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The role of stem cells and vitamin D in the attenuation of liver and kidney functions in STZ-induced diabetic rats: Biochemical study

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Abstract

Background: Diabetes is one of the most important causes of mortality and morbidity all over the world. Renewal of functional pancreatic islets has been a goal of stem cell biologists since early 2000. Since that time, many studies have reported successful creation of glucose-responsive pancreatic beta-cells. **Aim of work:** This work aimed to study the effect of MSC.s alone and/or in combination with vitamin D_3in Streptozotocin (STZ) - induced diabetic male albino rats to detect its potential therapeutic effect and its possible application to humans. **Material and methods:** Forty-eight male albino rats (150 – 170 grams) were included in this study. They were divided into four equal groups; each group have twelve rats: Group I (Normal: control of healthy), group II (STZ: control of diabetes), group III (diabetic group post-treated with MSC.s) and group IV (diabetic group post-treated with MSC.s in combination with vitamin D_3). Diabetes was induced by intraperitoneal injection of STZ (50 mg/kg); MSC.s were injected intravenously into the rat tail vein in group III and group IV; vitamin D (cholecalciferol) was administered orally at 150 ng (500 IU/kg) each other day at three times per week during the experiment. 6 rats from each group of animals were sacrificed at the interval 4 and 6 weeks. At the end of the experiment sera of blood sample were obtained for estimated some of liver and kidney markers. **Results:** Diabetic group (group II) showed significant increase of ALT, AST and ALP as well as kidney function rather than t. protein and albumin at the interval 4 and 6 weeks compared to control of non-diabetic group. Group III as well as group IV showed significant amelioration of liver and kidney functions in compared to group II. **Conclusion:** treatment with MSC.s and/or in combination with vitamin D_3improvement of liver and kidney functions compared to diabetic group.

Keywords: STZ (Streptozotocin), MSC.s (Mesenchymal stem cells) and Vit D (Vitamin D_3).

1. Introduction

Diabetes mellitus (DM) is a major and rapidly growing public health concern. Diabetes is a major health problem in different societies. The prevalence of diabetes in all age groups worldwide was estimated to be 2.8% in 2000 and is estimated to be 4.4% in 2030. The total number of patients with diabetes worldwide is expected to increase from 171 million in 2000 to 366 million by 2030 and constitutes one of the major threats to global health **(Rathmann & Giani., 2004).**

Specifically, in Egypt, the published figures showed that the prevalence of diabetes in persons over 20 years is increasing from 9.9% in 1995 to 10.2% in 2000 and expected to reach 13.3% in 2025 **(Ahmed *et al*., 2017).**

Diabetes is a group of diseases characterized by abnormally high levels of the sugar glucose as well as lack of insulin leads to hyperglycemia in the bloodstream and this excess glucose is responsible for devastating complications of diabetes, which include blindness, kidney failure, cardiovascular disease, stroke, neuropathy and amputations **(Liao *et al*., 2007).**

Diabetes mellitus has been characterized by hepatopathy and nephropathy **(Kokou *et al*., 2013).** The complications of diabetes mellitus have significant health, economic and social impacts on individuals, families, health systems and countries **(Lv *et al*., 2014).**

Experimental induction of DM in animal models is essential for the understanding of the various aspects of its pathogenesis and for screening potential therapies for the treatment of this condition. Induction of experimental diabetes in rats using streptozotocin (STZ) is a very convenient and simple technique. STZ (*N*-nitro derivative of glucosamine) is a naturally occurring broad-spectrum antibiotic and cytotoxic chemical that is particularly toxic to the pancreatic insulin-producing β cells in mammals **(Abeeleh *et al*., 2009).**

There is currently no cure for diabetes. People with type 1 diabetes must take insulin several times a day and test their blood glucose concentration three to four times a day throughout their entire lives **(Bonner *et al*., 2000).** Insulin replacement represents the current therapy for type 1 diabetes. However, its metabolic control remains difficult, as exogenous insulin cannot precisely mimic the physiology of insulin secretion. Exogenous insulin supply is not fully capable of achieving tight control of glucose regulation, leading to long-term complications **(Hori, 20009).**

Over the past several years, doctors have attempted to cure diabetes by injecting patients with pancreatic islet cells of the pancreas that secrete insulin and other hormones. However, the requirement for steroid immunosuppressant therapy to prevent rejection of the cells increases the metabolic demand on insulin-producing cells and eventually they may exhaust their capacity to produce insulin. The deleterious effect of steroids is greater for islet cell transplants than for whole-organ transplants **(Itkin-Ansari *et al*., 2001).** The current gold standard therapy for pancreas transplantation has limitations because of the long list of waiting patients and the limited supply of donor pancreas **(Hantuchova *et al*., 2015).**

Stem cell therapy holds a great promise for the repair of injured tissues and organs. Stem cells are undifferentiated cells that undergo both self- renewal and differentiation into one or more cell types **(Abeeleh *et al*., 2009).** Among stem cells, mesenchymal stem cells (MSCs) have several advantages for therapeutic use such as ability to migrate to the sites of tissue injury, strong immunosuppressive effects **(Xu *et al*., 2008)** and better safety after infusion of allogeneic MSCs **(Guo & Hebrok., 2009).**

