Biochemistry Letters, 19(1) 2023, pages 64-77

Scientific Research & Studies Center-Faculty of Science- Zagazig University- Egypt

Biochemistry Letters

Journal home page:

Protective effects of *Balanites aegyptiaca* extract, MSCs and Exosome against Diabetic nephropathy induced in male albino rats

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ARTICLE INFO

Received : 28/7/2023
Accepted : 24/8/2023
Available online: 25/8/2023

Keywords:
Diabetic nephropathy, Exosome, MSCs, *Balanites aegyptiacae*

ABSTRACT

Background: One of the most severe consequences of diabetes mellitus and end-stage renal failure is diabetic nephropathy (DN), which is regarded as such globally. Exosomes, which are released by a variety of cells, are one of the cutting-edge, hopeful treatments for chronic kidney damage.

Objective: This study assessed the effects of exosomes made from mesenchymal stem cells and an aqueous extract of the fruit of the *Balanites aegyptiacae* plant on DN in a diabetic rat model. It was suggested that balanites and exosomes could regulate diabetic kidney problems and function as a potential novel regulator in DN therapy. **Methods:** Seven groups of 70 mature male albino rats, weighing 180–200 g, were created: **Groups I and II** are the negative control groups, **Group III** is the **Balanites**-treated group, **Group IV** is the **MSCs**-treated group, **Group V** is the **Exosome**- treated group, **Group VI** is the **Balanites** + **MSCs**-treated group, and **Group VII** is the **Balanites** + **exosome**- treated group. The following measurements were made: plasma glucose, serum insulin, total cholesterol, triacylglycerol, and a kidney function test. Also performed was a histopathological evaluation of pancreatic tissue. **Results:** *Balanites aegyptiacae* co-administration with MSCs or exosomes showed a significant reduction in the concentrations of plasma glucose, total cholesterol, triacylglycerol, urea and creatinine. Furthermore, there was a significant rise in insulin levels in the groups co-administered *Balanites aegyptiacae* with MSCs or exosome as well as, enhanced regeneration of beta cell of the pancreas. **Conclusion:** These results clearly revealed that *Balanites aegyptiacae* co-administration with exosome has synergistic effect for each other and gives a higher renoprotective effect in rats with DN. In addition, *Balanites aegyptiacae* fruit aqueous extract exhibited potential anti-hyperglycemic and anti-hyperlipidemic effects.

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INTRODUCTION

With a mortality rate of 30–40%, one of the most serious microvascular consequences of diabetes, diabetic nephropathy (DN) is the most common cause of end-stage renal disease (ESRD) (1). Nowadays, pharmacological DN approaches are primarily focused on achieving an adequate control of blood pressure and glycemic levels, which is effective to slow the progression of diabetic kidney disease (2), but not in the later establishment of ESRD, where DN patients need hemodialysis or a renal transplant (3). Novel therapy options are thus required to maintain renal function in diabetes individuals.

The plant *Balanites aegyptiaca (L.)* Delile is a member of the Zygophyllaceae family, which is also known as the "desert date" (Heglig in Arabia). It is an evergreen tree that can be found in savannah and untamed places in South Asia and Africa. *Balanites aegyptiacae* phytochemical research have identified various kinds of metabolites, many of which have biological effects, including cumarins, flavonoids, and steroidal saponins. Fruits (mesocarp) of B. aegyptiaca have been shown to contain balanitoside (furostanol glycoside), 6-methyldiosgenin, balanitin-3 (spirostanol glycoside), and balanitin-6 and -7 (4). Its fruits' mesocarp extract has a long history of usage in Egyptian folk medicine as a hypoglycemic medication (5) and an anti-diabetic medication (6).

