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RESEARCH ARTICLE

ASSESSMENT OF THE POTENTIAL AMELIORATING EFFECTS OF BM-MSCsOR INSULIN ON THE ALTERED METABOLIC STATUS OF PANCREAS, LIVER AND KIDNEYIN STZ-DIABETIC RATS.

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Abstract

The current study was designed to investigate the probable hypoglycemic, hypolipidemic and hepato-renal protective effects of bone marrow derived mesenchymal stem cells (BM-MSCs) in comparison with insulin treatment in diabetic rats. Animals were classified into 4 groups; control group, diabetic group (D) received a single IP STZ dose (45 mg/kg b.w), D + insulin (0.75 IU/100 gm bw,SC daily for 4 weeks) group and D + BM-MSCs (single IV dose of 10^6 cell/rat). Herein, both insulin and BM-MSCs administration significantly improved the hyperglycemic status resulting fro m diabetes induction, as evidenced by lowered blood glucose, HbA1c and AGEs levels, while enhanced serum insulin, C-peptide and HO-1 levels, compared to the diabetic group. Regarding lipid metabolism, the increased levels of lipid fractions (TL, TG, TC and LDL-C) and the reduced HDL-C level in diabetic rats were reverted back to near normal values as a consequence to BM-MSCs treatment; indicating further, its hypolipidemic effect; in addition to enhancing the protein metabolism in diabetic rats. Furthermore, BM-MSCs was found to has hepato-renal protective effects via improving liver functions in diabetic rats; confirmed by decreased serum AST, ALT, ALP and γ-GT activities associated with decreased total bilirubin but increased total protein and albumin levels; and improving kidney status; indicated by the decreased serum levels of creatinine, uric acid and urea; compared to the diabetic group. Current findings clearly point out the health benefits of BM-MSCs; more than insulin; in ameliorating various metabolic disorders and hepato-renal diabetic complications.

Introduction:-

Recent times have witnessed large-scale human dramatic progress in diabetes mellitus (DM) patients count; making it a huge problem and a major health concern across the globe, particularly in the Middle East. It accounts for the 5% of all deaths around the world annually **(Alsairafi*et al*., 2018).** Approximately 400 million patients worldwide suffer from DM and number is rapidly increasing **(Lu *et al*., 2017).**

Type 1 DM (T1DM) is a heterogeneous chronic metabolic disorder due to irregularities in glucose metabolism, as a result of insulin dysregulation that characterized by blood glucose levels elevation with carbohydrate, lipid and

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protein metabolic abnormalities; accompanied with various serious long-term complications **(Chen *et al*., 2017)**. It is insulin-dependent resulting from insulin-secreting pancreatic β cells autoimmune destruction **(Monfrinia*et al*., 2017 and Subramanian and Hirsch, 2018).** The absence of insulin is life-threatening and generally lethal unless treated with daily exogenous insulin injections by SC route; which is crucial for metabolic optimization **(Thakkar *et al*., 2017).**

Lifelong exogenous insulin treatment is still the gold standard and the primary treatment of for these diabetic patients to replaces missing hormone in order to alleviate the symptoms, and nor diet neither exercise can prevent or reverse this type **(Xv *et al*., 2017).** However, exogenous injected insulin cannot adequately mimic β cell function sometimes resulting in hypoglycemia, ketosis and coma, that leads to mortality and severe diabetic complications development; such as retinopathy, hepatopathy, nephropathy, neuropathy and multiple cardiovascular problems **(Roche *et al*., 2017).**

With no current cure, this disease management focusing now on limiting complications via optimizing blood glucose control **(Dewar and Heubergerb, 2017).** Because in T1DM patients, β cells majority are lost by an autoimmune attack; injection of insulin only focuses in reversing hyperglycemia, not to increase β cells count **(Lu *et al*., 2017)**. However, blood glucose optimal control alone could not prevent complications; therefore, to overcome diabetes, the best strategy might be through β cell mass replenish**;** which promote essentiality using an alternative treatment approaches **(Mohan and Nandhakumar, 2014).** New β cells generation is an important target in T1DM treatment **(Amer *et al*., 2018);** thus, stem cells usage development could be the ideal choice for this disease therapy **(Thakkar *et al*., 2017).**

Mesenchymal stem cells (MSCs) found nearly in all tissues; with differentiation capability into various different cell types; holding notable promise in repairing of tissues in a cell replacement manner **(Qi *et al*., 2017).** In the area of regenerative medicine, researchers have revealed the BM-MSCs reprogramming potency to become functional insulin producing cells (IPCs), hence, normalizing hyperglycemia in streptozotocin (STZ)-induced diabetic rats **(Zang *et al*., 2017).** MSC-related research had demonstrated exciting tissue repair and glycemic control therapeutic effects both *in vitro* and *in vivo***(Davies *et al*., 2016).** A plenty of evidence illustrated that MSCs can delay T1DM onset and relieve hyperglycemia via improving pancreatic β cell regeneration, differentiating into IPCs, ameliorating insulin resistance, increase insulin production and promoting the conversion of α cells to β **(Zang et al., 2017)**, thus preventing a lot of long-term complications, improving life quality and minimizing immunosuppression-related side effects.

Taken together, T1DM is a potential candidate disease that may benefit from stem cell replacement protocols**.** Hence, in this article, we discuss BM-MSCs as an alternative cell source for DM treatment by alleviation and suppression of experimentally-induced diabetic complications; owing to its trans-differentiating capacity into IPCs; and to observe their probable hypoglycemic and hypolipidemic capacities.

Materials And Methods:

Chemicals:

STZ was purchased from MP Biomedicals Company. (Bp 50067, Lllkrich, France). While, Insulinagypt containing insulin (100 IU/ml) was produced and supplied by Medical Union Pharmaceuticals Company, Egypt.

