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Biochemical Evaluation of Bone Marrow Mesenchymal Stem Cells and GLP-1 Therapy as a New Approach for Type II Diabetes Treatment in Rat Model

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Abstract | Diabetes therapy discovery is a focus point for alleviating micro and macrovascular complications. Here, the current study conduct to investigate whether the efficacy of long-acting GLP-1 derivatives or Bone Marrow mesenchymal stem cells as a novel potential glucose-lowering therapy was comparable to metformin, the most often prescribed oral treatment for T2DM in male rats with STZ-induced T2DM.70 healthy male rats were divided into two groups one control (10 rats) fed with commercially available normal diet and other diabetic (60 rats) received high fat diet for 7 weeks then injected with STZ single dose (25mg/kg b.w/i.p) for T2DM induction. The diabetic one was allocated to 6 groups (10 each) based on their treatment: untreated group, metformin (Metformin HCl, 500mg/kg b.w/day), GLP-1 (Trulicity,1.5mg/kg b.w/week), BM stem cells (IV injected with $1x10^6$ units/once), GLP-1 plus metformin and the last group BM stem cells plus metformin for 4 weeks. After 4 weeks, rats were sacrified, blood samples are collected to measure FBG, FINS, C-peptide, and HOMA IR. TNF, IL-6 beside Kidney and liver function parameters were also measured. Liver and kidney tissues examinated for histopathological and Immunohistochemistry. Untreated diabetic group showed significant (P≤0.05) hyperglycemia, hyperinsulinemia, and elevation in inflammatory cytokines with hepatic and kidney tissues disruption. At the same time, treatment alleviated hyperglycemia and IR with a significant (P≤0.05) decrease in inflammatory biomarkers levels and tissue expressions, especially GLP-1 and Stem cell groups combined with metformin. The study concluded that whether BM stem cells or GLP-1 able to decline hyperglycemia and inflammatory conditions associated with diabetes and have additive or even synergistic effects with metformin in alleviating T2DM complications.

Keywords: BM stem cells, GLP-1, Type II diabetes, Inflammation

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INTRODUCTION

DM is likely one of the oldest diseases humans have encountered. Egyptian manuscripts were the first to document it approximately three thousand years ago (Ahmed, 2002). Although the cause and aetiology of diabetes vary significantly, they always involve problems in insulin synthesis or response at some time during the disease. T1DM

is immune-mediated or idiopathic, whereas T2DM (formerly known as non-insulin-dependent DM) is the most frequent type of DM (Maitra and Abbas, 2005).

In 1988, T2DM was initially identified as a component of metabolic syndrome, defined by chronic hyperglycemia and insulin resistance, as well as varying degrees of impairment in the metabolism of carbohydrates, lipids, and proteins (Patlak, 2002). Most instances of T2DM are identified either due to complications or, unintentionally, due to a heightened susceptibility to extensive artery atherosclerosis, often linked to hypertension, hyperlipidemia, and obesity. The principal causes of death for most persons with T2DM are cardiovascular illness and end-stage renal disease (Pittas, 2009).

The most often recommended biguanide medication for treating hyperglycemia in people with T2DM is metformin. Furthermore, it has been documented that the addition of synergistic medication improves numerous markers of diabetes complication risk with acceptable glycaemic control by metformin alone. (Forst, 2012). Metformin is expected to exert these potent effects largely by counteracting insulin resistance, which in turn reduces hepatic glucose production and increases insulin-stimulated glucose absorption in muscle and fat. Insulin resistance and decreased insulin secretion are two primary abnormalities in type 2 diabetes. Considering that the use of metformin in conjunction with insulin secretagogues and even with rigorous insulin treatment is advised (Nathan *et al*., 2009).

Incretins are peptides that originate from the intestines and are released in reaction to meals, particularly following the absorption and availability of nutrients in the intestinal lumen. Gonadotropin-like peptide-1 (GLP-1) is the principal incretin. Upon food intake, the L-cells of the intestinal epithelium release GLP-1, a 30 amino acid peptide, due to the proteolytic breakdown of proglucagon, the most potent insulinotropic hormone (Holst, 1994). Multiple studies have demonstrated that administering GLP1 can significantly reduce elevated blood sugar levels in diabetic animals. Following GLP1 therapy, animal models demonstrated beta cell regeneration, proliferation, and neogenesis, enhancing and restoring beta cell mass (Miao *et al*., 2013).

Based on the finding that GLP-1 can enhance glucose-dependent insulin production from pancreatic β cells, GLP-1 based medicines have recently been authorised as additional therapy alternatives for patients with type 2 diabetes (Marso, 2016) GLP-1 has additional extra-pancreatic actions in addition to reducing glucose like inhibition of glucagon secretion in islet cells, reduction of gastric emptying, and subsequent decrease in absorption of insulinotropic nutrients (Drucker *et al*., 2017).

Mesenchymal stem cells (MSCs) are well recognized as highly important multipotent adult stem cells. They are evaluated based on their capacity to secrete various factors, modulate their local environment, activate endogenous progenitor cells, and differentiate into any cell in damaged tissues (Stanekzai *et al*., 2012). MSCs primarily function by secreting soluble factors during the antigen-presenting and succeeding T-cell-activation stages (Bartholomew *et al*., 2002). The latest progress in regenerative medicine, particularly stem cell therapy, has postulated several innovative and promising treatments for DM (Hao and Ram, 2014). MSCs assist in restoring damaged pancreatic islet cells to a nearly normal state. These cells also have beneficial therapeutic effects in T2DM, such as lowering the amount of insulin required daily, reducing the number of medications required, improving the level of glycosylated haemoglobin (HbA1C), and lowering blood glucose levels (El-Badawy and El-Badri, 2016).

Consequently, the current investigation will investigate the possible role of GLP-1 and Mesenchymal stem cells in treating STZ-induced T2DM in rats in comparison with metformin.

MATERIAL AND METHODS

Animals

The current investigation was conducted on 70 healthy male rats weighing 110-120g, procured from the Laboratory Animal House at the Faculty of Veterinary Medicine (FVM) at Suez Canal University (SCU).