Bone marrow and mesenchymal tissues include mesenchymal stem cells (MSCs), which are multipotent, self-renewing, and capable of differentiating into cells of the mesodermal lineage(7). MSCs generated from bone marrow can be given intravenously to treat DN(8). Due to their ability to produce extracellular vesicles (Evs), which make up the majority of these cells' secretory capacity, mesenchymal stem cells (MSCs), which are present in virtually all tissues, have been widely used in clinical trials and acknowledged as an ideal choice for cell-based therapies (9). Exosomes, microparticles, and apoptotic bodies are examples of the common forms of Evs (10). Exosomes are the most prevalent Evs, ranging in size from 30 to 120 nm, and are found in large quantities in a variety of bodily fluids (11). They are capable of mediating intercellular communication by transporting RNAs and proteins between cells or distant organs. Since they play a significant part in tissue regeneration and inflammation inhibition, MSCs have significant therapeutic potential in renal disorders like DN (12). However, the precise functions of MSC-derived exosomes in the progression of DN and the chemicals involved are too complex to be fully understood.

This study aimed to evaluate the roles of combination between exosomes derived from MSCs and *Balanites aegyptiacae* fruit extract in DN pathogenesis and histological alterations.

MATERIALS AND METHODS

Plant material

Balanites aegyptiaca fruits were purchased from a neighborhood market in Cairo, Egypt. The Department of Botany Taxonomically, Faculty of Science at Zagazig University, Egypt, recognized and verified the plant.

Chemicals:

Streptozotocin and nicotinamide were bought from Sigma Chemicals Co. in St. Louis, Missouri, in the United States.

Preparation of MSCs derived exosomes:

MSCs obtained from rat bone marrow were synthesized at the Medical Experimental Research Center (MERC), Faculty of Medicine, Mansoura University, Egypt. In Dulbecco's Modified Eagle Medium (DMEM), which contains no fetal bovine serum and 0.5% human serum albumin, MSCs were cultured overnight. The overnight developed cells would have a vitality of more than 99%, according to trypan blue exclusion. A cell density of 4000 cells per cm2 was used for seven days of cell plating. After being trypsinized with 0.25% trypsin in 1 mmol/L EDTA for 5 minutes at 37°C, cells were counted and then replated in growth media at a density of 2000 cells per cm2 for an additional 7 days (end of passage 1) (13). Collection and

analysis of the conditioned media and stored at -80°C.

Isolation of MSC-Derived Exosomes

Up until 80% confluence, MSCs were grown in a standard growth medium before switching to a medium devoid of serum. After 48 hours, the cell culture medium was collected and centrifuged three times—300 x g for 10 min. at 4°C, 2000 x g for 10 min., and 10000 x g for 30 min.—to remove any lingering cells. 20 cc of the supernatant was centrifuged at 100,000 x g (Sorvall SureSpin 630) for 70 minutes at 4 °C to separate the exosomes. The resulting pellet, which contained the EVs, was then rinsed with an equal volume of ice-cold PBS before being centrifuged for 70 minutes at 100,000 x g at 4 °C. The exosome-containing pellet was then resuspended in (50 to 100 l) of PBS (14).

Making an aqueous extract from the fruits of *Balanites aegyptiacae*

The mesocarp of the recovered *Balanites aegyptiacae* fruits was then peeled using a clean, dry knife after the fruit's protective epicarp was carefully removed manually. The seeds then separated from the fleshy outer layer known as the mesocarp. The mesocarp in the air was then ground in the lab using a coffee grinder. After that, even after it was used, it was kept in a dry plastic container in the refrigerator. After the seeds were taken out, 4 kg of dried fruit were submerged in distilled water for 24 hours (100 g of powder was extracted using 200 ml of distilled water). A thick, dark brown extract was produced from this freshly manufactured filtrate after it successfully underwent freeze-drying using a labconco, model 18 freeze drier. A daily dose of 80 mg/kg body weight of prepared Balanites aegyptiacae fruit aqueous extract was given orally using an oral gastric tube for four weeks (15).

Laboratory Animals

At the Faculty of Science at Zagazig University's Experimental Animal House, 70 adult male albino rats weighed between 180 and 200 g. Rats were housed in a controlled setting that had a constant temperature of 25 °C, a relative humidity of 65%, or a 12-hour cycle of light and dark. Rat food and water from commercial pellet sources were available to the rats at all times. The study's techniques and design were authorized by the Zagazig University Ethical Committee (ZU-IACUC/1/F/80/2019).