BM-MSCs preparation:

Isolated BM-MSCs were obtained from 6–8-week-old rats (the femurs and tibias) and suspended in DMEM media (contain streptomycin/penicillin as an antibiotic and 10% fetal bovine serum); in an atmospheric state of 5% carbon dioxide. Then, morphological characterization was carried out using an inverted microscope to confirm the BM-MSCs identity **(Hamza *et al*., 2016)**.

BM-MSCs flow cytometric characterization:

BM-MSCs flow cytometric analysis was performed to verify retaining of their phenotype following their expanding in the cell culture. $CD44^+$, $CD45^+$ and $CD90^+$ antibodies were placed against their surface markers: 30 min for the $CD45^+$ antibody and for 4 min for the $CD44^+$ and $CD90^+$ antibodies, all at -20 C; before flow cytometry was assessed **(Hamza *et al*., 2016).**

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Animals:
100-120 g *Rattus rattus* male albino rats were obtained from the National Research Center, Dokki, Giza, Egypt. They housed in plastic cages and were maintained under conventional laboratory conditions throughout the study, maintained at 22°C under 12 h light/12 h darkness photoperiod. Rats were fed standard pellet chow and water *ad libitum*. After acclimatization for one week, rats were divided into 4 groups each of 6 animals. All experimental procedures were approved by the Ethics Committee in the Faculty of Science, Mansoura University, Mansoura, Egypt.

Induction of diabetes:
Overnight fasting rats were injected with a single IP dose of freshly prepared STZ solution (45 mg/kg bw) dissolved in citrate buffer, pH 4.6. Two days after, diabetes was confirmed by examining blood glucose level using ACCU–CHEKGo apparatus (Roche Company, Germany**).** Rats with fasting blood glucose level over 200 mg/dl are considered as diabetic **(Koroglu*et al*., 2015).**

Experimental design
1. **Control group:** Received IP single dose of citrate buffer (pH 4.6).
2. **Diabetic (D) untreated group:** Received IP single dose of STZ (45 mg/kg bw) dissolved in citrate buffer (pH 4.6).
3. **Diabetic insulin-treated group:** Diabetic rats received SC insulin injection dose (0.75 IU/100 g bw) **(Abdel-Razek, 2010),** once daily for 4 weeks**.**
4. **Diabetic BM-MSCs-treated group:** Diabetic rats received IV single dose of BM-MSCs ($1x10^6$ cell/rat) **(Hamza *et al*., 2016)**.

Samples collection
At the end of the experimentation period (4 weeks), overnight fasted rats were anesthetized using diethyl ether before being dissected and blood samples were immediately withdrawn directly from the heart. Only few droplets of blood samples were placed in clean heparinized tubes for measuring glycosylated hemoglobin. In clean non-heparinized centrifuge tubes, the remaining of blood samples were collected and let to stand for 15 min, after which they were centrifuged at 3000 rpm for 15 min. Blood sera were carefully separated, labeled and kept at -20 °C for subsequent biochemical analysis. On the other hand, pancreas specimens were quickly separated and an appropriate part was weighed and homogenized forming 10% (w/v) homogenate in distilled water, labeled and kept at -20 °C for subsequent biochemical examinations, while the remnant part labeled and kept at -80 °C for subsequent flowcytometric analysis.

Biochemical determinations
Fasting serum glucose and serum HDL-C concentration were estimated using SPINREACT diagnostics kit, Spain. Meanwhile, HbA1c, AGEs and HO-1were estimated by using kits obtained from Teco Diagnostics, USA. Serum insulin was measured by ELISA kit purchased from Boehringer Mannheim, Germany, using Boehringer Analyzer ES 300; while C-peptide measurement occurred by enzyme immunoassay (EIA) kit purchased from Bio Vision, USA. Serum lipids (TL, TG, TC, LDL-C and HDL-C) and total proteins were estimated using kits from Biodiagnostic Company, Egypt; while serum albumin level was estimated using Diamond Company kit, Egypt. Using kits from ELITech Company, France, serum AST, ALT and γ-GT activities were estimated**,** while serum ALP activity and total bilirubin, creatinine, urea and uric acid contents were detected using kits from SPINREACT diagnostics kit, Spain.

Statistical analysis
Obtained data were statistically evaluated with SPSS 17.5 software. *P* values equal or less than 0.05 were considered the minimal level of significance. All the results were expressed as the mean ± SE for six animals in each group. Percentage of change in the treated groups was calculated.

Results:-
Table 1 illustrate that diabetic group showed a significant increase in serum glucose, HbA1c and AGEs while showed a marked decline in insulin, C-peptide and HO-1 levels when compared to normal control one. The results revealed that treatment of diabetic rats with either insulin or BM-MSCs showed significant amelioration in all tested parameters; except insignificant increase in C-peptide in case of insulin treatment; when compared to the diabetic group. While non-significant changes compared to control group were observed except for C-peptide and HO-1

levels in diabetic rats treated with insulin which were still significantly lower than control. There were no remarkable changes between the results of insulin and BM-MSCs treatments of diabetic rats except for C-peptide and HO-1 which showed a marked enhancement with BM-MSCs than insulin treatment.

The data summarized in **table 2** showed a significant increase in all lipid profile parameters in diabetic group; except for HDL-C which showed a marked decrease when compared to normal control one. On the other hand, diabetic rats treated with either insulin or BM-MSCs showed a significant decrease in serum TL, TG, TC and LDL-C levels with a marked elevation in HDL-C level when compared to the diabetic group. While serum TG and TC levels in case of insulin treatment still significantly higher when compared to the control group. Diabetic rat's treatment with BM-MSCs obtained results displayed non-significant variations in the mentioned parameters compared to diabetic rats treated with insulin.

The represented results in **table 3** showed that significant decreases of total proteins, albumin and globulins were seen in diabetic group when compared to normal control one. However, diabetic insulin or BM-MSCs treated groups showed significant increases in all tested parameters levels when compared to the diabetic group. Non-significant changes were shown in the above tested parameters in case of diabetic rats treated with BM-MSCs when compared to the control group; in contrast to diabetic rats treated with insulin results which were still significantly lower. No detectable changes were recorded in both serum TP and albumin levels between the two diabetic rats-treated groups; while a marked enhancement was shown in serum globulins level in BM-MSCs treated group comparing to insulin-treated group.