They were housed in the FVM's SCU Animal House. The animals were kept in separate metal inclosures with controlled climatic and nutritional conditions, including a temperature of 20-24°C and a relative humidity of 55-60%. They were maintained on a conventional balanced ratio for two weeks (Tae-Yoal *et al*., 1995; Brichard *et al*., 1996). The animals were granted unrestricted access to sustenance and water. The animal study was approved by the Ethics Committee, FVM's SCU, Egypt. The rats were handled in accordance with the guide for the care and use of laboratory animals prepared by the Ethics Committee. Then, the rats were divided into two groups based on their dietary regimens. During the first seven weeks, the control group (10 rats) received a conventional balanced ration, while the remaining 60 rats received high fat diet (HFD) (58% fat, 25% protein, and 17% carbohydrate, respectively), (Reed *et al*., 2000). The composition and preparation of HFD are similar to those disclosed by Srinivasan *et al*. (2005).

Drugs and Chemicals

Steptozotocin (STZ): U.S.-based Sigma-Aldrich Company offered STZ in a granular form. It possesses a

purity level above 99%. The solution must be dissolved in a recently made sodium citrate buffer with a pH of 4.5.

Metformin: Cidophage, a specialty firm in the pharmaceutical and chemical sectors, provides tablets containing 500mg of metformin hydrochloride, a biguanide derivative. It is freely soluble in water. It underwent dissolution in distilled water prior to its application.

Dulaglutide (GLP-1 Agonist): Dulaglutide was obtained from Trulicity, Eli Lilly (Company Pharmaceuticals, United States). A GLP-1 receptor agonist is a 0.5mL solution within each pre-filled injectable pen containing 1.5mg of the drug.

Bone marrow-derived mesenchymal stem cells (BM-derived MSCs): Stem cell proliferation occurred in the stem cell facility in the Biochemistry Department of the Faculty of Medicine at SCU.

Experimental Design

The rats were randomly assigned to six groups, each containing ten rodents.

Group (1): Functioned as a negative control group and was provided with a standard, balanced diet for the 12-week experimental period.

Group 2: Was administered HFD for seven weeks, followed by STZ injections (25 mg/kg b.w/i.p), and served as the positive control untreated diabetic group for the 12-week experimental period.

Group 3: Was administered HFD for seven weeks, followed by STZ injections (25mg/kg b.w./i.p) and Metformin treatment (Metformin HCl 500 mg/kg b.w./day) for 4 weeks.

Group 4: Underwent a 7-week HFD, followed by an injection of STZ (25mg/kg b.w/i.p.) and treatment with Trulicity (1.5mg/kg BW/week) for 4 weeks.

Group 5: Subjects were administered HFD for seven weeks, followed by an injection of STZ (25mg/kg b.w/i.p.) and treatment with BM-derived MSCs (IV/one million units/once).

Group 6: Underwent a 7-week HFD, followed by an injection of STZ (25 mg/kg b.w./i.p.) and treatment with Metformin (Metformin HCl 500mg / kg b.w/day) plus Trulicity (1.5mg/kg BW/week) for 4 weeks.

Group 7: Rats were administered HFD for seven weeks, injected by STZ (25mg/kg b.w/i.p.) followed by an intravenous injection of one million units of BM-derived MSCs and Metformin HCl (500mg / kg b.w.) for 4 weeks Figure 1.

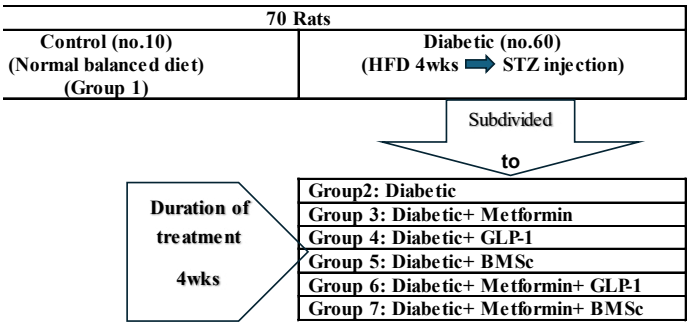

Figure 1: Simple illustration of experimental design.

Induction of T2DM

A modest single dose of STZ (25 mg/kg b.w.) was administered intraperitoneally (ip) to 60 rats that had been on HFD for 7 weeks (Latt *et al*., 2013; Srinivasan *et al*., 2005). STZ was dissolved in a cold citrate buffer (pH 4.4) at 15mg /mL concentration. Before the injection, all animals were required to fast overnight. Citrate buffer was administered intraperitoneally to 10 rats fed a standard balanced diet (0.2 mL citrate buffer/rat/i.p.).

Rats exhibiting elevated fasting blood glucose (FBG) levels (>11.1 mmol / l, 200 mg / dl) were selected for the following interventions. FBG levels were tested one week after STZ injection with a glucometer (Accu-Chek Metre, Roche Diagnostics GmbH, Germany).

Drug Administration

Body weight was measured weekly for all groups for drug dose adjustment.

- Metformin was administered orally to fasting rodents via distilled water daily (Lauro *et al*., 2012).
- Trulicity was injected s/c once weekly (Tuttle *et al*., 2017).
- BM-derived MSCs (BMSCs): After being processed and cultured for 14 days as previously described (Mageed et. al., 2007), BM-derived MSCs were administered intravenously in a rat tail vein in a single dose (1×10^6 cells/mL) dispersed in 1 mL of phosphate buffer saline (PBS) (Ngoc *et al*., 2011).

Biochemical Parameters Estimation

Determination of FBG, fasting blood insulin (FINS) and serum C-peptide: FBG, measured by UV test, the enzymatic reference method with hexokinase according to Tietz (2006). The estimation of FINS is conducted using The ALPCO Rat Insulin ELISA Kits, Catalogue Number: 80-INSRT-E01, E10, as described by Finlay and Dillard (2007). The quantification of C-Peptide is performed using the Abnova C-Peptide ELISA Kit, Catalogue Number KA1259, as described by Bonger and Garcia (1984).

HOMA-IR Calculation: The HOMA-IR is calculated by multiplying the fasting insulin level (FINS) by the fasting blood glucose (FBG) ratio and dividing the quotient by the constant 22.5. HOMA-IR is calculated as (FINS X FBG)/22.5 (Wallace *et al*., 2004).

Determination of serum lipid profile: Total cholesterol (TC), Triglycerides (TG), and High-density lipoprotein-cholesterol (HDL-c) levels were measured following the methods described by Richmond (1973), Allain *et al.* (1974) and Lopez-Virella *et al.* (1977).