DN in adult male albino rats is induced

A single freshly produced injection of streptozotocin (60 mg/kg b. wt, STZ), dissolved in (100mM, pH 4.5) cold citrate buffer, was used to induce DN intraperitoneally after 15 minutes after intraperitoneal (i.p) NIC injection (120 mg/kg body weight, NIC) dissolved in 0.9% (wt /v) sodium chloride (16). An hour after receiving injections of streptozotocin and NIC, rats were given an overnight% glucose solution to avoid hypoglycemia. Following that, the levels of fasting blood glucose were monitored for 72 hours following the injection and on the seventh day using a portable glucose meter. diabetic rats with fasting blood sugar levels more than 250 mg/dl (17). At the conclusion of the sixth week following induction, male adult albino rats showed significantly elevated serum levels of creatinine and urea as well as histological changes that proved nephropathy. These substantial values (18) were obtained from rats in this study.

Design of an experiment:

Seven groups of ten male adult albino rats each were created from the seventy animals.

Group I: The negative control group consisted of healthy rats.

Group II: Rats with DN, a positive control group.

Group III: Balanites treatment group: DN was induced, and rats received an oral dose of 80 mg/kg body weight each day for four weeks of the produced *Balanites aegyptiacae* fruit aqueous extract using an oral gastric tube (15).

MSCS-treated group in group IV: DN rats were given 200 L of phosphate buffer saline (PBS) suspended in 1.0 104 MSCs per gram of body weight twice every four weeks at four weeks after receiving an injection of STZ (19).

Group V: Exosome-treated group: Rats with DN were given two injections of exosomes (100 g per kg intravenously dissolved in 200 L of phosphate buffer saline (PBS) (20). The first was given during the study's eighth week, and the second was given at the conclusion of the tenth (18).

Group VI: Balanites + MSCs treated group: Rats with DN were given MSCs (1.0 104 cell/g) twice every four weeks via the tail vein (19) and made *Balanites aegyptiacae* fruit aqueous extract of (80 mg/kg) orally for four weeks daily (15).

Group VII: **Balanites + exosomes treated group**: For four weeks, DN was given an oral aqueous extract of the *B. aegyptiacae* fruit (80 mg/kg) as well as two injections of exosomes (100 g per kg per dose diluted in 200 ml PBS) (20). The first was given during the study's eighth week, and the second was given at the conclusion of the tenth (18).

Samples collection:

After fasting for 10 hours, the rats were given light ether anesthesia while blood samples were taken from the retro-orbital venous plexus. Whereas blood samples were taken in two different types of tubes—one with sodium fluoride for measuring blood sugar and another with empty tubes for centrifuging serum for 20 minutes at 4000 rpm. These types were all employed for different biochemical assays. For histopathological analysis, pancreatic samples from various rat groups were dipped in 10% neutral buffered formalin.

Biochemical parameters:

Fasting blood glucose

According to the procedure described in the literature (21) a commercial kit made by Elitech Clinical Systems, France, was used to assess plasma glucose.

Serum insulin

Using a commercial kit made by SIEMENS Company, USA and following the procedure described in the literature, serum insulin was measured (22).

Lipid profile

Using a commercial kit from the Spin React Company in Spain (23) and the vitro Scient Company in Germany (24) respectively, serum cholesterol and triacylglycerol were measured.

Kidney function tests

Serum samples were checked for kidney function tests such urea and creatinine using commercial kits from Diamond Diagnostic Company in Germany (25) and Spin React Company in Spain (26), respectively.

Histopathological study

The rats were executed at the conclusion of the experiment. Pancreatic tissues were placed right away in 10% buffered neutral formalin for tissue fixation. Metallic blocks were utilized to carefully insert the tissues in the melted paraffin. The tissues were then placed beneath freezing plates to solidify the paraffin after being covered in flexible plastic casts. The fixed tissues were sliced into cross sections that were five millimeters thick. Hematoxylin and eosin staining of these tissue sections was followed by light microscopy analysis to reveal the microscopic organization of the pancreas. Light microscopy was used to evaluate the pathological changes in the tissues in accordance with the Lillie technique (27).