Previous studies have shown that MSCs are able to differentiate into several cell types, including cardio-myocytes, vascular endothelial cells, neurons, hepatocytes, epithelial cells, and adipocytes, making them a potentially important source for the treatment of debilitating human diseases including DM. An increasing number of data has showed that the therapeutic effects of MSCs not only rely on their differentiation ability to repair damaged tissue, but also depend on their potency to modulate local environment, activate endogenous progenitor cells, and secrete various factors **(Volarevic *et al*., 2011).** Recently, some studies have shown that MSCs can improve the metabolic profiles of diabetic animal models, providing evidence for the potential therapeutic efficacy of MSC therapy in diabetes **(Wagner *et al*., 2010).**

Such multipotent differentiation characteristics coupled to their capacity for self-renewal and capability for the regulation of immune responses, described MSCs as potentially new therapeutic agents for treatment of the complications of diabetes mellitus (DM) **(Abdi *et al*., 2008).** Focusing on MSCs therapy in most clinical applications they are isolated from bone marrow (BM) **(Kern *et al*., 2006).** Depending on their intended purpose, experimental or therapeutic use, the main functional characteristics of MSCs are their immune-modulatory ability make them a promising therapeutic tool for severe refractory autoimmune diseases. They suppress T-cell proliferation and significantly reduce the expression of certain activation markers on stimulated lymphocytes, the other main functions are self-renewal, and differentiation into tissues of mesodermal origin **(Addi *et al*., 2004).**

During the past decade, theories on the functions of vitamin D_3 have been proposed. In addition to its well-established role in calcium and bone homeostasis, vitamin D_3 has been shown to exert autocrine or

paracrine immunomodulatory effects **(Gao *et al.*, 2009).** Much epidemiological research has shown that vitamin D_3 deficiency is associated with a large number of autoimmune and inflammatory diseases, such as rheumatoid arthritis, lupus, inflammatory bowel disease, transplant rejection, cardiovascular disease, infections, and diabetes **(Guillot *et al.*, 2010).** Thus, an understanding of the underlying anti-inflammatory mechanisms of vitamin D_3 may extend its clinical applications.

Recently, the relationship between vitamin D_3 and DM has become a focus of study upon reduced the elevation of liver and kidney functions in STZ induced diabetic rats and to clarify the underlying mechanisms involved in this effect beside the paracrine signals that mediate MSC.s action.

2. Aim of the work:

The study aimed to detect the therapeutic effect of mesenchymal stem cells alone and/or in combination with vitamin D_3on the DM complications of STZ induced diabetic rat model upon the attenuation of liver and kidney functions.

3. Materials and Methods

Animal: The study was carried out on forty-eight (12 weeks old) adult male albino rats (*Rattus norvegicus*) were used for the experiment with an average weight from 150 to 180 g ± 20 g (mean ± SD: 160 ± 1.11). They were obtained from the Animal House of the Al_Nile Company of Pharmaceutical Products (Cairo, Egypt). They were housed in a temperature at 25 ± 2°C and light-controlled room (12-h light/dark cycle) with free access to standard diet pellets (El-Nasr company, Cairo, Egypt), and tap water. Animals were housed in metallic cages and left to acclimatize for one week to the laboratory condition before starting the experiment. The study was conducted at the animal house at faculty of science, Al-Azhar University according the Guidelines of Ethics for the Care and Use of Laboratory Animals.

Chemicals: Streptozotocin (STZ) was purchased from Sigma Chemical Company (St Louis, Missouri, USA) in the form of powder. Vitamin D was purchased from local market, Elnasr, co, Cairo, Egypt.

Research design and methods

The animals (rats) were randomly divided into four groups; each group has 12 rats as the following:

Group I: Non- diabetic rats (Served as control of healthy). This group included 12 rats, they were injected intraperitoneally with citrate buffer and were sacrificed at the different interval 4 and 6 weeks asin the other experimental groups.

Group II: Diabetic non-treated group (Control of diabetes) using Streptozotocin (STZ). This group included 12 rats that were fasted for 12 h before induction of diabetes. Diabetes was induced by means of a single intraperitoneal injection of STZ at a dose of 50 mg/kg body weight **(Bhansali *et al.*, 2015).**

Group III: Diabetic post-treated group (STZ +MSC.s). This group included 12 rats in which diabetes was induced by means of a single intraperitoneal injection of STZ; followed by intravenous injection in a single dose of 0.5×10^6MSC.s (which were processed and cultured for 14 days) per rat through the tail vein**(El Aziz *et al.*, 2011)**

Group IV: Diabetic post-treated group (STZ +MSC.s + Vitamin D). This group included 12 rats in which diabetes was confirmed; they were injected with MSC.s and their administrated vitamin D3 per oral; cholecalciferol (Doxercalciferol) was administered orally at 150 ng (500 IU/kg) each other day at three times per week **(Choi *et al.*, 2011).**

Six rats from each group were scarified at the different interval 4^{th} and 6^{th} weeks post- first week of streptozotocin-induced diabetic rats.