Calculation of Serum Low-Density Lipoprotein-Cholesterol (LDL-c)

LDL- cholesterol was calculated using a formula (Friedewald *et al*., 1972).

$$\text{LDL} - \text{c}\left(\frac{\text{mg}}{\text{dl}}\right) = \text{Total cholesterol} - \{(\text{triglycerides} \div 5) - \text{HDL} - \text{c}\}$$

Determination of Kidney and Liver Function Tests

The quantification of serum creatinine and urea concentrations, together with the measurement of alanine aminotransferase (ALT / SGPT) and Aspartate aminotransferase (AST / SGOT) activities, is conducted using the kinetic colorimetric assay protocols developed by Jaffé *et al.* in (1886), Rock *et al.* (1987) and Breuer (1996).

Immunological Parameters Estimation

Interleukin-6 (IL-6) and Tumor Necrosis Factor-alpha (TNF-α) determination: The quantitative sandwich enzyme immunoassay technique is implemented in this assay, as per Feldmann and Maini (2001). Quantikine® ELISA Rat TNF-α, Catalog Number: RTA00, was employed following Taga and Kishimoto, (1997). The BD™ ELISA for rat IL-6, Catalog Number: R6000B, and Immunoassay, Canada, respectively.

Histopathology Examination for Liver, Kidney and Pancreas Tissue

The liver, kidney, and pancreas tissue samples were dissected correctly and cleaned with normal saline. The specimens were directly immersed in a fixative and processed for histopathological and immunohistochemical procedures. The appropriate fixative was 10% paraformaldehyde for about 24-48 hours. The tissue specimens were then dehydrated in ethanol in progressive grades, cleaned in xylene, and embedded in paraffin wax. Then, sectioning at 4-6 μm thickness and mounting were completed. The Hematoxylin and Eosin (HandE) stain was utilized. (Bancroft, 2008). Stained section were examined via using Olympus (Bx-53) at 40 x.

Immunohistochemistry for TGF-β and NF-Kb in Liver and Kidney tissues

Utilizing monoclonal antibodies (Abcam, Anti-TGF-β1 antibody [EPR21143], Abcam, Anti-NF-kB p65 antibody [E379]) following the methodologies outlined by Ming-Xian *et al.* (2012); Gulubova *et al.* (2013). Paraffin sections were mounted on positively charged slided by using avidinbiotin peroxidase complex (ABC) method. Sections from each group were incubated with previous antibodies , then the reagents required for ABC method were added (Vectastain ABC -HRP Kit, Vector Labortatories). Marker expression was labeled with perioxidase and colored with diaminobenzidine (DAB,produced by Sigma) to detect antigen -antibody complex .negative controls were included using non immune serum in place of the primary or secondary antibodies. IHC examined via using Olympus microscope (BX-53).

Statistical Analysis

It was performed using SPSS 17.0 software. The mean±SEM was employed to depict data that follows a normal distribution. Following a one-way analysis of variance (ANOVA), a Bonferroni post hoc tukey multiple comparison tests was conducted. The threshold for statistical significance was set at $P < 0.001$.

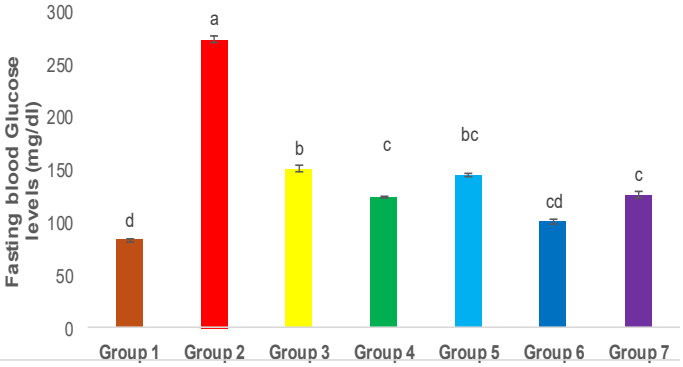

Figure 2: FBG levels in different treated groups, Means bars for all groups with error bars (± SE) for glucose (mg/dL), The P≤0.05 indicates that the means with varying superscripts are significantly distinct. Group 1: Control, Group 2: Diabetic, Group3: Diabetic + Metformin, Group 4: Diabetic + GLP1, Group 5: Diabetic + BMSCs, Group 6: Diabetic + Metformin + GLP1, Group 7: Diabetic + Metformin + BMSCs.

RESULTS AND DISCUSSION

Effect of Metformin, GLP-1 and BMSCs and Their Coadministration on FBG and FINS Levels Among all Treated Groups

Figure 2 and 3, The diabetic group (group 2) had significantly higher FBG and FINS levels ($P < 0.05$) compared to the control and treated groups. Compared to diabetics, all

treatment groups showed significant decreased FBG and FINS levels (P < 0.05). Glucose levels in Groups (4,6,7) outperformed the metformin group (group 3) and BMSCs group (group 5).

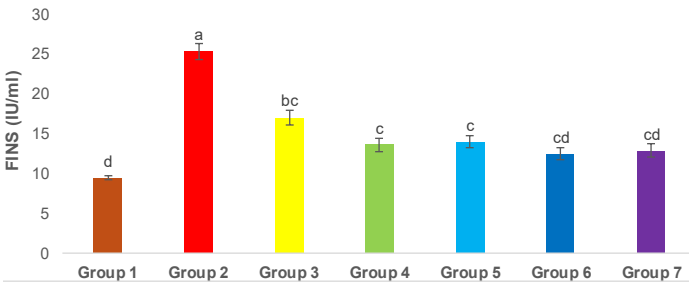

Figure 3: FINS levels in different treated groups. Means bars for all groups with error bars (± SE) for insulin (IU/ml) The P≤0.05 indicates that the means with varying superscripts are significantly distinct. Group 1: Control, Group 2: Diabetic, Group3: Diabetic + Metformin, Group 4: Diabetic + GLP1, Group 5: Diabetic + BMSCs, Group 6: Diabetic + Metformin + GLP1, Group 7: Diabetic + Metformin + BMSCs.

Effect of Metformin, GLP-1 and BMSCs and Their Coadministration on HOMA IR and C-Peptide Level Among all Treated Groups

Figure 4 and 5, HOMA IR indicates significant insulin resistance (P≤0.05) in Diabetic groups while significantly (P≤0.05) improved in all treated groups, especially groups (4,6,7), as well as results related to C-peptide levels, all treated groups have significant (P≤0.05) reduction in level of C-peptide rather than diabetic one.

Effect of Metformin, GLP-1 and BMSCs and Their Coadministration on Lipid Profile Among all Treated Groups

Substantial elevation (P≤0.05) in serum TG, TC, and LDL-c levels, together with a substantial drop in HDL-c levels (Table 1**)** was observed in the lipid profile of the diabetic group. Conversely, all treated groups showed significant improvement in lipid profile, particularly groups (4,5,6,7), with a notable increase in HDL-C.