Statistical Analysis

SPSS for Windows 25 (version 28), a statistical program for social research, was used to code and enter the data. One-way analysis of variance (one-way ANOVA) has been used to examine quantitative data from more than two groups in order to make comparisons between the research groups. The significance level was set at a p-value of less than 0.05. With n = 10, the values are shown as mean SEM. Significant differences are indicated by different superscript letters for values (P0.05). The following P values are against the control group: * P 0.05, ** P 0, and *** P 0.001. P values against the positive control group were 0.05, 0.01, and 0.001. The percentage change of the positive control was calculated based on the negative controls.

RESULTS

Effect on plasma glucose of Exosome, MSCs, and *Balanites aegyptiacae* in all examined groups.

The results of the fasting plasma glucose of all groups under investigation are shown in **Table 1**. DN rats showed a substantial rise in plasma glucose levels compared to healthy normal rats. *Balanites aegyptiaca* co-administration with exosome or MSCs, meantime, significantly improved (P<0.001) the unfavorable alterations in plasma glucose compared to DN rats.

Influence of exosome, MSCs, and *Balanites aegyptiacae* on serum insulin in all groups under investigation

The results of the serum insulin levels for each group under study are shown in **Table 2**. Serum insulin levels were found to be significantly lower (P<0.001) in diabetic nephropathy rats compared to normal healthy rats. In comparison to DN rats, co-administration of *Balanites aegyptiacae* with exosome or MSCs significantly (P<0.001) ameliorated the unfavorable alterations in insulin.

Effects of exosome, MSCs, and *Balanites aegyptiacae* on triacylglycerol and total cholesterol in all groups under study

The values of triacylglycerol and total cholesterol for each group under study are shown in **Table 3**. In comparison to the normal, healthy rats, diabetic nephropathy rats had significantly higher levels of total cholesterol and triacylglycerol. Comparing *Balanites aegyptiacae* co-administration to exosome or MSC co-administration, the unfavorable alterations in total cholesterol and triacylglycerol levels were significantly (P 0.001) ameliorated.

Effects of MSCs, exosomes, and *Balanites aegyptiacae* on kidney function tests in all examined groups

According to our findings, diabetic nephropathy rats had significantly higher levels of urea and creatinine than normal, healthy rats. As indicated in **Table 4**, urea and creatinine levels significantly decreased in DN animals groups that had received exosomes or MSCs together with *B. aegyptiacae*.

Effect of Exosome, MSCs and *Balanites aegyptiacae* on Pancreatic Function and Photomicrographs of Pancreatic Tissue

Histopathological changes to the pancreas were measured as shown in **Figure 1** to study the therapeutic effects of exosome on the enhancement of pancreatic performance in the STZ-induced diabetic model.

DISCUSSION

One of the most prevalent side effects of Diabetes Mellitus (DM) is diabetic nephropathy (DN) (29). The current work examines the pathophysiology of DN using the rat STZ-induced diabetes model, a type 2 diabetes model. Streptozotocin is the drug of choice to induce experimental diabetes mellitus, which results in hyperglycemia and diabetic complications like nephropathy (32), due to its capacity to cause specific necrosis of the pancreatic beta cells that leads to degranulation and loss of capacity to secrete insulin (30,31). There are many traditional DM therapies available. Traditionally, isolated compounds and extracts from diverse natural resources, particularly plants, have been a powerful toolbox for the management and treatment of DM problems and complications (33). The anti-diabetic, anti-hyperlipidemic, and anti-oxidant compounds flavonoids, amino acids, gallotannins, and numerous other related polyphenols are abundant in plants (34). In Egyptian traditional medicine, the herb *Balanites aegyptiaca* is frequently employed as a hypoglycemic agent (5).