Results of **table 4** demonstrated that diabetic rats showed significant increases in kidney function markers when compared to normal control one. Meanwhile, diabetic rats treated with either insulin or BM-MSCs showed significant decreases in all tested parameters when compared to the diabetic group, although values of urea and uric acid levels in insulin treated group were still significantly higher when compared to normal control group. A non-significant change was detected in creatinine level while a significant decrease in urea and uric acid levels in case of BM-MSCs treated group in comparing to insulin treated group.

Results illustrated in **table 5** showed that regarding to diabetic group, significant increases were obtained in liver function markers when compared to normal control one. In contrary, both diabetic treated groups showed significant decline in all tested parameters when compared to the diabetic group. However. values of ALT, γ-GT and total bilirubin still significantly higher in comparing to normal control group. The results reveal a non-significant change in AST, ALP, γ-GT and total bilirubin values between both diabetic treated groups, while ALT level in BM-MSCs treated rats was significantly lower compared to insulin treated rats.

Discussion:-

DM is a global health care issue; with various life-threatening complications; resulting from marked uncontrolled hyperglycemia **(Chien*et al*., 2015),** as a consequence of β-cell insufficiency leading to impairment insulin production or function which highlighting the exogenous insulin need **(Kaliraiet *al*., 2017).** Because of theirself-renewal ability, stem cell therapy holds great promise for the damaged tissues and organs repair; making them a candidate for use in regenerative medicine as one of the most promising DM therapies **(El Barky *et al*., 2018).**

1. Carbohydrate and glycemic control

The results of the present study showed that a single injection of 45 mg/kg bw of STZ to the rats caused a significant increase in fasting blood glucose (FBG) and blood HbA1c levels as well as in serum advanced glycation end products (AGEs) in contrast to significant decrease in serum insulin and C-peptide levels as well as serum heme oxygenase-1 (HO-1) as compared to the control group. Similarly, participants with diabetes presented with significantly higher FBG and HbA1c, and lower insulin and C-peptide when compared with control subjects **(Lin*et al*., 2017 and Amer *et al*., 2018).** These results are, also, in accordance with the findings of **Mayyas*et al*. (2018)**and **Nia *et al*. (2018).**

In addition to provoking hyperglycemia, **Jaen *et al*. (2017)** cleared that in diabetic dogs there was total or near total loss of insulin secretion, as number of cells producing insulin were reduced. The experiment done by **Ghosh *et al*. (2015)**suggested DNA alkylatoin as the major cause for β-cell death induced by STZ resulting in significant insulin decrease and marked elevation in serum glucose level.Such suggestion was in harmony with the study of **Adam *et al*. (2016)** who observed that deviations in control of glucose level, due to STZ injection, is sufficient to trigger an

array of maladaptive processes including decreased serum insulin level with huge signs of pancreatic destruction and glucose transporter-4 (GLUT-4) depletion in the pancreas of diabetic rats compared to control group. However, C-peptide is excised from proinsulin to generate biologically active insulin; it is used to assess endogenous insulin secretion, as its decline is indicative for the insulin production decrease and DM progression **(Chen *et al*., 2017).**

HbA1c and AGEs elevated levels; in the present study; in STZ-induced diabetic rats are in accordance with the results revealed a significant HbA1c elevated levels with a marked decrease in the total Hb, which may be attributed to the higher blood glucose levels and its impaired utilization; as HbA1c is produced in a non-enzymatic glycation manner when Hb exposed to excess glucose, serving as an average blood glucose levels marker; and the rate of glycation is proportional to the blood glucose concentration **(Muruganathan *et al*., 2017 and Mayyas*et al*., 2018).**

It was clear from **Arcaro *et al*. (2014)** and **Hamza *et al*. (2016)** data that extended hyperglycemia causes blood and tissue AGEs accumulation in diabetic rats**;** which may be due to increased ROS production; having a pivotal role in the long-term diabetic complications development**.** In hyperglycemia, oxidative stress increased production leads to tissues increase of AGEs and their receptors (RAGE) formation and deposition; inducing NFκB activation and IL increased production **(Miyata and Dan, 2012).** However, there was a significant decrease in the serum HO-1activity; a rate-limiting enzyme catalyzes heme breakdown yielding cytoprotective products including carbon monoxide, ferritin and bilirubin; in the diabetic group relative to control **(Zhang *et al*., 2017 and Hamza *et al*., 2016).**

On the other hand; in the current study; marked hypoglycemic effects were shown by either insulin or BM-MSCs treatments in STZ-diabetic rats. Also, their administration raised serum insulin and C-peptide levels as well as HO-1 levels, while significantly lowered blood glucose, HbA1c and AGEs levels to reach nearly normal control values in comparison with diabetic rats. The results, herein, are in harmony with the findings devoted that insulin treatment clearly reversed serum glucose and HbA1c to normal levels in STZ-treated mice **(Raccah, 2017)**, in dogs **(Jaen *et al*., 2017)** and in diabetic patients **(Katz*et al*., 2018);** causing recurrent hypoglycemia**.** It was known that, insulin lowers the increased blood glucose level by increasing glycolysis and glucose uptake by insulin-sensitive peripheral tissues like muscle, liver and fat cells; inhibits glycogenolysis and gluconeogenesis in the liver; and inhibits lipolysis in adipose tissue **(Jaen *et al*., 2017).** Furthermore, insulin injection could directly affects glucose metabolism in STZ-diabetic rats via glucokinase and pyruvate kinase gene expressions up-regulation; the key rate-limiting enzymes mediating glucose oxidation and ATP generation **(Jamshidi*et al*., 2018).**