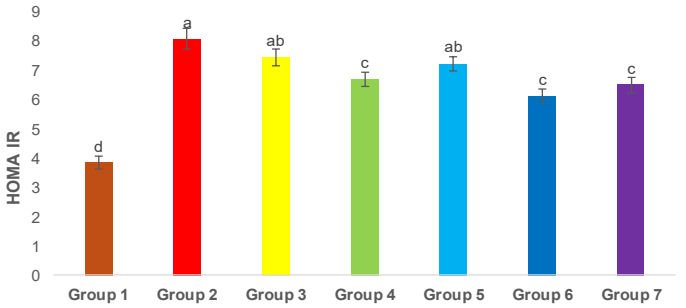

Figure 4: HOMA -IR in different treated groups. Means bars for all groups with error bars (± SE) for calculated HOMA-IR. The P≤0.05 indicates that the means with varying superscripts are significantly distinct. Group 1: Control, Group 2: Diabetic, Group3: Diabetic + Metformin, Group 4: Diabetic + GLP1, Group 5: Diabetic + BMSCs, Group 6: Diabetic + Metformin + GLP1, Group 7: Diabetic + Metformin + BMSCs.

Effect of Metformin, GLP-1 and BMSCs and Their Coadministration on Kidney and Liver Function Among all Treated Groups

Significantly higher mean value of ALT and AST activity and increased blood creatinine and urea levels were seen in the diabetic group with insulin resistance (P ≤ 0.05). The treated groups exhibited a further enhancement in their previous parameters compared to the diabetic group (Table 2).

Effect of Metformin, GLP-1 and BMSCs and Their Coadministration on Immunological Parameters Among Different Treated Groups

In Table 3, TNF-α and IL-6 levels in diabetic groups weresignificantly elevated (P≤0.05) if compared with control one. Treated groups rather than metformin groups showed the best improvement in these immunological parameters.

Histological Changes in Pancreas, Liver and Kidney by (HandE) Stain and Immunohistochemistry Expression of TGF-β and NF-Kb

Microscopic examination of pancreatic islets in diabetic rats clarified reduction of its cellular components than normal when compared to the control group, the exocrine

Table 1: Effect of Metformin, GLP-1 and BMSCs and their coadministration on lipid profile among all treated groups.

Groups/ Measurement	Group1	Group2	Group3	Group4	Group5	Group6	Group7
Triglycerides (mg/dL)	$54.0^{d} \pm 3.58$	$229.0^{a} \pm 2.25$	$137.0^{b} \pm 0.57$	$120.9^{c} \pm 1.15$	$132.7^{b} \pm 0.53$	$115.0^{cd} \pm 1.23$	$125.4^{bc} \pm 1.04$
Total Cholesterol (mg/dL)	$56.5^{d} \pm 0.85$	$148^{a} \pm 2.30$	$123.1^{b} \pm 1.62$	$105.1^{bc} \pm 1.03$	$106^{bc} \pm 11.89$	$98.9^{c} \pm 0.69$	$111.6^{bc} \pm 1.34$
HDL-C (mg / dL)	$44.8^{a} \pm 0.65$	$19.6^{d} \pm 0.30$	$30.3^{c} \pm 0.37$	$38.7^{b} \pm 0.47$	$34.2^{bc} \pm 0.49$	$41.7^{b} \pm 0.50$	$37.2^{bc} \pm 0.55$
LDL-C (mg / dL)	$28.8^{d} \pm 1.92$	$82.6^{a} \pm 2.61$	$68.4^{b} \pm 0.65$	$53.0^{c} \pm 0.83$	$59.6^{bc} \pm 0.56$	$46.6^{cd} \pm 0.43$	$56.2^{bc} \pm 0.80$

Data are expressed as M±SE, The P≤0.05 indicates that the means with varying superscripts are significantly distinct. Group 1: Control, Group 2: Diabetic, Group3: Diabetic + Metformin, Group 4: Diabetic + GLP1, Group 5: Diabetic + BMSCs, Group 6: Diabetic + Metformin + GLP1, Group 7: Diabetic + Metformin + BMSCs.

Table 2: Effect of Metformin, GLP-1 and BMSCs and their coadministration on kidney and liver function among different treated groups.

Groups/ Measurement	Group1	Group2	Group3	Group4	Group5	Group6	Group7
Creatinine (mg/dL)	$0.67^{d} \pm 0.03$	$1.94^{a} \pm 0.09$	$1.40^{b} \pm 0.03$	$1.28^{bc} \pm 0.02$	$1.14^{c} \pm 0.02$	$0.99^{cd} \pm 0.02$	$0.88^{cd} \pm 0.03$
Urea (mg / dL)	$23.75^{d} \pm 0.50$	$65.60^{a} \pm 1.00$	$46.41^{b} \pm 0.87$	$43.09^{bc} \pm 0.70$	$38.63^{c} \pm 0.35$	$36.84^{c} \pm 0.32$	$33.80^{cd} \pm 0.47$
ALT (U / L)	$69.67^{d} \pm 1.05$	$106.57^{a} \pm 2.95$	$89.75^{b} \pm 0.62$	$86.89^{bc} \pm 0.48$	$83.22^{bc} \pm 0.46$	$80.70^{c} \pm 0.45$	$76.78^{cd} \pm 0.62$
AST (U / L)	$90.5^{d} \pm 2.53$	$163.4^{a} \pm 2.19$	$134.5^{b} \pm 1.20$	$131.0^{b} \pm 1.08$	$125.4^{bc} \pm 0.99$	$127.7^{bc} \pm 1.01$	$117.0^{c} \pm 0.62$

Data are expressed as M±SE, The P≤0.05 indicates that the means with varying superscripts are significantly distinct. Group 1: Control, Group 2: Diabetic, Group3: Diabetic + Metformin, Group 4: Diabetic + GLP1, Group 5: Diabetic + BMSCs, Group 6: Diabetic + Metformin + GLP1, Group 7: Diabetic + Metformin + BMSCs.

Table 3: Effect of Metformin, GLP-1 and BMSCs and their coadministration on immunological parameters among all treated groups.