Balanites aegyptiacae succeeds in causing a hypoglycemic effect in hyperglycemic diabetic rats but fails to do so in normoglycemic rats, which is a desired characteristic because hyperglycemia was discovered to lead to a chain reaction of negative effects (35). Balanites extract is administered to promote the regeneration of - cells since it contains a lot of flavonoids (36). It was discovered that the isoflavone genistein, a flavonoid produced from soy, reduced islet

apoptosis, preserved islet bulk, and encouraged islet -cell survival (37). There aren't many scientific studies that detail the hypoglycemic potential of different *Balanites aegyptiacae* aqueous extracts (38). The hypoglycemic impact was believed to be caused by enhanced peripheral glucose metabolism, an increase in insulin release from -cells, and a decrease in gastrointestinal glucose absorption (39, 40).

MSCs play a key role in the management of hyperglycemia and the decrease of renal damage, which are two treatment approaches for DN. MSCs can cure high blood sugar and minimize the kidney damage it causes by promoting islet cell regeneration, reducing insulin resistance, and improving islet function. MSCs can also directly repair kidney damage in a number of different ways (41). Exosomes produced by MSCs, which are lipid membrane micro-vesicles with a size range of 30 to 150 nm, have been discovered to be crucial in MSC therapy. RNA (42) and proteins (43) are examples of genetic molecules that can influence an organism's microenvironment and epigenetic processes under normal or abnormal circumstances. Thus, exosomes can transmit signals or materials and mediate microenvironmental communication in a variety of disorders (45), as they shuttle between cells and tissues while carrying a number of these substances (44). According to other research, the cargo contained in exosomes made from MSCs mediates therapeutic methods for a variety of disorders, including tumors (46), infections (47), metabolic diseases (48), and immunological diseases (49).

Exosomes are one of the likely therapeutic approaches for enhancing DN because to their prospective applications in a variety of scientific studies and clinical settings (50). According to the current study, DN rats had much lower serum insulin levels than healthy normal rats, and their fasting plasma glucose levels had significantly increased. The observed hyperglycemia and hypo-insulinemia in DN rats are in line with several earlier studies (51, 52), but the co-administration of *Balanites aegyptiacae* with MSCs or exosome significantly lessened (P 0.001) the undesirable changes in plasma glucose and serum insulin than in DN rats. According to the findings (53,54) that supported the anti-hyperglycemic actions of the *Balanites aegyptiaca* fruit, the increased plasma glucose levels have decreased. Fruit from *B. aegyptiaca* contains a lot of bioactive substances (55, 56). Flavonoids extracted from various plant sources were used to demonstrate their anti-diabetic action in animal models (57). *Balanites aegyptiaca* contained six distinct flavonoids, according to research (36). In diabetic rats, the fruit saponin of *Balanites aegyptiaca* was found to have a hypoglycemic effect (58).

MSC intravenous transplantation has been shown in numerous trials to drastically lower blood glucose levels in diabetic rats (59,60). MSC infusion decreased blood glucose levels in diabetic mice by a number of methods, including enhancing insulin sensitivity and perhaps upregulating GLUT4 expression as well as boosting phosphorylated Insulin receptor substrate 1 (IRS-1) and Akt levels in insulin target tissues (59). Exosomes, which are known to include a variety of components that support the anti-inflammation, repair, and regeneration of renal tissues, are abundantly present in MSC-conditioned medium (MSC-CM) (61). Furthermore, MSC-Exos have additional benefits than MSCs, including decreased immunogenicity, greater stability, and simpler storage (62). MSC-Exos show a substantial protective impact against acute and chronic kidney injury, according to prior research (63,64).