Dose titration of STZ in induction of hyperglycemia

After fasting rats for 18 h, Streptozotocin (STZ) was purchased from Sigma Chemical Company (St Louis, Missouri, USA) was freshly dissolved in 0.1 M citrate buffer (pH = 4.5) and administrated at the dose of 50 mg/kg B.W. intraperitoneally within 15 minutes of dissolution **(Bhansali *et al.*, 2015).** The non-diabetic control rats (group I) also received an injection of the citrate buffer. Following the injections, the rats had free access to (5%) glucose solutions for 24 hours in order to avoid the anticipated hypoglycemic shock. 72 hours following the injection, tail blood samples from

overnight fasting rats were obtained to measure blood glucose level **(Montilla *et al.*, 1998).** Diabetes was confirmed by the determination of fasting glucose concentration on the third day post administration of streptozotocin. Rats with glucose levels was higher than 200 mg/dl were considered to be diabetic and chosen for the experiment while those with blood glucose level outside this range were excluded **(Furman, 2015).**

Preparation of bone marrow- derived mesenchymal stem cells

The six-week-old male white albino rats were sacrificed after administration of sodium pentobarbital intraperitoneally at a dose of 30 mg/kg. Bone marrow was harvested by flushing the tibiae and femurs of rats with Dulbecco's modified Eagle's medium (DMEM, Gibco/BRL, Gibco BRL, Karlsruhe, Germany) supplemented with 10% fetal bovine serum (Gibco/BRL). Nucleated cells were isolated with a density gradient (Ficoll/Paque; Pharmacia) and re-suspended in complete culture medium supplemented with 1% penicillin–streptomycin (Gibco/BRL). Cells were incubated at 37°C in 5% humidified CO_2 for 12–14 days until formation of large colonies (80–90% confluence). The culture was washed with PBS and released with 0.25% trypsin in 1 mM/l EDTA (Gibco/BRL) for 5 min at 37 °C. After centrifugation, the cells were re-suspended with serum- supplemented medium and incubated in a 50-cm^2 culture flask (Falcon). The resulting cultures were referred to as first-passage cultures **(Alhadlaq & Mao., 2004).** MSCs in culture were characterized by their adhesiveness and fusiform shape **(Rochefort *et al.*, 2005).**

Treatment of diabetes mellitus by mesenchymal stem cells

Blood samples were obtained from the retro-orbital veins plexus to confirm that the animals had become diabetic. Thereafter, MSCs were injected post- first week of streptozotocin-induced diabetic rats by injecting one million units of cells suspended in 0.5 ml puffer per animal through the caudal tail vein **(El Aziz *et al.*, 2011).**

Preparation of Biological Samples

At the different interval 4 and 6 weeks of the experiment, rats were fasted for 12 hr, blood samples were collected from each animal under diethyl ether anesthesia from retro-orbital venous plexus puncture using blood capillary tube. Blood samples were left to clot at room temperature for 15 minutes. Serum samples were separated by centrifugation at 3000 rpm at 20°C for 15 minutes where the clear serum was obtained and kept frozen at -80°C for various biochemical analyses.

Biochemical parameters

Serum ALT and ASTwere measured using the kinetic method according to **Reitman & Frankel (1957)**, Activity of Alkaline phosphatase (ALP) enzyme was determined according to the method of **Moss (1982**), Colorimetric determination of serum total protein was calculated according the method of **Tietz (1994)** and serum albumin according the method of **Doumas *et al.*, (1971)** using available commercial kits obtained from *spectrum*, Egypt.

Serum urea level was estimated according to the colorimetric method described by **Fawcett & Soctt (1960)**, Serum creatinine level was determined according to the colorimetric method described by **Larsen (1972)** and uric acid level was determined according to the method described by **Barham and Trinder (1972)**

Statistical analysis

The Statistical Package for the Social Sciences (SPSS, version 22) was used in data analysis. Data were expressed as mean ± S.E.M One-way analysis of variance (ANOVA) test was used to compare between groups followed by Fisher's least significant difference (LSD) analysis. *P* values less than 0.05 were considered significant **(Armitage & Berry., 1994).** Data were tabulated as it was represented.

4. Results

The results of STZ induced-diabetic rats showed very high significant elevations ($P<0.001$) in serum ALT and AST activities in compared with non-diabetic rats. Moreover, the represented data from diabetic rats post-treated with MSC.s alone and/or in combination with vitamin D revealed that no significant change ($P<0.05$) found when compared with the corresponding values of control group. On the other hand, this enzyme activity was significantly decreased ($P<0.01$) might be restored to normal levels in diabetic rats post-treated with MSC.s alone and/or in combination with vitamin D as compared with the corresponding values of diabetic-untreated rats after four weeks of treatment.