Groups/ Measurement	Group1	Group2	Group3	Group4	Group5	Group6	Group7
TNF-α (pg/mL)	$23.13^{d} \pm 0.70$	$56.7^{a} \pm 0.33$	$37.48^{b} \pm 0.51$	$30.77^{c} \pm 0.30$	$29.49^{cd} \pm 0.20$	$30.74^{c} \pm 0.35$	$28.30^{cd} \pm 0.09$
IL – 6 (pg/mL)	$60.67^{d} \pm 1.76$	$149.60^{a} \pm 3.56$	$120.00^{b} \pm 1.70$	$108.00^{bc} \pm 1.05$	$96.00^{c} \pm 1.41$	$102.00^{bc} \pm 1.18$	$90.00^{cd} \pm 1.73$

Data are expressed as M±SE, The P≤0.05 indicates that the means with varying superscripts are significantly distinct. Group 1: Control , Group 2: Diabetic,Group3: Diabetic + Metformin, Group 4: Diabetic + GLP1, Group 5: Diabetic + BMSCs, Group 6: Diabetic + Metformin + GLP1, Group 7: Diabetic + Metformin + BMSCs.

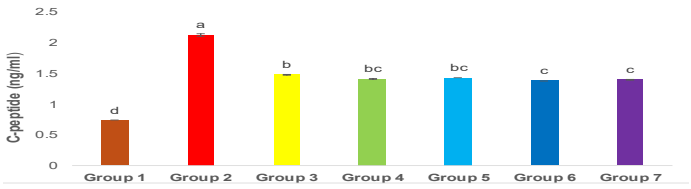

Figure 5: C-Peptide levels in different treated groups. Means bars for all groups with error bars (± SE) for C-Peptide (ng/ml). The P≤0.05 indicates that the means with varying superscripts are significantly distinct. Group 1: Control, Group 2: Diabetic, Group3: Diabetic + Metformin, Group 4: Diabetic + GLP1, Group 5: Diabetic + BMSCs, Group 6: Diabetic + Metformin + GLP1, Group 7: Diabetic + Metformin + BMSCs.

pancreatic tissue had dilated ducts, localised necrosis, and perivascular cellular infiltration. Groups 3, 4, 5, and 6 that were treated showed that some acini had necrotic alterations in beta cells along with vacuolar degeneration.Group 7 (stem cell plus metformin) demonstrated beta cell and pancreatic acini structural regeneration Figure 6.

In diabetic rats, the hepatic tissue displayed necrobiotic alterations, hepatocyte congestion and inflammation, and portal fibrosis with fatty liver and enlargement symptoms. Group 7 had normal histological structure of the portal region and hepatocytes, while treatment groups 3, 4, 5, and 6 showed signs of moderate portal fibrosis, newly created bile ductules, and infiltration by a small number of mononuclear inflammatory cells Figure 7.

Diabetic rats exhibited increasing in urinary space of renal glomeruli and presence of necrobiotic changes in renal tubules. Some renal tubules in treated groups (3,4,5,6), had nuclear pyknosis and necrobiotic changes while Group 7 the structure of renal glomerulai and tubules were normal Figure 8.

Immunohistochemistry expression of TGF-β and NF-K β in hepatic and renal tissue as seen in (Figure 9,10,11 and 12) as diabetic groups and treated group with metformin and GLP-1(group 2,3,4) had strong sever expression of TGF-β and NF-K β in this tissues while groups 5,6,7 shown moderate to negative expression of TGF-β and NF-K β.

T2DM arises from a complex interplay of genetic, environmental, and lifestyle elements. These variables collectively contribute to IR and β-cell malfunction, resulting in elevated blood sugar levels (Trinh *et al*., 2021). Consistently, the diabetic group exhibited a notable increase in FBG levels compared to the normal rats. Chronic consumption of HFD contributes to IR development, followed by compensatory hyperinsulinemia (Kraegen *et al*., 1986). Due to their high secretary activity, β-cells are continuously subjected to two types of stress: glucolipotoxicity and oxidative stress. (DeFronzo, 2004). This leads to the demise of β-cells, a common feature of T2DM marked by elevated insulin and glucose levels (Rhodes, 2005). The diabetic rats also showed a notable rise in the HOMA-IR index and intolerance to glycolipids. Collectively, our findings validate the accurate initiation of diabetes in accordance with documented literature (Abdel-Latif *et al*., 2018).

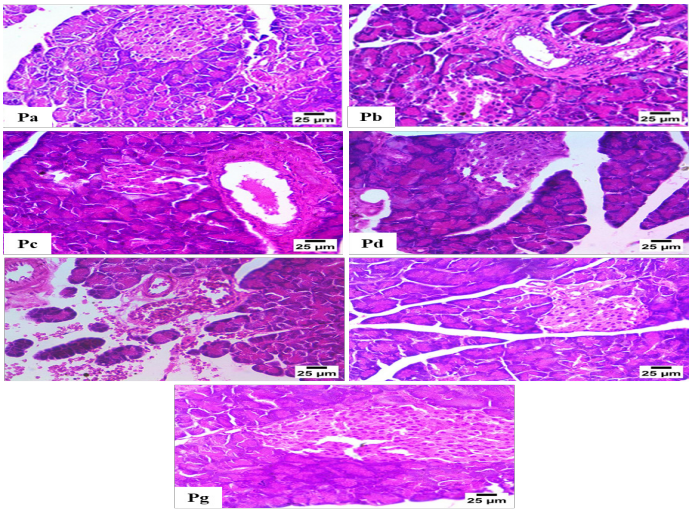

Figure 6: Histopathological Examination for Pancreas (H and E); Figure Pa: normal structure of pancreatic acini and few beta cells (Group 1). Figure Pb: pre-acinar fibrosis with infiltration by many mononuclear inflammatory cells, presence of nuclear pyknosis, and decreasing number of beta cells (Group 2). Figure Pc: pre-acinar fibrosis with infiltration (Group 3). Figure Pd: necrobiotic changes in some beta cells, including vacuolar degeneration and pyknotic nuclei. (Group 4). Figure Pe: pancreatic hemorrhage between acini and presence of necrobiotic changes in some acini (Group 5). Figure Pf: Some beta cells' nuclear pyknosis and vacuolar degeneration (Group 6). Figure Pg: normal histological structure of pancreatic acini and beta cells (Group 7).

Palmer *et al*. (2004) identified that T2DM is linked to IR and usually exhibits either normal or increased levels of C-peptide. This observation aligns with our preliminary results, which are more apparent in the group with diabetes than in the control group. Furthermore, C-peptide readings have been used to indicate the activity of pancreatic β-cells.