When compared to the negative control group, the serum levels of total cholesterol and triacylglycerol in diabetic nephropathy rats significantly increased (p 0.001). When diabetic mice were given the fruit of the *Balanites aegyptiaca* plant along with MSCs or exosomes, the serum's altered total cholesterol and triacylglycerol levels significantly improved. The *B. aegyptiaca* fruit and seed aqueous extracts include various constituents that have a wide range of biological activity. These include anti-inflammatory, hepatoprotective, cancer-

preventive, and hypocholesterolemic properties for 9, 12-octadecadienoic acid (Z, Z) (65, 66). In diabetic nephropathy rats, hypoinsulinemia was demonstrated to be the primary contributor to dyslipidaemia, hypertriglyceridemia, and hypercholesterolemia. These results concur with those of (67), who discovered a notable rise in the levels of serum triacylglycerol, cholesterol, and LDL cholesterol in diabetic animals. This rise may be a result of the reduced lipoprotein lipase (LPL) activity brought on by low insulin levels (68). While combining MSCs or exosomes with *Balanites aegyptiaca* enhanced lipid profiles by lowering total cholesterol and triacylglycerol. Insulin may boost lipid mobilization from blood vessels to the liver and decrease hepatic lipogenesis, which would otherwise result in less lipid mobilization to the blood, by increasing lipase activity in the pancreatic acinar cells (69). *B. aegyptiaca* has been shown to have anti-hyperlipidemic and anti-hyperglycaemic properties in the past (70,71). Our findings demonstrated that the STZ-induced diabetic rats had impaired kidney function, which was evidenced by a notable increase in blood urea and creatinine. Rats that received *Balanites aegyptiaca* together with MSCs or exosomes had significantly lower concentrations of urea and creatinine (P 0.001) than DN rats.

Exosome and *Balanites aegyptiacae* + exosome groups' histological images of the pancreas resembled those of the negative control group **(Figure 1(A))** in **Figures 1(F)** and **1(G).**These results are consistent with a different study (72) which showed that exosome and balanitas treatment resulted in normal pancreatic parenchyma, pancreatic lobules, and pancreatic acini and acinar cells. When compared to the Negative control group **(Figure 1(A))**, the Positive control group's islets cells showed severe degenerative and necrotic alterations **(Figure 1(B))**. Infiltration of leucocytic cells as well as dilated and clogged blood arteries were also observed. **Figure 1(C)** shows hyperplasia in the pancreatic islets of langerhans with normal cells and nuclei following the administration of *Balanites aegyptiaca* fruits aqueous extract only to diabetic rats, whereas **Figure 1(D)** shows dilatation in the pancreatic ducts, blood vessel congestion, and interlobular edema in the Balanites + MSCS treated group. Compared to the MSCS-treated group **(Figure 1(E))**, the pancreatic tissue in the Balanites + MSCS treated group **(Figure 1(D))** displayed a greater improvement. In actuality, flavonoids are abundant in *Balanites aegyptiaca* and many other plant preparations (36).

In pancreatic islets, the flavonoid genistein decreased cell apoptosis, raised the proportion of insulin-positive cells, encouraged islet cell survival, and conserved islet bulk (37). MSCs significantly reduced STZ-induced pancreatic islet damage and elevated insulin protein expression in pancreatic cells (73). These results are consistent with another study (74) which demonstrated that the infusion of MSCs could effectively reduce hyperglycemia in rats treated with alloxan and partially restore islet function. The underlying mechanism for MSCs' ability to treat hyperglycemia may also entail islet regeneration, including direct differentiation into functionally capable cells.

CONCLUSION

This work demonstrated the anti-diabetic and nephroprotective effects of the aqueous extract of *Balanites aegyptiaca*, MSCs, and exosome on the diabetic nephropathy in rats. *Balanites aegyptiaca* may be used as a supplement to dietary and pharmaceutical treatments to help better control diabetes. The encouraging outcomes support the use of *B. aegyptiaca* fruit aqueous extract in conventional folk medicine, MSCs, and exosome for the treatment of type 2 diabetes and its consequences.