There is no doubt that BM-MSCs have therapeutic effects on diabetes; as they are able to stimulate damaged pancreatic β-cells regeneration and becoming an alternative β cell source after induction; making them an ideal choice for DM treatment**. Wang *et al*. (2014) and Sood*et al*.(2015)** proved that BM-MSCs treatment resulted in HbA1c levels and insulin requirement reductions in diabetic rats, and in 9 out of 11 diabetic patients **Bhansali *et al*.(2014)**. Hypoglycemic therapeutic potential of MSC transplantation may be a direct effect of their intrinsic regenerative capacity and differentiation into IPCs, insulin release in a glucose-dependent manner, improving diabetic symptoms in T1DM animal (less likely) and preserve residual β-cell mass; or an indirect effect of immunomodulators secretion, thus, arresting autoimmune T cells from inducing destruction to the pancreatic β-cell **(Monfrinia*et al*., 2017 and Thakkar *et al*., 2017).** This is confirmed, also, by **Zang *et al*. (2017)** whoreported thatMSCs exert beneficial effects on glycemic control as insulin requirementsdecreased by 50%; by ameliorating insulin resistance and restoring islet function; through promotion of islet cell regeneration, differentiation into IPCs, protection of endogenous islet cells and promote trans-differentiation of α cells into β cells followed by β cell mass restoration and dramatic hyperglycemia amelioration, in mice with STZ-induced T2DM**.**

Recently confirming this regard**, Amer *et a*l. (2018)** revealed that transplanted IPCs differentiated from Ad-MSCs in STZ-diabetic rats; showed marked pancreatic β cell markers expression, apparent islet cells regeneration and proliferation and significant increase in C-peptide with increased insulin secretion in glucose dependent manner. Interestingly, MSCs infusion during the early phase (7 days) could ameliorate pancreatic islets destruction, restore β-cell function, reduce insulin resistance and promote MSCs recruitment to the damaged tissues; whereas late phase infusion (21 days) merely ameliorated insulin resistance **(Si *et al*., 2017).** Meanwhile, FBG, insulin requirement and HbA1c levels were decreased while C-peptide level was increased, in diabetic rats after BM-MSCs therapy for 3 months **(Thakkar *et al*., 2016),** 6 months **(Liu *et al*., 2014)** or12 months **(Wang *et al*., 2015),** compared with the diabetic control therapy; due to prevent islet cell loss, elevated insulin secretion from existing β-cells and insulin biosynthesis marked increase**,** suggesting improvement in the islet β cellsnumber and/or function **(Li *et al*., 2016).**

Herein, the insignificant increase of serum C-peptide level in insulin treated group, in contrast to its huge significant increase in BM-MSCs treated group, clearly confirms the importance of BM-MSCs as a therapeutic adjunct for diabetes cure. The most probable explanationfor this was that MSCs rapid infusion could improve remnant β cell regeneration and proliferation,leading to an endogenous insulin secretionelevation; minimizing the exogenous insulin injection need.

2. Lipid profile

Insulin deficiency and dysregulated lipid metabolism are major causes of DM **(Chen, 2016).** For lipid metabolism, STZ diabetic rats in the present work showed a great disturbance in lipid profile as they exhibited significant increase in serum TL, TG and TC in addition to LDL-C levels while a significant decrease in serum HDL-C level was seen compared to the control group. However, progression of dyslipidemia was related to glycemic control **(Katz*et al*., 2018),** asincreased lipid levels with huge signs of pancreatic destruction were observed in diabetic subjects **(Adam *et al*., 2016).** These results are in agreement with those obtained by **Antony *et al*. (2017)** and **Lin*et al*. (2017)** who reported that, in STZ diabetic rats, the primary quantitative lipoprotein disturbance defects are glycation of apolipoproteins, elevated TG, VLDL-C and LDL-C levels with HDL-C decline, and elevated ability of LDL-C to oxidation**.**Nonetheless, LDL is more likely to be oxidized and glycated, while HDL undergoes increased catabolism**(Cariou *et al*., 2017).**

In fact, lipoidosis considered the primarily pathological change in DM complications, as some experts preferred that there might be more rewarding to approach it as "lipocentric" than "glucocentric".Dyslipidemia development is associated with insulin resistance; and once hyperglycemia is present, increased hepatic free fatty acid synthesis and influx, driven by concomitant glycemic control and insulin sensitivity loss, cause dyslipidemia to deteriorate further **(Li *et al*., 2012).**

Both inflammation and insulin signal pathways have key roles in deficiency of insulin and accumulation of fats **(Chen, 2016).** As a consequence of insulin deficiency; since insulin inhibits lipase hormone; serum fatty acids excess promotes its conversion into hepatic cholesterol and phospholipids, which along with formed hepatic TG excess, may be discharged into the blood in the form of lipoproteins. Unoxidized long-chain fatty acids over accumulation causes a 'spill over' of lipids into non-adipose tissues, like heart, muscle, liver as well as pancreatic-islets; promoting programmed cell-death (lipoapoptosis) and metabolically relevant cellular dysfunction (lipotoxicity) in these tissues **(Krijnen *et al*., 2009).** In addition, according to**Titchenell*et al*. (2017)**; during DM; insulin fails to suppress hepatic glucose production but promotes lipid synthesis leading to hyperglycemia and hypertriglyceridemia. Deviations in control of glucose level is sufficient to trigger an array of maladaptive processes including changes in the oxidation of free fatty acid **(Huynh *et al*., 2014).** In DM, liver exhibits abnormally high levels of triglyceride and cholesterol synthesis, resulting in marked dyslipidemia (**Giralt *et al*., 2018).**