Dyslipidemia, characterized by elevated serum TG, TC, and LDL and decreased HDL levels in the blood, is a consequence of the protracted hyperglycemia observed in diabetic rats. These results were confirmed by additional research conducted by (Veneti and Tziomalos, 2020). The insulin-inhibited hormone-sensitive lipase is the main factor responsible for the excessively high levels of serum lipids in DM, perhaps because it enhances the release of free fatty acids from the peripheral fat reserves. This could result from either an increase in the absorption of TGs or a decrease in their peripheral uptake (Magalhaes *et al*., 2019).

Diabetic nephropathy is identified biochemically and histologically in diabetic rodents in the current study. Elevated serum urea and creatinine levels, together with the biochemical findings of the kidney, are indicative of renal failure. Consistent with the ongoing enquiry, Abdel Aziz *et al*. (2014) found that elevated levels of creatinine and urea in the bloodstream after the injection of STZ are very sensitive and distinctive markers of renal damage.

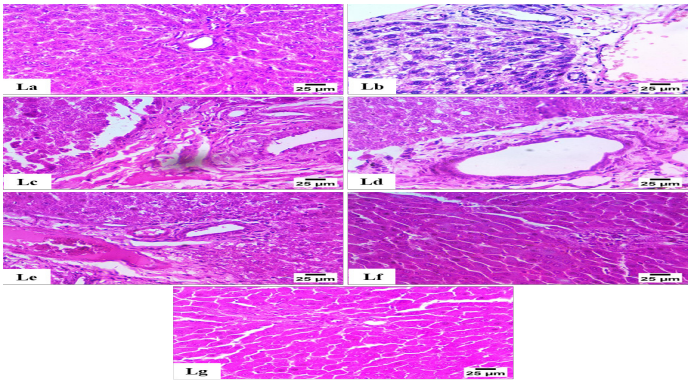

Figure 7: Histopathological Examination for Liver (H and E); Figure La: shows a normal histological structure of the portal area and hepatocytes (Group 1). Figure Lb: shows necrobiotic changes in hepatocytes with a few number of inflammatory cells between hepatocytes and the presence of portal fibrosis (Group 2). Figure Lc: shows portal fibrosis with newly formed bile ductules (Group 3). Figure Ld: shows portal fibrosis with infiltration by a small number of mononuclear inflammatory cells (Group 4). Figure Le: Demonstrating portal fibrosis characterized by the infiltration of a small number of mononuclear inflammatory cells and the accumulation of blood vessels in the portal region (Group 5). Figure Lf: shows mild portal fibrosis with infiltration by a small number of mononuclear inflammatory cells (Group 6). Figure Lg: show the normal histological structure of the portal area and hepatocytes (Group 7).

Metformin is the conventional primary therapy for lowering blood glucose levels in T2DM. It targets multiple pathways, such as reducing hepatic gluconeogenesis and intestinal glucose absorption, improving pancreatic β-cell activity, and increasing insulin sensitivity. Metformin also lowers lipogenesis in the liver, muscles, and adipocytes, which improves glucose consumption and GLP-1 release (Derosa *et al*., 2020). Figueiredo *et al*. (2020), reported that metformin affected hyperglycemia in rats by significantly decreasing serum insulin, glucose, TGs, TC, LDL, creatinine, and urea. This explains the result obtained in diabetic rats treated with metformin.

The present study found a significant ($P < 0.05$) improvement in FBG levels in treatment groups receiving GLP-1 alone or combined with Metformin. This improvement was greater than that observed in the diabetic rats (Group 2) than in the other groups. The GLP-1 agonist, when used alone, was able to alleviate IR and glucose intolerance. Additionally, the combined treatment enhanced the protective effects of GLP-1 agonists. Empirical evidence has shown that GLP-1 agonists enhance insulin production, mitigate resistance, and maintain glucose homeostasis (Abdel-Latif *et al*., 2018).

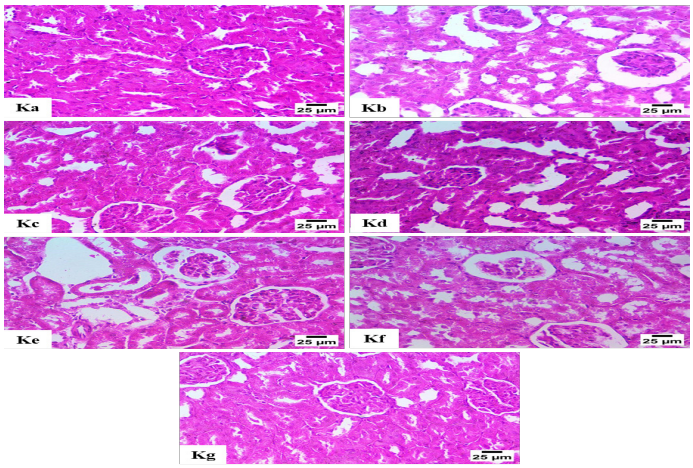

Figure 8: Histopathological Examination for Kidney (H and E); Figure Ka: Presents a typical histological arrangement of the glomeruli and renal tubules (Group 1), Figure Kb: shows an increased urinary space of renal glomeruli and the presence of necrobiotic changes in some renal tubules (Group 2), Figure Kc: shows some glomeruli collapsed, increase urinary space in glomeruli and presence of necrobiotic changes in some renal tubules (Group 3), Figure Kd: showing nuclear pyknosis in the epithelium of some renal tubules (Group 4). Figure Ke: shows marked degeneration in diverse glomeruli and the presence of nuclear pyknosis in the epithelium of some renal tubules (Group 5). Figure Kf: shows an increase in urinary space in renal glomeruli and necrobiotic changes in some renal tubules (Group 6). Figure Kg: shows a normal histological structure of glomeruli and renal tubules (Group 7).

In comparison to the diabetic control group, GLP-1 treatment alone or in combination with Metformin improved the HOMA-IR level as well as the lipid profile, as demonstrated by lower serum LDL and higher HDL levels in the blood. These findings were derived from the investigations conducted in this study. This study also discovered that GLP-1 was more effective than metformin in boosting serum HDL levels. The dyslipidemia significantly improved in diabetic rodents who were treated. GLP-1 inhibits intestinal chylomicrons' synthesis and diminishes hepatic lipoproteins' release (Drucker, 2016). Furthermore, it indirectly stimulates white adipose tissue, improves lipid clearance, and reduces triglycerides (Kooijman *et al*., 2015).