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Table 1: MSC, Exosome and *Balanites aegyptiacae* effects on glucose (mg/dl) in all studied groups

Groups	Glucose (mg/dl)	% change	P Value
Negative control group	91.5±1.16^{c}	---	< 0.001
Positive control group	460±17.6***	403.2%	
Balanites treated group	104.9±0.49^{c}	-77.17%	
MSCs treated group	107.2±4.1^{c}	-76.57%	
Exosome-treated group	100.2±1.6^{c}	-78.10%	
Balanites + MSCs treated group	83.5±0.34^{c}	-81.85%	
Balanites + exosomes treated group	90.5±1.3^{c}	-80.26%	

Table 2: Exosome, MSCs and *Balanites aegyptiacae* effect on Insulin (µIU/ml) in all studied groups

Groups	Insulin (µIU/ml)	% change	P Value
Negative control group	7.27±0.29[c]	---	< 0.001
Positive control group	4.94±0.01***	-32.0%	
Balanites treated group	6.75±0.06[c]	36.63%	
MSCs treated group	7.12±0.14[c]	44.13%	
Exosome-treated group	7.9±0.06[c]	59.91%	
Balanites + MSCs treated group	8.02±0.23[c]	62.34%	
Balanites + exosomes treated group	8.37±0.13[c]	69.43%	

Table 3: Exosome, MSCs and *Balanites aegyptiacae* effect on total cholesterol and triacylglycerol levels in all studied groups

Groups	Cholesterol (mg/dl)	% change	Triacylglycerol (mg/dl)	% change	P Value
Negative control group	45.3±0.7[c]	---	42±0.49[c]	---	< 0.001
Positive control group	93±1.6***	105.29 %	74.5±1.1***	77.38%	
Balanites treated group	63.2±0.8***[c]	-32.04%	57.2±1.2***[c]	-23.22%	
MSCs treated group	56.7±0.83***[c]	-39.03%	35.4±0.94***[c]	-52.48%	
Exosome-treated group	62.9±1.4***[c]	-32.36 %	38.6±1.0*[c]	-48.18%	
Balanites + MSCs treated group	47.4±1.4[c]	-49.03 %	44.3±0.81[c]	-40.53%	
Balanites + exosomes treated group	53.8±0.64***[c]	-42.15%	44.1±0.52[c]	-40.80%	

Table 4: Exosome, MSCs and *Balanites aegyptiacae* effect on kidney function tests in all studied groups

Groups	Urea (mg/dl)	% change	Creatinine (mg/dl)	% change	P Value
Negative control group	13.4±0.16[c]	---	0.48±0.02[c]	---	< 0.001
Positive control group	38.8±0.32***	189.55%	0.9±0.00***	87.5%	
Balanites treated group	14.8±0.41*[c]	-61.85%	0.74±0.02***[b]	-17.77%	
MSCs treated group	15.7±0.33*[c]	-59.53%	0.67±0.01***[c]	-25.55%	
Exosome-treated group	15.1±0.37*[c]	-61.08%	0.63±0.01***[c]	-30%	
Balanites + MSCs treated group	15.2±0.32*[c]	-60.82%	0.65±0.01***[c]	-27.77%	
Balanites + exosomes treated group	14.6±0.45*[c]	-62.37%	0.57±0.03[c]	-36.66%	