Additionally, the results of serum ALP activity in diabetic un-treated rats showed a significant increase

(P<0.05) when compared with the corresponding values of non-diabetic rats. However, ALP activity in diabetic rats post-treated with MSC.s alone and/or in combination with vitamin D revealed that, no significant change (P<0.05) found as compared with the corresponding values of non-diabetic rats and diabetic-untreated rats.

Serum total protein and albumin concentrations showed a very high significant reduction (P<0.001) in STZ induced-diabetic rats when compared with the corresponding values of non-diabetic rats. On the other hand, the level of albumin was significantly increased (P<0.001) might be restored to normal levels in diabetic rats post-treated with MSC.s alone and/or in combination with vitamin D as compared with the corresponding values of diabetic-untreated rats.Moreover, the represented data of serum total protein level in diabetic rats post-treated with MSC.s in combination with vitamin D showed a high significant elevation (P<0.001) rather than rats post-treated with MSC.s alone revealed that no significant change (P<0.05) found when compared with the corresponding values of diabetic-untreated rats after four weeks of treatment.

The results of serum ALT activity in diabetic-untreated rats showed significant elevation (P<0.001) in compared to non-diabetic rats. However, the represented data of serum ALT from diabetic rats post-treated with MSC.s alone and/or in combination with vitamin D revealed that no significant change found (P<0.05) when compared with the corresponding values of non-diabetic rats. However, this enzyme activity was significantly decreased (P<0.001) might be restored to normal levels in diabetic rats post-treated with MSC.s alone and/or in combination with vitamin D as compared to diabetic-untreated rats after six weeks of treatment.

Serum AST activity in diabetic-untreated rats and diabetic rats post-treated with MSC.s alone and/or in combination with vitamin D showed very high significant elevations (P<0.001) as compared with the corresponding values of non-diabetic rats. Additionally, serum ALP activity in diabetic-untreated rats showed a very significant increase (P<0.001) when compared with non-diabetic rats. However, the represented data from diabetic rats post-treated with MSC.s alone and/or in combination with vitamin D revealed that, no significant change (P<0.05) found in AST as well as ALP enzymes activity as compared with the corresponding values of non-diabetic rats and diabetic-untreated rats after six weeks of treatment.

Serum total protein, albumin and globulin concentrations in diabetic-untreated rats and diabetic rats post-treated with MSC.s alone and/or in combination with vitamin D revealed that no significant change (P<0.05) found when compared with the corresponding values of non-diabetic rats. However, the level of albumin in diabetic rats post-treated with MSC.s in combination with Vitamin D showed very high significant elevations (P<0.001) as compared with the corresponding values of diabetic un-treated rats after six weeks of treatment.

The results of serum urea, creatinine and uric acid levels of STZ induced diabetic-untreated rats showed a very high significant elevation (P<0.001) as compared to non-diabetic rats. Unlike, the represented data from diabetic rats post-treated with MSC.s alone and/or in combination with vitamin D revealed that no significant change found (P<0.05) in urea and creatinine levels when compared with the corresponding values of non-diabetic rats. However, serum uric acid level in diabetic rats post-treated with MSC.s in combination with vitamin D after four weeks of treatment showed a significant increase (P<0.05) when compared with the corresponding values of non-diabetic rats.

On the other hand, the represented data of urea, creatinine and uric acid levels in diabetic rats post-treated with MSC.s alone and/or in combination with vitamin D showed a very high significant decrease (P<0.001) as compared with the corresponding values of diabetic-untreated rats after four weeks of treatment.

Serum urea concentration in diabetic untreated rats of STZ-induced diabetes showed a high significant increase at (P<0.01) as well as serum uric acid and creatinine levels were elevated (P<0.001) as compared with the corresponding values of non-diabetic rats. On the contrary, there was no significant change (P<0.05) found in serum urea, creatinine and uric acid levels in diabetic rats post-treated with MSC.s alone and/or in combination with vitamin D as compared to non-diabetic rats. On the other hand, the represented data of serum creatinine and uric acid levels in diabetic rats post-treated with MSC.s alone and/or in combination with vitamin D showed a very high significant decrease (P<0.01) when compared with the corresponding values of diabetic-untreated rats. However, serum urea level in diabetic rats post-treated with MSC.s alone and/or in combination with vitamin D presented no significant change (P<0.05) in compared to diabetic-untreated rats after six weeks of treatment.

Table (1): Effect of mesenchymal stem cells transplantation and vitamin D administration on serum AST, ALT and ALP enzymes activity as well as T. Protein, Albumin, Globulin and A/G ratio levels after four weeks of treatment in STZ-induced diabetic rats.