On the other hand, in the present study, either insulin or BM-MSCs administration to diabetic rats greatly counteracted lipid profile as compared to diabetic group. T1DM Patients under good glycemic control often have a 'supernormal' lipid profile, and subcutaneous insulin administration could increase lipoprotein lipase activity leading to VLDL-C particles turnover **(Cariou *et al*., 2017).** These results agree also with those of **Jaen *et al*. (2017)** who stated that throughout the 8-year follow-up period, diabetic dogs treated with insulin showed normal serum levels of TL, TG and TC comparing to diabetic group, that reduce the risk for cardiac disease in diabetic subjects. In addition to its antiatherogenic action, HDL-C may also have an antidiabetic function, through its protective role for insulin resistance and DM incidence exacerbation **(Tabara*et al*., 2017).** Moreover, **Katz*et al*. (2018)** reported thatinsulin therapy dampened the increase in TL, TG, TC and LDL-C**.**

However, **Lin *et al*. (2013)** found that WT-MSC transplantations resulted in lipoprotein lipase upregulation and reduced fatty acid synthase mRNA levels in the adipose tissues of high-fat/sucrose diet-fed mice, indicating a marked hypolipidemic ability. Meanwhile, BM-MSCs at 8 weeks of treatment; in high fat diet- and STZ-diabetic mice; prevented excessive lipid accumulation and reversed insulin resistance **(Nagaishi *et al*., 2014).** Furthermore, the results of **Gao *et al*. (2014)** suggested that MSC could attenuate the diabetic adipocytes abnormal function, via up-regulation of GLUT4 expression and PI3K/AKT insulin signaling pathway associated with an increased MSCs-insulin-like growth factor-1 (IGF-1) secretion.

3. Protein metabolism

Evidence shows that DM metabolism disturbances not applies to glucose and lipid only but protein metabolism as well **(Gougeon, 2013).** Concerning proteins, the present study demonstrated that serum total proteins, serum albumin and globulins levels were significantly decreased in diabetic rats. These results are comparable with those of other studies illustrating that the marked changes in the level of total proteins reflect disorders in the synthesis and metabolism of proteins **(El- Kholy*et al*., 2011 and Manjushaa*et al*., 2012).**

Many reports had suggested a marked muscle and lean tissue strength loss with aging in DM **(Gougeon, 2013).** It could be observed that the average weight of diabetic rats, serum albumin, globulins and total protein levels decreased significantly after STZ injection, with respect to the control group **(Hamza *et al*., 2016and Antony *et al*., 2017).** The results also are in accordance with those of **Arya*et al*. (2012)** who reported that there were significant decreases in plasma protein, albumin and globulin levels in STZ-induced diabetic rats as a consequence to acute liver damage leading to the decrease of their synthesis in liver.

The reduced use of glucose as a source of energy and oxidative stress, besides liver metabolic abnormalities, could induce an energy depletion; resulting in substantial protein and weight loss **(Jamshidi*et al*., 2018).** However, the decrease in total proteins as well as albumin and globulin levels in serum of diabetic rats may be attributed to several reasons such as increased gluconeogenesis, decreased amino acid uptake, hepatic damage anddisruption and/or dissociation of polyribosomes from endoplasmic reticulum **(Singh and Kakkar, 2013).** Furthermore, it seems likely to suggest that insulin lack would decrease amino acids incorporation into proteins and/or may decrease protein synthesis; asinsulin deficiency *in vivo* is associated with enhanced protein breakdown, amino acids levels elevation and negative nitrogen balance in diabetic rats **(Ene*et al*., 2007).** According to **Hebert and Nair (2010)** protein metabolism is markedly altered during insulin deprivation, since there was a net protein loss; because of the great whole-body protein breakdown increase than protein synthesis.

Herein, diabetic rat's treatment with insulin or BM-MSCs cause a significant increase in serum total proteins, albumin and globulins in comparable to diabetic group. Insulin has a critical role in maintaining proteostasis, via regulation of protein degradation and synthesis as well as post-translational modifications at the tissue and the organism level **(James *et al*., 2017**). Insulin treatment clearly causes protein and weight gain, perhaps through controlling the elevated serum glucose level **(Herman*et al*., 2017).** Thus, we can assume that insulin injection and BM-MSCs administration in our study consequently leads to a marked elevation in serum insulin levels which help in restoration of appropriate protein metabolism balance.

4. Kidney functions

In DM, kidney is an important target organ, as hyperglycemia, activated inflammation and innate immunity are relevant factors in the microvascular diabetic complications development, as they can stimulate free radical's production and renal cells apoptosis induction leading to diabetic nephropathy (DN) **(Elmarakby *et al*., 2012).** Our results indicate that, in cases of diabetes induced by STZ injection, a marked increase in the serum urea, uric acid and creatinine levels have been noted.

The results of the present experiment coincide with those in the study conducted by **Hamza *et al*. (2016)** and **Antony *et al*., (2017)** in which they determined that high glucose levels and ROS leads to serious kidney damage that revealed significant increase in serum uric acid, urea and creatinine, in diabetic group with respect to the control group**.**According to **Feig *et al*. (2008),** a high concentration of uric acid has been associated with T2DM, as insulin resistance causes acidic urine; as uric acid stones tend to form in the low pH urine. One of the reasonable explanation for the DN is that hyperglycemia-induced apoptosis in various renal cell types especially to the glomerular podocyte (epithelial cells attached to the glomerular basement membrane), leading to albuminuria; which are DN critical early events (**Davey *et al*., 2014 and Jiang *et al*., 2016).** Another explanation is that HO-1 reduction can seriously results in DN; while its induction is protective in many chronic and acute renal insults; as renal HO-1 expression up-regulation could improve both renal and function vascular (**Pitlovanci*et al*. 2013)**. These findings supported those of **Elmarakby*et al*. (2012).** More recently,**Xie*et al*. (2018)** reported that chronic kidney disease (CKD) is characterized by glucose and insulin homeostasis disturbances, increases insulin resistance and; in advanced stages; results in β-cell dysfunction and defective insulin secretion, and this combination led to observation of a higher glucose intolerance prevalence. Although experimental evidence identifies urea as a putative culprit, higher urea level may suppress insulin secretion and increase insulin resistance**.** In this regard, **Thomas *et al*.**

(2015) and Koppe*et al*. (2016) suggest that insulin secretion defection in CKD advanced stages is mechanistically caused by increased serum urea level.