Results of the present work suggested that the urea and creatinine serum rates of diabetic rodents treated with GLP-1 decreased compared to those of the diabetic control group. These findings are consistent with the results of Leon-Jimenez *et al.* (2020), who indicated that the renal advantages of GLP-1 were primarily due to the preservation of glomerular filtration and a decrease in microalbuminuria. GLP-1 is a nephron-protective substance effective in treating T2DM patients (Kristensen *et al*., 2019).

Bone marrow-derived mesenchymal stem cells (BM-MSCs) are flexible stromal cells with the potential to treat diabetes effectively. However, the specific mechanism by which they work is still debatable (Dong *et al*., 2008).

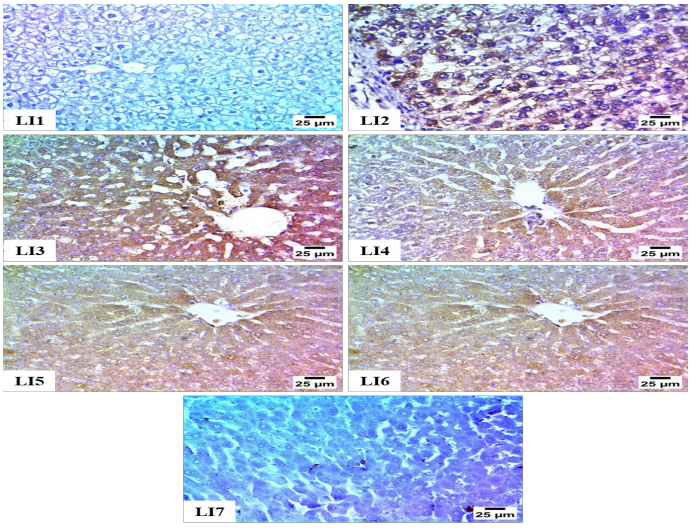

Figure 9: Immunohistochemistry for Hepatic Expression of TGF-β; Figure LI1. Negative expression for TGF-β in hepatocytes (Group 1). Figure LI2. Sever positive expression for TGF-β in the cytoplasm of hepatocytes (Group 2). Figure. LI3. Sever positive expression for TGF-β in the cytoplasm of hepatocytes (Group 3). Figure LI4. Moderate positive expression for TGF-β in the cytoplasm of hepatocytes (Group 4). Figure LI5. Moderate positive expression for TGF-β in the cytoplasm of hepatocytes (Group 5). Figure LI6. Moderate positive expression for TGF-β in the cytoplasm of hepatocytes (Group 6) Figure LI7. negative expression for TGF-β in hepatocytes (Group 7).

The principal objective of the current study was to experimentally assess the efficacy of MSCs derived from BM in treating diabetes. This study demonstrated that rodents that were injected with STZ developed significant hyperglycemia. The STZ-treated rats improved significantly after receiving a single infusion of MSCs. These findings are consistent with previous studies (Abdel Aziz *et al*., 2008).

Compared to diabetic rodents, the treated group with MSCs substantially decreased FBG, FINS, and C-peptide levels. Compared to other groups, MSCs combined with Metformin resulted in significant ($P<0.05$) improvements in FBG, FINS, and C-peptide levels. Previous research has demonstrated that the infusion of MSCs in T2DM rodents alleviates IR, thereby ameliorating hyperglycemia (Tsatsoulis, 2018). The findings revealed that the early transplantation of MSCs has the potential to regulate the progression of T2DM at an early stage and delay its related complications.

Compared to diabetic rodents, the treatment with stem cells substantially reduced cholesterol, TGs, and LDH lev-

els and increased HDL. The capacity of MSCs to alleviate lipid metabolic dysfunction was reported by Hamza *et al.* (2018).

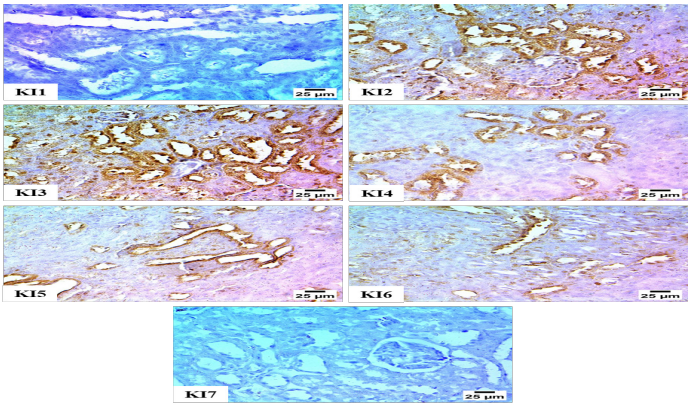

Figure 10: Immunohistochemistry for Renal Expression of TGF-β; Figure KI1: Negative expression for TGF-β in renal tubules (Group 1), Figure KI2: Sever +ve expression for TGF-β in cytoplasm of renal tubules (Group 2). Figure KI3. Severe +ve expression for TGF-β in the cytoplasm of renal tubules (Group 3). Figure KI4: Moderate +ve expression for TGF-β in renal tubular cytoplasm (Group 4). Figure KI5: Moderate +ve expression for TGF-β in renal tubular cytoplasm (Group 5). Figure KI6L: Moderate +ve expression for TGF-β in renal tubular cytoplasm (Group 6). Figure KI7: -ve expression for TGF-β in renal tubules (Group 7).

The present work determined that administering BM-MSCs to diabetic rodents substantially improved kidney function (urea and creatinine) compared to the diabetic group. This was also validated by (Mousa *et al.*, 2016), who found an improvement in kidney function after administering stem cells. This improvement may be attributed to the direct differentiation ability of MSCs, which causes the regeneration of injured tissue, or to the paracrine factors secreted by MSCs.

In the paradigm, the rats induced to ingest an HFD by STZ were accompanied by an increase in ALT / AST. The results of this work indicate that the use of MSCs in the first phases of T2DM can significantly decrease insulin/glucose levels and effectively treat lipometabolic problems and liver dysfunction.

The study found that the diabetic group had significantly higher serum TNF-α and IL-6 levels than the control group. The results of this work are in direct opposition to those of another study (English and Wood , 2013). Additionally, the rodents administered Metformin exhibited a statistically significant increase in serum TNF-α and IL-6 levels compared to the other treated groups. Experimental studies have indicated that either GLP-1 or MSCs inhibited the upregulation of TNF-α and IL-6 (Zhou *et al.*, 2014; Wang *et al.*, 2019b). The treatment with MSCs results in the secretion of hepatocyte growth factor and the downregulation of TNF-α and cytokine expression (Li *et al.*, 2018). Conversely, DM is an inflammatory condition that does not directly affect cells or organs. Instead, it activates the immune system, mainly through monocytes and macrophages, which release various pro-inflammatory cytokines, including TNFα (Fiske *et al.*, 2001).