Biochemical parameters	Liver function profile after fourweeks of treatment						
	ALT (U / L)	AST (U / L)	ALP (U / L)	T. Protein (g/dl)	Albumin(g/dl)	Globulin(g/dl)	A/G ratio (g/dl)
Experimental groups	**Mean±SE**	**Mean±SE**	**Mean±SE**	**Mean±SE**	**Mean±SE**	**Mean±SE**	**Mean±SE**
Group I	19.0 ± 2.14	46.40±3.74	114.60±1.25	7.03±0.63	4.28±0.32	2.75±0.71	2.03±0.53
Group II	81.33 ± 2.72 [a]	123.12±4.85 [a]	249.88±32.27 [a]	4.28±0.58 [a]	1.95±0.09 [a]	2.33±0.61	1.28±0.39
Group III	17.70 ± 1.27 [b]	68.62±7.77 [b]	211.96± 60.87	4.75±0.24 [a]	3.61±0.29 [b]	1.14±0.13 [a]	3.39±0.59
Group IV	13.84 ± 2.40 [b]	71.46±6.36 [b]	235.44± 41.02	5.81±0.25 [b]	4.05±0.16 [b]	1.76±0.31	2.73±0.63

Table (2): Effect of mesenchymal stem cells transplantation and vitamin D administration on serum AST, ALT and ALP enzymes activity as well as T. Protein, Albumin, Globulin and A/G ratio levels after six weeks of treatment in STZ-induced diabetic rats.

Biochemical parameters	Liver function profile aftersix weeks of treatment						
	ALT (U / L)	AST (U / L)	ALP (U / L)	T. Protein (g/dl)	Albumin(g/dl)	Globulin(g/dl)	A/G ratio (g/dl)
Experimental groups	**Mean±SE**	**Mean±SE**	**Mean±SE**	**Mean±SE**	**Mean±SE**	**Mean±SE**	**Mean±SE**
Group I	15.55±1.22	53.58±4.55	135.78±13.71	8.11±1.16	3.84±0.24	4.27±1.07	1.47±0.53
Group II	41.33±5.29 [a]	129.62±11.9 [a]	393.48±50.6 [a]	5.38±0.34 [a]	3.05±0.28	1.93±0.40	2.70±1.21
Group III	16.04±1.98 [b]	100.48±14.4 [a]	270.10±48.66 [a,b]	6.49±0.83	4.06±0.44	2.44±1.01	6.88±3.80
Group IV	16.00±1.86 [b]	110.23±6.03 [a]	276.82±34.79 [a,b]	6.59±0.27	4.58±0.44 [b]	2.01±0.56	4.36±1.61

Each value represents mean of 5 records ± S.E

Means with dissimilar superscript letter are significantly different at (P < 0.05), where: [a] significance vs. control group; [b] significance vs. STZ group.

Group I (Control: Normal), **Group II** (Diabetic: STZ), **Group III** (STZ + M.S. Cs) and **Group IV** (STZ + MSC.s + Vit D)
STZ= Streptozotocin;**MSC.s=** Mesenchymal stem cells;**Vit-D=** Vitamin D

Table (3): Effect of mesenchymal stem cells transplantation and vitamin D administration after four weeks of treatment on serum urea, creatinine and uric acid levels in STZ-induced diabetic rats.

Biochemical Parameters	kidney function markers								
	Urea (mg/dl)			Creatinine (mg/dl)			Uric acid (mg/dl)		
Experimental groups	Four weeks of treatment			Four weeks of treatment			Four weeks of treatment		
	Mean±SE	%change	*P-Value*	Mean±SE	%change	*P-Value*	Mean±SE	%change	*P-Value*
Group I	47.03±4.61			0.66±0.07			7.04±0.37		
Group II	77.45±5.79[a]	**64.7%**	***p<0.001***	1.84±0.06[a]	**178.3%**	***p<0.001***	14.78±0.88[a]	**109.8%**	***p<0.001***
Group III	39.24±1.73[b]	**-16.6%**	***N.S***	0.77±0.08[b]	**16.4%**	***N.S***	8.54±0.38[b]	**21.2%**	***N.S***
Group IV	35.81±3.44[b]	**-23.9%**	***N.S***	0.69±0.04[b]	**4.5%**	***N.S***	8.78±.20[a,b]	**24.7%**	***p<0.05***

Table (4): Effect of mesenchymal stem cells transplantation and vitamin D administration after six weeks of treatment on serum urea, creatinine and uric acid levels in STZ-induced diabetic rats.

Biochemical Parameters	kidney function markers								
	Urea (mg/dl)			Creatinine (mg/dl)			Uric acid (mg/dl)		
Experimental groups	Six weeks of treatment			Six weeks of treatment			Six weeks of treatment		
	Mean±SE	%change	*P-Value*	Mean±SE	%change	*P-Value*	Mean±SE	%change	*P-Value*
GroupI	40.8±2.28			0.66±0.08			7.3±0.39		
GroupII	71.5±7.41[a]	**75.2%**	***p<0.01***	2.15±0.19[a]	**224.7%**	***p<0.001***	15.4±1.53[a]	**111.8%**	***p<0.001***
Group III	55.6±9.91	**36.3%**	***N. S.***	0.83±0.10[b]	**25.2%**	***p<0.001***	8.2±0.43[b]	**12.6%**	***p<0.001***
Group IV	52.1±3.86[b]	**27.7%**	***p<0.001***	0.74±0.13[b]	**12.3%**	***p<0.001***	8.5±0.88[b]	**15.9%**	***p<0.001***

Each value represents mean of 5 records ± S.E.
Means with dissimilar superscript letter are significantly different at (P < 0.05), where: [a] significance at vs. control group; [b] significance vs. STZ group.
Means, which have the same superscript symbol (N.S.), are not significantly different.
Percent of changes (%) are calculated by comparing treated groups with normal control group.