On the other hand, BM-MSCs contribute to cell turnover and repair in various tissue types, including kidneys. Because nephrons are of mesenchymal origin**,** MSCs are attractive candidates for renal repair**.** Diabetic rat's treatment with either insulin or BM-MSCs, herein, resulted in significant reduction in serum urea, uric acid and creatinine levels comparing to the diabetic control. In harmony with our findings, the study of **Hamza *et al*. (2016)** revealed that BM-MSCs therapy was found to have a positive effect on the kidney functions as it showed significant serum creatinine, urea and uric acid levels reduction; compared to the untreated groups**;** since treatment recovered the organizational structure of both pancreas and kidney, as demonstrated by the histopathological analysis**.** The result of systemic administration of MSCs after STZ-induction of DM indicated reversed hyperglycemia associated with pancreatic β-islets regeneration and kidney function improvement **(Zhou *et al*., 2016)**. **Li *et al*. (2014)** found that mice treated with MSCs exhibited reduced proteinuria and BUN and ameliorated renal pathological damage as MSCs migrated to the kidney, compared with glomerulonephritis mice**.** In consistent with these results, **Pitlovanci*et al*. (2013)** stated that treatment of diabetic rats with MSCs could maintain serum urea and creatinine near the normal levels suggesting their beneficial role in providing protection against DN; either directly or indirectly.

Immunomodulators expression modification was proposed as a possible mechanism for MSCs reno-protective effects. **Fang *et al*. (2012)** cleared that MSC implantation significantly reduce renal tissues damage, via oxidative stress and inflammatory response suppression. Similar results were obtained by **Wang *et al*. (2013)** who found that administration of BM-MSCs via the left renal artery of diabetic rats prevented albuminuria, creatinine clearance rate, urinary albumin to creatinine ratio development and preserve normal renal histology, suggesting a paracrine mode of action with MSCs exerting their beneficial effects by increasing of the podocyte survival factor BMP-7 expression. In this line, MSC treatment ameliorated DN; through inhibiting MCP-1expression, thus reducing macrophage infiltration and down-regulating TNF-α, Il-1B and IL-6 expressions in diabetic rat's renal tissue **(L*et al*., 2013)**. Furthermore**,** MSC can produce a large number of soluble factors (chemokines, cytokines and growth factors); in response to inflammatory mediators; capable of regulating inflammation and tissue remodeling; suggesting that the transient presence of MSCs within the injured kidneys may provide a paracrine support rather than a direct effect for the healing process and cell damage repair **(Jiang *et al*., 2016** and**Sordia*et al*., 2017)**.

These results support the suggestions that BM-MSCs may home to injured glomerular endothelium, differentiate into endothelial cells and participate in glomerular microvasculature regeneration **(Rookmaaker*et al*., 2007)**. However, BM-MSCs contribute to the renal tubular cell regenerating, via its capability in accelerating tubular proliferation, improve renal function and repair renal injury as well as HO-1 expression up-regulation; which has been shown, in animal models, to exert a renal function protective effect **(Yener*et al*., 2008).** These findings are in good agreement with our results.

5. Liver functions

There is a particularity of liver diseases during diabetes, including hepatitis C virus infection, cirrhosis, and metabolic steatosis **(Petit, 2017).** Diabetes is a metabolic disease which leads to significant increase in free radicals prompting liver diseases development via fibrogenesis, inflammatory response and hepatocyte apoptosis induction **(Ghosh *et al*., 2015).** Liver histopathological evaluation showed the high blood glucose level deleterious effects in the forms of fatty degeneration and microvascular steatosis **(Jamshidi*et al*., 2018).**

The data obtained by the present study showed that serum AST, ALT, ALP, γ-GT activities as well as total bilirubin level obviously increased in diabetic rats compared with normal control. These results are compatible with the findings obtained by **Ramesh *et al*. (2012); Sirasanagandla*et al*. (2013)** and **Xie*et al*. (2014)** who foundthese enzymes leakage into the blood stream from the liver cytosol; indicating hepatocytes damage due to increased oxidative stress, liver dysfunction, enzymes biosynthesis disturbance with liver membrane permeability alteration.This is confirmedby **Antony *et al*., (2017)** and **Muruganathan *et al*. (2017)** who stated that serum AST, ALT and γ-GT activities increased in STZ-diabetic rats in response to marked hepatic abnormalities.Furthermore, severe hepatic damage in the STZ groups was noticed through serum AST, ALT and ALP fluctuating levels; which result from these mitochondrial, cytosolic, and extracellular enzymes leakages to the blood stream **(Jamshidi*et al*., 2018).**

In accordance with **El-Sharaky*et al*. (2007)** results, diabetes was found to increase the serum total bilirubin perhaps due to the decreased liver uptake and/or conjugation or increased bilirubin production due to accelerated RBCs hemolysis indicating presence of both hematological and liver problems. The excessive production of free radical in diabetes may be another explanation, since the clearance of serum bilirubin was associated with free radical production **(Oche*et al*., 2014).** Lastly, plasma bilirubin level elevation may be attributed to periportal necrosis confirming liver damage incidence **(Newairy*et al*., 2009).**

On the other hand, different liver diseases may involve different nutritional management, glycemic monitoring, and the use of antidiabetic therapies **(Petit, 2017)**. BM-MSCs therapy can be mediated by enhancing endogenous hepatocyte regenerative processes, which can improve liver function in advanced chronic liver disease **(Kumar *et al*., 2011).** The data, herein, showed that diabetic rats treatment with either insulin or BM-MSCs resulted in a significant amelioration in the serum enzyme levels and this improvement demonstrated the protective effect of them on hepatocytes structure and function in diabetic rats.