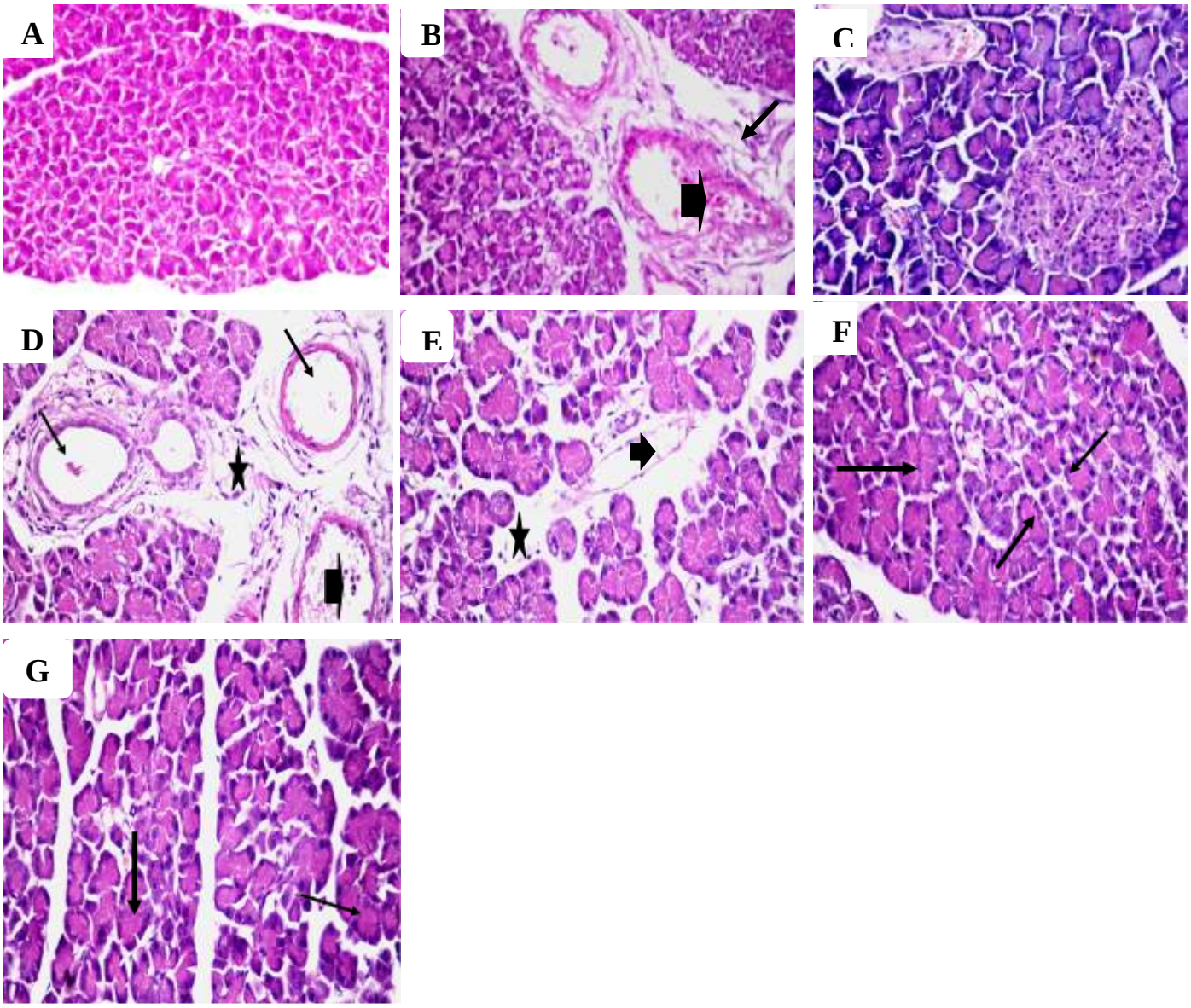

Figure 1: Photomicrographs of pancreatic tissue from all groups examined are shown in Figure 1. **(A)** Normal pancreatic acini were visible in the pancreas of the negative control group (Score lesion 0). **(B)** An inflammatory response was visible in the pancreas portion from the positive control group; take note of the enlarged and congested blood arteries (arrow head) and the infiltration of leucocytic cells (arrow). This lesion received a score of ++++. **(C)** The pancreas from the Balanites-treated group showed hyperplasia in the islets of Langerhans, together with normal cells and nuclei (arrows), and a score lesion of +++. **(D)** The pancreas from the Balanites + MSCS treated group exhibited interlobular edema (*), score lesion ++, blood vessel congestion (arrow head), and dilatation in the pancreatic ducts (arrow). **(E)** The pancreas from the MSCS-treated group exhibited interlobular edema (*), score lesion +++, and blood vessel dilatation (arrow head). **(F)** The pancreas from the *Balanites aegyptiacae* + exosome group displayed normal pancreatic lobules, normal pancreatic acini and acinar cells, together with normal pancreatic ducts and islets (Score lesion 0). **(G)** The exosome group's pancreas had normal pancreatic lobules, normal pancreatic acini and acinar cells (arrows), as well as normal pancreatic ducts and islets (Score lesion 0). **(H&E Stain, Magnification Power= x400)**.