Group I (Control: Normal), **Group II** (Diabetic: STZ), **Group III** (STZ + M.S. Cs) and **Group IV** (STZ + MSC.s + Vit D)
STZ= Streptozotocin;**MSC.s=** Mesenchymal stem cells;**Vit-D=** Vitamin D

5. Discussion

Diabetes mellitus has been characterized by hepatopathy and nephropathy **(Kokou *et al*., 2013).** Induction of streptozotocin in rats with pancreatic neoplasm induces hepatotoxicity **(Noorafshan *et al.*, 2005)**, therefore we believe that the changes observed were due to the induction of diabetes and not only due to the toxic effect of streptozotocin.

The hepatic impairment of streptozotocin was confirmed through elevate in the activities of liver marker enzymes **(Ghosh and Suryawansi, 2001).** While, serum aminotransferases (ALT & AST) are cytosolic enzymes located in hepatocytes and an increase in their activities reflecting the increase in plasma membrane permeability which in turn are associated with cell death **(Rosen &Keeffe, 2000).**

AST, ALT and ALP activities in serum were increased owing to outflow of those enzymes from the liver cytosol into the blood stream **(Navarro *et al.*, 1993)** that was a sign of hepatotoxic nature of streptozotocin. The presence of elevated values of ALT and AST is indicative of liver damage according to STZ was reported to have a direct damaging effect on some organs, such as kidney and liver which is supported in our study **(Elsner *et al.*, 2000).** Moreover, ALP attributed to defense mechanisms against the damaged occur in the structural integrity of hepatic cells upon the induction of STZ in diabetic untreated rats **(Shahjahan *et al.*, 2004)** due to the large of bile duct obstruction, intrahepatic cholestasis or infiltrative diseases of the liver **(Giboney, 2005).** This is buttressed with high level of liver-function enzymes.

In this field of study, the increment of the activities of ALT, AST and alkaline phosphatase (ALP) were elevated in diabetic untreated rats, upon STZ- induced diabetes as compared with the corresponding values of non-diabetic rats. The results indicated that the increase in the activities may be due to the induction of hepatic dysfunction by diabetes **(El-Demerdash *et al.*, 2005).** Regarding to the results of ALP activity, the obtained data revealed that, ALP showed a very high significant increase in diabetic untreated rats in compared with the corresponding values of non-diabetic rats. The results suggested that, serum ALP activity was increased in liver and released into the bile, which might lead to loss of the secretory function of the liver **(Baraona *et al.*, 1980).**

Furthermore, the represented data at the interval four and six weeks post one week of STZ induction indicate a significant elevation of AST and ALT activities in diabetic untreated rats which are concomitant with the results of **Arulselvan *et al*. (2006).** The diabetic effect of STZ is due to an excess in the production of reactive oxygen species (ROS). This excess leads to toxicity in pancreatic cells, which, in turn, reduces the synthesis and release of insulin while concurrently affecting other organs, such as liver **(Sakuria *et al.*, 2001).** The represented data are in accordance with the study of **Eskander *et al.* (1995)** found that liver was necrotized in diabetic rats followed by the elevation of liver enzymes. These findings have further confirmed by **Rawi *et al.* (1998).**

However, the other documented data marked the elevation of serum AST and ALT and ALP enzymes activity in diabetic untreated rats may also reflect the damage of the hepatic cells thought to be consistent with their greater need for gluconeogenic substrates **(Nanbora *et al.*, 1990).** However, other researchers have postulated that diabetes could induce defects in sarcolemmal enzymatic activities **(Micheal *et al.*, 1985)** which lead finally to such effects.

Although, diabetes mellitus describes a metabolic disorder of multiple etiology, which is characterized by chronic hyperglycemia **(Negre Salvayre *et al.*, 2008).** Recurrent or persistent hyperglycemia during diabetes causes non- enzymatic glycosylation of body proteins **(Kennedy &Baynes., 1984).** Hence, the result of serum T. protein and albumin concentrations were significantly decreased after four weeks of STZ-induced diabetic rats as compared with the corresponding values of non-diabetic rats. Hence, increase of protein catabolism is accompanied by gluconeogenesis and urea formation in diabetic condition that may be reason for raise of transaminases in vital tissues.

In the contrary, stem cell therapy and vitamin D supported by reduction in serum AST, ALT and ALP enzymes activity, as well as increase in total protein and albumin levels (probably due to decrease in gluconeogenesis) meanwhile, AST, ALT and ALP activities were return back to near normal levels in diabetic post-treated rats after four weeks of treatment when compared to diabetic control rats. Interestingly, MSC.s transplantation alone and/or in combination with vitamin D administration was significantly normalized the blood glucose levels and ameliorated the increase of serum ALT, AST and ALP activities.