Insulin-loaded nanoparticles decreased hepatic inflammations via suppressing the necro-inflammatory process in the diabetic liver histopathology, which is in line with the reduction of the liver enzymes activities (ALP, ALT, and AST); as liver degenerative changes marker **(Jamshidi*et al*., 2018). Ning *et al*. (2016)** hypothesized that insulin augments and protects the hepatocyte sensitivity to saturated fatty acids-induced lipotoxicity, based on the decline in LDH activity and caspase-3 expression, mechanistically through alleviating ER stress via a PI3K/Akt/p53 involved pathway, contributed to its hepatoprotective role.

Given their unique function in differentiation potential and self-renewal, MSCs might be used to regenerate damaged liver tissue. Recent studies have shown that MSCs-based therapies can improve liver function in a mouse model of hepatic failure **(Lee *et al*., 2018).** Similarly, assessment of serum parameters including AST, ALT and total bilirubin, at day 7 exhibited significant reduction, after BM-MSCs in an animal model of CCl_4-induced acute hepatic failure; compared with the control group (**Fathi-Kazerooni*et al*., 2017**). Additionally, **Ramanathan *et al*. (2017)** found that hepatic pathological changes were significantly restored in d-Galactosamine **i**nduced acute **l**iver **i**njury in mice that received WJ-MSCs; showed by the significant decline in AST, ALT, ALP and total bilirubin serum levels; compared to control; indicating hepatoprotective and probable regenerative property.

BM- MSCs was effective in rescuing experimental CCl_4-induced fulminant hepatic failure indirectly; via increasing hepatocyte survival and minimizing apoptosis. Such effects resulted from the secretion of large fractions of chemotactic cytokines or chemokines by MSCs **(Wang *et al*., 2017).** These findings are in consistence with those of **Christ *et al*. (2015)** who stated that MSCs, harbor pro-proliferative, immunomodulatory, anti-inflammatory and anti-apoptotic properties; which are desirable in liver diseases treatment. MSCs could release paracrine factors needed for functional liver restoration and both chronic and acute liver diseases treatment. Indeed, their pleiotropic actions include cell proliferation stimulation, immune reactions modulation and cell death responses attenuation; which are responsible for MSCs hepatocyte differentiation capacity.

In conclusion

Taken together, these studies indicated that MSCs therapy of diabetic metabolic abnormalities in addition to markedly hepato-renal dysfunction was superior to insulin treatment; which might have glycemic and metabolic control but was less effectively improve diabetic complications. BM-MSCs beneficial effects may involve individual or combinatorial effects of various protective processes, e.g., cells differentiation and regeneration, anti-inflammatory potency, immune modulation and protection capacity and control of hyperglycemia; however, the complete derivation of the exact mechanisms of action have yet to be elucidated.

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Table 1:-Serum glucose, insulin, C-peptide, HbA1c, AGEs and HO-1 levels.

puorG / retemaraP		Control	Diabetic (D)	D + Insulin	D + BM-MSCs
Glucose (mg/ 100 ml)	Mean ± SEM	92.83 ± 4.64	395.20 ± 19.76 [a]	115.00 ± 5.75 [b]	108.30 ± 5.41 [b]
	*		+ 325.72	+ 23.88	+ 16.66
	**			- 70,90	- 72.59
	***				- 5.82
Insulin (µ I U/ml)	Mean ± SEM	17.10 ±0.86	9.22 ±0.46 [a]	17.00 ±0.85 [b]	16.97 ±0.76 [b]
	*		- 46.08	- 0.58	- 0.76
	**			+84.38	+ 84.05
	***				- 0.17
C-peptide (ng/ml)	Mean ± SEM	0.85 ± 0.04	0.31 ± 0.02 [a]	0.37 ± 0.02 [a]	0.79 ± 0.04 [bc]
	*		- 63.52	- 56.47	- 7.05
	**			+ 19.35	+ 154.83
	***				+ 113.51
HbA1c (%)	Mean ± SEM	2.92 ± 0.15	4.94 ± 0.25 [a]	3.40 ± 0.17 [b]	3.10 ± 0.16 [b]
	*		+ 69.17	+ 16.43	+ 6.16
	**			- 31.17	- 37.24
	***				- 8.82
AGEs (AU/mg protein)	Mean ± SEM	2.86 ± 0.14	8.58 ± 0.43 [a]	3.48 ± 0.19 [b]	3.24 ± 0.16 [b]
	*		+ 200	+ 21.67	+ 13.28
	**			- 59.44	- 62.23
	***				- 6.89
HO-1 (P mol/mg)	Mean ± SEM	270.60 ± 13.53	72.00 ± 3.60 [a]	185.20 ± 9.26 [ab]	251.00 ± 12.55 [bc]
	*		- 73.39	- 31.55	- 7.24
	**			+ 157.22	+ 248.61
	***				+ 35.52

Values expressed as mean ± SEM (n = 6). **a, b** and **c** are Significant differences ($P \leq 0.05$) comparing to control, diabetic and diabetic insulin-treated groups respectively. ***. **** and ******* are % of change comparing to control, diabetic and diabetic insulin-treated groups respectively.