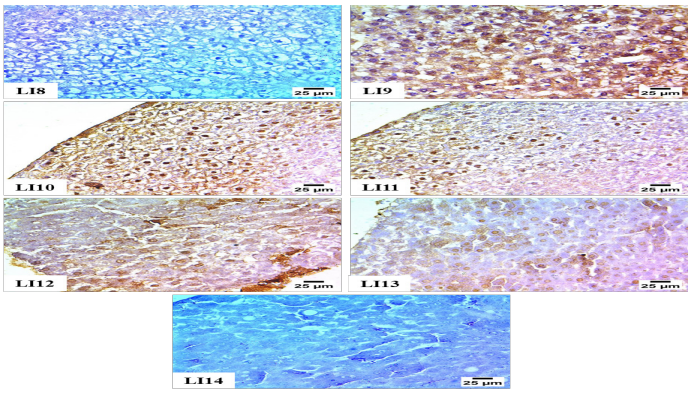

Figure 11: Immunohistochemistry for hepatic expression of NF-Kβ; Figure lI8: Negative expression for NF-Kβ in hepatocytes (Group 1), Figure lI9: sever +ve expression for NF-Kβ in nuclei and cytoplasm of hepatocytes (Group 2), Figure LI10: Sever +ve expression for NF-Kβ in nuclei and cytoplasm of hepatocytes (Group 3), Figure LI11: Sever +ve expression for NF-Kβ in nuclei of hepatocytes (Group 4). Figure LI12: Moderate +ve expression for NF-Kβ in nuclei of hepatocytes (Group 5). Figure LI13: Moderate +ve expression for NF-Kβ in nuclei of hepatocytes (Group 6), Figure I14: -ve expression for NF-Kβ in hepatocytes (Group 7).

Hyperglycemia, substantial alterations in glucose and lipid metabolism, and the activation of oxidative stress tremendously impact the development of problems associated with DM. This stress stimulates the upregulation of NF-κB, which then triggers the release of inflammatory cytokines like TNF-α and IL-6, along with cellular death (Ilyas *et al.*, 2017). This explanation explains the obtained result in immunohistochemistry expression of NF-κB hepatic and renal tissue, which was modulated in the treated groups, particularly those treated with stem cells.

Yener *et al.* (2007) found that hyperglycemia and hyperinsulinemia dramatically boost TGF-β expression via different pathways. TGF-β substantially advances glomerulosclerosis and interstitial fibrosis in end-stage renal disease, leading to diabetic nephropathy in mice (Gomes, *et al.*, 2014). According to an increasing body of evidence, growth factors may influence the development and progression of fibrosis in diabetic nephropathy. Particularly noteworthy are the functions of TGF-β in diabetic nephropathy (Antonello, 2012).

Here, renal TGF-β was observed to be overexpressed in diseased animals and was substantially reduced by either MSCs or GLP-1 agonists. Previous studies have shown that MSC therapies can reduce TGF-β expressions in diabetic rodent kidneys and livers (Lang and Dai, 2016; Paulini *et al*., 2016).

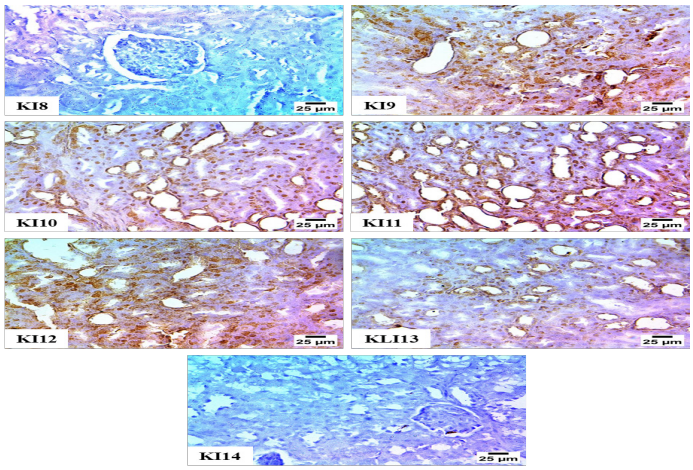

Figure 12: Immunohistochemistry for renal expression of NF-Kβ**;** Figure KI8: Negative expression for NF-Kβ in renal tubules (Group1). Figure KI9: Sever +ve expression for NF-Kβ in nuclei of the renal tubular epithelium (Group2). Figure KI10: Sever +ve expression for NF-Kβ in nuclei of the renal tubular epithelium (Group 3). Figure KI11: +ve expression for NF-Kβ in the renal tubular epithelium (Group 4). Figure KI12: Moderate +ve expression for NF-Kβ in the renal tubular epithelium (Group 5). Fig: KI13: moderate +ve expression for NF-Kβ in nuclei of the renal tubular epithelium (Group 6). Figure KI14: -ve expression for NF-Kβ in renal tubules (Group 7).

Treatment with MSCs significantly lowered levels of TGF-β and NF-κB. It is possible to infer that a paracrine mechanism mediates the protective effects of MSCs. The cytokine environment of the wounded tissues is modulated by BMMSCs, which leads to their functional repair (Sha-Sha *et al*., 2013).

CONCLUSIONS AND RECOMMENDATIONS

Metformin, GLP-1 and BMSCs are able to overcome hyperglycemia and IR associated with T2DM,while inflammatory condition which associated with diabetes and contribute in tissue complication GLP-1 and BMSc are effective more than metformin. consequently, GLP-1 and BMSCs have novality as diabetic therapy and synergistic therapy in combination with metformin. Further study need to support our result by study the efficacy of GLP-1 and BMSCs on PPARs receptors and thyroid gland function for proper glucose homstasis.

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NOVELTY STATEMENT

The current study is the first study in T2DM related research designed to evaluate and compare between traditional therapy (metformin) and new therapy (GLP-1 based drugs and BMSCs) to give new approach for T2DM treatment and overcome its chronic complications

AUTHOR'S CONTRIBUTIONS

Ashraf B. Said & Marwa A. El-Beltagy, Idea,Data collection , research design and write the manuscript. Hoda I. Bahr, sample collection and lab analysisIbrahim A. Ibrahim Revision.

Conflict of Interest

All authors have no conflict of interest to disclose.

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