This reduction may be owing to the discharge of these enzymes in liver.

Our findings suggest that, the ameliorative effect of liver enzymes activity upon the therapeutic effects of MSCs not only rely on their differentiation ability to repair damaged tissue, but also depend on their potency to modulate local environment, activate endogenous progenitor cells, and secrete various factors **(Zhang *et al*., 2007).** Similarly, reduction of ALP activity is suggestive of the ability of MSC.s alone and/or in combination with vitamin D treatment to protect the cell from cytotoxic injury **(Vitek, 2012).**

Diabetic nephropathy (DN) is the most common cause of end-stage renal disease in the world, and could account for disability and high mortality rate in patients with diabetes **(Yamagishi & Matsui., 2010).** D.N is thought to result from interaction between metabolic and hemodynamic factors. The pathologic changes in D.N include renal hypertrophy and renal failure through the tubular interstitial fibrosis **(Forbes *et al.*, 2007).**

Stem cell therapy holds a great promise for the repair of injured tissues and organs, including the kidney. Stem cells are undifferentiated cells that undergo both self- renewal and differentiation into one or more cell types **(Weissman, 2000).** Among stem cells, mesenchymal stem cells (MSCs) have several advantages for therapeutic use such as ability to migrate to the sites of tissue injury, strong immunosuppressive effects **(Volarevic *et al.*, 2010)**, and better safety after infusion of allogeneic MSCs (**Lee *et al.*, 2010).**

Serum urea and uric acid concentrations showed a very high significant increase after four weeks of STZ-induced diabetic untreated rats as compared to the non-diabetic rats. Increases these levels of kidney functional markers may have been due to STZ-induced metabolic disturbances, and renal dysfunction. These results are in accordance with the study of **Idonije *et al.* (2011)** showed that in addition to elevated blood sugar level in diabetes mellitus, plasma creatinine and urea concentration are also significantly increased in male and female diabetics when compared with their levels in apparently healthy non-diabetic male and female rats.

In contrary results to that of **Zhang *et al.* (2008)** in which urea level reduced in diabetic rats but support the study by **Guan *et al.* (2013)** where the urea level was significantly decreased after the 15th week of STZ injection. Low concentration of urea suggested by a reduced turnover of protein in diabetic rats **(He *et al.*, 2012).** Thus, it indicated a reflect in glomerular filtration rate and worsened the renal function **(Guan *et al.*, 2013).**

Four weeks after MSC.s injection of STZ induced diabetic nephropathy, urea and uric acid levels in diabetic rats post-treated with M.S. Cs in a combination with Vitamin D showed no significant differences compared to normal rats. Otherwise, the revealed data showed a significant decrease of those levels in diabetic rats post-treated with MSC.s in a combination with Vitamin D as compared to diabetic untreated rats. These data indicate that MSC.s transplantation improved kidney function in diabetic rats. This successful MSC.s treatment of diabetic nephropathy could be explained by MSC.s competence to differentiate into insulin-producing beta cells followed by decrease of glycemia and glycosuria, factors important for damaging renal cells **(Ezquer *et al.*, 2008).** The results are in harmony with **Hammam *et al.* (2015)** who found that injection of MSC.s ameliorated the renal function.

The results of serum urea and uric acid levels in diabetic untreated rats at six weeks showed a very high significant elevation as compared with the corresponding values non-diabetic rats. This elevation of urea and uric acid levels in streptozotocin induced diabetic rats, might be due to disturbance in metabolic activity that increased activities of lipid peroxidation, triglyceride and cholesterol **(Madianov *et al.*, 2000).** Besides that, **Zhang *et al.* (2008)** reported a significantly or moderately increased level of urea in diabetic rats and this relationship suggests enhanced amino acids-fueled gluconeogenesis in diabetic rats that lead to increased nitrogen load to the liver where urea is formed.

On the contrary, there was no significant change found in serum urea, creatinine and uric acid levels in diabetic rats post-treated with MSC.s in a combination with vitamin D after six weeks of treatment as compared to non-diabetic rats. Meanwhile, MSC.s migrated to injured tissue might act by paracrine effects and/or differentiation. MSC.s have been proved to integrate into damaged tubules and differentiate into renal epithelial cells in cases of cisplatin and glycerol induced acute kidney injury **(Morigi *et al.*, 2004).** However, other studies showed protection from injury by exogenous MSCs with little or no tubular incorporation. This discrepancy may be explained in

part by different analytical methods and injury models **(Broekema *et al*., 2005).**

In addition, the represented data which obtained from diabetic rats post-treated with M.S. Cs in a combination with vitamin D showed a very high significant decrease in serum urea and uric acid levels when compared with the corresponding values of diabetic-untreated rats. From the previous findings demonstrated in the present study, it could be suggested that, intravenously injected MSC.s have migrated to renal glomeruli and tubules and improvement the function of renal injury. The role of exogenous BM-MSC.s might be also attributed to production of substances that stimulate renal stem cells. This was consistent with other researchers who suggested that exogenous MSC.s paracrine activity may stimulate the endogenous renal stem cell population, leading to cellular recovery and renal injury repair **(Bussolati *et al*., 2008).**