Table 2:-Serum TL, TG, TC, LDL-C and HDL-C levels.

puorG / retemaraP		Control	Diabetic (D)	D + Insulin	D +BM-MSCs
Serum TL (mg/dl)	Mean ± SE	258.20 ±12.91	544.00 ±27.20 [a]	316.80 ±15.99 [b]	278.75 ±13.94 [b]
	*		+110.68	+ 22.69	+ 7.95
	**			- 41.76	- 48.75

	***				- 12.01
Serum TG (mg/dl)	Mean ± SEM	59.16 ± 2.90	120.00 ±6.00 [a]	84.60 ±4.23 [ab]	70.00 ±3.50 [b]
	*		+ 102.83	+ 43.00	+ 18.32
	**			- 29.50	- 41.66
	***				- 17.25
Serum TC (mg/dl)	Mean ± SEM	78.00 ±3.89	150.20 ± 7.51[a]	101.00 ±5.15 [ab]	88.25 ±4.41 [b]
	*		+ 92.56	+ 29.48	+ 13.14
	**			- 32.75	- 41.24
	***				- 12.62
LDL-C (mg/dl)	Mean ± SEM	107.66 ± 0.45	144.86 ± 0.44 [a]	119.68 ± 0.66 [b]	110.65 ± 1.01 [b]
	*		+ 34.55	+ 11.16	+ 2.77
	**			- 17.38	- 23.61
	***				- 7.54
HDL-C (mg/dl)	Mean ± SEM	41.50 ± 2.07	18.66 ± 0.96 [a]	35.60 ± 1.78 [b]	36.40 ± 1.82 [b]
	*		- 55.03	- 14.21	- 12.28
	**			+ 90.78	+ 95.06
	***				+ 2.24

Values expressed as mean ± SEM (n = 6). **a, b** and **c** are Significant differences ($P \leq 0.05$) comparing to control, diabetic and diabetic insulin-treated groups respectively. ***. **** and ******* are % of change comparing to control, diabetic and diabetic insulin-treated groups respectively.

Table 3:-Serum total proteins, albumin and globulins levels.

puorG / retemaraP		Control	Diabetic (D)	D + Insulin	D + BM-MSCs
Serum total proteins (g/dl)	Mean ± SEM	7.25 ±0.36	3.58 ±0.18 [a]	6.20 ±0.31 [ab]	6.98 ±0.35 [b]
	*		- 50.62	- 14.48	- 37.24
	**			+ 73.18	+ 94.97
	***				+ 12.58
Albumin (g/dl)	Mean ± SEM	4.30 ±0.22	2.17 ±0.11 [a]	3.68 ±0.18 [ab]	3.96 ± 0.21 [b]
	*		- 49.53	- 14.41	- 7.90
	**			+ 69.58	+ 82.48
	***				+ 7.60
Globulins (g/dl)	Mean ± SEM	2.95 ±0.26	1.41 ±0.17 [a]	2.52 ±0.30 [ab]	3.02 ±0.34 [bc]
	*		- 52.20	- 14.57	+ 2.37
	**			+ 78.72	+ 114.18
	***				+ 19.84

Values expressed as mean ± SEM (n = 6). **a, b** and **c** are Significant differences ($P \leq 0.05$) comparing to control, diabetic and diabetic insulin-treated groups respectively. ***. **** and ******* are % of change comparing to control, diabetic and diabetic insulin-treated groups respectively.

Table 4:-Serum creatinine, urea and uric acid levels in different animal groups.

puorG / retemaraP		Control	Diabetic (D)	D + Insulin	D + BM-MSCs
Creatinine (mg/dl)	Mean ± SEM	0.46 ±0.02	1.43 ±0.07 [a]	0.52 ±0.03 [b]	0.49 ±0.02 [b]
	*		+ 201.86	+ 13.04	+ 6.52
	**			- 63.63	- 65.73
	***				- 5.76
Urea (mg/dl)	Mean ± SEM	20.60 ±1.03	76.75 ±3.84 [a]	40.00 ±2.00 [ab]	25.75 ±1.29 [bc]
	*		+ 272.57	+ 94.17	+ 25.00
	**			- 47.88	- 66.44
	***				- 35.62
Uric acid (mg/dl)	Mean ± SEM	1.46 ±0.07	3.10 ±1.16 [a]	2.15 ±0.11 [ab]	1.48 ±0.07 [bc]
	*		+ 112.32	+ 47.26	+ 1.36
	**			- 30.64	- 52.25
	***				- 31.16

Values expressed as mean ± SEM (n = 6). **a, b** and **c** are Significant differences ($P \leq 0.05$) comparing to control, diabetic and diabetic insulin-treated groups respectively ***. **** and ******* are % of change comparing to control, diabetic and diabetic insulin-treated groups respectively.

Table 5:-Serum AST, ALT, ALP and γ-GT activities and total bilirubin level.

puorG / retemaraP		Control	Diabetic (D)	D + Insulin	D + BM-MSCs
AST (u/l)	Mean ± SEM	58.80 ±2.94	113.60 ±5.68 [a]	75.00 ±3.75 [b]	70.67 ±3.53 [b]
	*		+ 93.19	+ 27.55	+ 20.18
	**			- 33.97	- 37.79
	***				- 5.77
ALT (u/l)	Mean ± SEM	32.00 ±1.60	67.80 ±3.39 [a]	52.00 ±2.60 [ab]	35.50 ±1.78 [bc]
	*		+ 111.87	+ 62.50	+ 10.93
	**			- 23.30	- 47.64
	***				- 31.73
ALP (u/l)	Mean ± SEM	232.60 ±111.63	401.00 ±20,05 [a]	287.60 ±14.38 [b]	259.40 ±12.97 [b]
	*		+ 72.39	+ 23.64	+ 11.52
	**			- 28.27	- 35.31
	***				- 9.80
γ-GT (u/l)	Mean ± SEM	32.00 ±1.60	80.60 ±4.03 [a]	44.20 ±2.21 [ab]	37.20 ±1.86 [b]
	*		+ 151.87	+ 38.12	+ 16.25
	**			- 45.16	- 53.84
	***				- 15.83

Bilirubin (mg/dl)	**Mean ± SEM**	0.59 ±0.03	2.09 ±0.04 [a]	0.73 ±0.04 [ab]	0.64 ±0.03 [b]
	*		+ 254.23	+ 23.72	+ 8.47
	**			- 65.07	- 69.37
	***				- 12.32

Values expressed as mean ± SEM (n = 6). **a, b** and **c** are Significant differences ($P \leq 0.05$) comparing to control, diabetic and diabetic insulin-treated groups respectively. ***. **** and ******* are % of change comparing to control, diabetic and diabetic insulin-treated groups respectively.