CTT CCT AC	58
	TGC AGT AGT TCT CCA GCT GC	
Insulin II	CAA CA TGG CCC TGT GGA TGC	60
	AGT TGC AGT AGT TCT CCA GC	
GLUT 2	CATCAAAACGTAGAGCACGGTAA	63.4
	TATGGGCATTTAGTCTGCACGTA	
GLUT4	GCTTGGCTCCCTTCAGTTTG	63.4
	CCTACCCAGCCAAGTTGCAT	
β-actin	GTG GGC CGC TCT AGG CAC CAA	64.5
	CTC TTT GAT GTC ACG CAC GAT TTC	

GK: Glucokinase, G6Pase: Glucose-6-phosphatase, GLUT2: Glucose transporter type 2, GLUT4: Glucose transporter type 4.

Table 2: Effect of oral administration of *Balanites sp* and *Balanites sp* nanoparticles on plasma AST and ALT in normal and diabetic male rats.

Treatment	AST (U/dl)	ALT (U/dl)
Control	32.7 ± 0.8^{c}	51.2 ± 2.1^{c}
DM	74.3 ± 2.3^{a}	94.1 ± 3.6^{a}
DM+Insulin	47.8 ± 1.6^{bc}	61.8 ± 3.1^{bc}
DM+ *Balanites sp 2*0	58.4 ± 1.4^{b}	74.2 ± 2.4^{b}
DM+ *Balanites sp 42*	42.6 ± 1.6^{c}	64.1 ± 3.2^{bc}
DM+ *Balanites sp-NP*s 20	40.8 ± 2.1^{c}	62.9 ± 3.6^{bc}
DM+ *Balanites sp-NP*s 42	35.2 ± 2.4^{c}	54.2 ± 2.3^{c}

AST :Aspartate aminotransferase, ALT: Alanine aminotrasferase

Table 3: DNA fragmentation in liver tissues of male DM-rats treated with different doses of *Balanites sp or Balanites sp nanoparticles* analyzed by diphenylamine reaction procedure.

Treatment	% of DNA Fragmentation	
	Range	Mean±SEM
Control	06 – 15	08.6 ± 0.3^{d}
DM	34 – 46	32.5 ± 2.6^{a}
DM+Insulin	28 – 34	24.3 ± 2.1^{b}
DM+ *Balanites sp 2*0	26 – 31	28.1 ± 0.6^{bc}
DM+ *Balanites sp 42*	21 – 29	22.3 ± 1.4^{bc}
DM+ *Balanites sp-NP*s 20	20 – 26	19.3 ± 1.5^{c}
DM+ *Balanites sp-NP*s 42	18 – 24	16.9 ± 1.2^{bc}

Conclusion:-

In conclusion, our results provide novel promising mechanisms for the plasma glucose-lowering action of nanoparticles of *Balanites sp*-NPs. The extract produced its anti-hyperglycemic effect. Further it is confirmed that the extract suppressed the transcription of genes involved in pancreatic insulin and hepatic glucose production. Moreover, oral administration of nanoparticles of *Balanites sp*-NPs restored the altered plasma enzyme (aspartate aminotransferase and alanine aminotrasferase) levels to near normal. Altogether, it can be concluded that the nanoparticles of *Balanites sp*-NPs extract could be used as a drug to bring about normo-glycemic impact.

Acknowledgement:-

The skilful assistance of all staff members of the Biology Department, King Abdulaziz University, Jeddah, Saudi Arabia is gratefully acknowledged.

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