Beside MSC.s, the results suggested that the action of vitamin D as reno-protective effect in diabetic nephropathy may be due to the role of vitamin D receptors (VDR), and VDR-mediated **(Zhang *et al*., 2008).** However, another experimental study, proposed that, vitamin D/VDR signaling in podocytes (marker gene expression) played a critical role in the protection of the kidney from diabetic injury and reduce albuminuria in rats with D.N **(Wang *et al*., 2012).**

The results are in accordance with the study of **Sadek *et al*. (2013)** assumed that administration of BM-MSC.s resulted in improvement of renal injury, both morphologically and functionally. Although studies have reported that vitamin D (cholecalciferol) decreases albuminuria **(Molina *et al*., 2013).**

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الملخص العربي

تقييم وظائف الكبد والكلى في الجرذان المصابة بالسكرى المحدث بواسطة الإستربتوزوتوسينوالمعالجه بالخلايا الجذعية المشتقة من النخاع العظمي و فيتامين د: دراسة كيميائية حيوية

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المقدمة: السكرىمرض مزمن وغير معد ويصيب جميع الأشخاص بمختلف الأعمار وتزداد معدلات الإصابة بتقدم العمر وهو مرض شائع يؤثر على معظم سكان العالم وتمتلك مصر نسبة كبيره من المرض على طول الإحصائيات المعده،وهو عبارة عن ارتفاع معدل السكر الطبيعي في الدم ،نتيجة لنقص نسبي أو كامل في الأنسولين أو لخلل في تأثير الأنسولين على الأنسجة , مما ينتج عنه مضاعفات مزمنة في أعضاء مختلفة من الجسم.
وفى الاونة الاخيرة .. وفيما اطلق عليه ثورة فى علاج مرض السكر،تم الاعلان عن علاج جديد من شأنه القضاء على المرض وهو الخلايا الجزعية إلى جانب دور فيتامين د وعلاقتة بمرض السكرى .
الهدف من الدراسه: أجريت هذه الدراسة لتقييم الأضرار الناجمة عن مرض السكرى كما تهدف أيضاً هذه الدراسة إلي إلقاء الضوء علي دور للخلايا الجذعيه وفيتامين د ضد التغيرات البيوكيميائية في دمذكور الجرذان البيضاء المصابه بمرض السكرى المحدث بواسطة الإستربتوزوتوسين على مدار 4 و6 أسابيع من العلاج.
المواد والطرق المستخدمه: الدراسة الحالية، استخدمت عدد48 من ذكور الجرذان البيضاء وزنها 150-180جم تم تقسيم الفئران إلى أربع مجموعات. (تحتوي على عدد 6جرذ في كل مجموعة) وصممت على النحو التالي:
1- **المجموعة الأولى:** (مجموعة التحكم)، المجموعة الضابطة: تم تجريع الجرذان على ما يعادل 1مل / كجممره واحده من المحلول الملحى الفسيولوجي (0.9٪ كلوريد الصوديوم) عن طريق الفم.
2- **المجموعة الثانية:** (مجموعة السكر)، المجموعة الضابطة حقنت جرذان هذه المجموعة بمادة الإستربيتوزيتوسينداخل التجويف البريتوني مره واحده (50 ملجم / كجم من وزن الجسم)طول فترة التجربة.
3- **المجموعة الثالثه:** مجموعة (الخلايا الجذعيه), تم حقن الجرذان المصابه بمرض السكرى فى هذه المجموعة بالخلايا الجذعيه بجرعه مليون خليه/ 0.5 مل مره واحده لكل جرذ من جرذان المجموعه على طول مدة التجربه.
4- **المجموعة الرابعه:** مجموعة (الخلايا الجذعيه وفيتامين د), حقنت الجرذان المصابه بمرض السكرى فى هذه المجموعة بالخلايا الجزعيه كما هو الحال في المجموعة الثالثه وقد تم إعطاء فيتامين د بجرعه 1جم / كجم من وزن الجسم عن طريق الفم يوم بعد يوم طوال فترة التجربة.
النتائج: ارتفاع كلا من إنزيمات الكبد ودلالات الكلى في مجموعة السكربنسبه كبيرة على عكس الحال في في المجموعات المعامله بالخلايا الجذعيه على حده أو مع فيتامين دبالموازنة مع المجموعة الضابطة. في حين أظهرت نتائج القياسات الدمويه تحسنا ملحوظاً في كلا من تلك القيم السابقه في المجموعات المعامله بالموازنه معمجموعه السكرى.
الخلاصه: بناءعلى ماتوصلت إليهنتائجالدراسةالحالية،فقد أظهرتالنتائجأنالعلاج بالخلايا الجذعيه على حده أو مع فيتامين د له القدره على علاجمرضى السكرى بنسبه كبيره حيث تعمل الخلايا الجذعيه على إصلاح العضو التالف علاوة على تحسين وظائف خلايا البنكرياس لإنتاج الإنسولين وحرق الجلوكوز.

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