



Dual Assessment of Antidiabetic Efficacy and Adverse Outcomes of Semaglutide and Tirzepatide in Type 2 Diabetic Swiss Albino Male Mice

Elham M. Alzahrani^{1,2}, Badr A. Aldhmarsh¹, Doaa M. Elnagar¹, Mohammed Batis³, Mukhallad Almutairi¹, Aisha H. Alqarni¹, Wajdan S. Alqahtani¹, Mansour Almansour¹, Ahmed Rady¹, Amjad Khan⁴ and Ahmed Ateya^{5*}

¹Zoology department, college of Science, King Saud University, Riyadh, Saudi Arabia.

²Department Biology, college of Science, Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia.

³Family and Community Medicine Department, College of Medicine, King Saud University, Riyadh, Saudi Arabia.

⁴Kangbuk Samsung Hospital, Samsung Medical Centre, School of Medicine, Sungkyunkwan University (SKKU), Seoul, Korea

⁵Department of Development of Animal Wealth, Faculty of Veterinary Medicine, Mansoura University, Mansoura 35516, Egypt.

Abstract

SEMAGLUTIDE (S) is a GLP-1 receptor agonist; Tirzepatide (T) has dual GLP-1/GIP receptor agonism. This research compared their therapeutic effects and adverse reactions in a type 2 diabetes mellitus (T2DM) mouse model. Body weight, blood glucose, lipid profile, insulin, and IGF1 were estimated, as well as histopathological examination and gene expression. S and T independently led to prominent body weight reduction in diabetic mice, as well as enhancements of glucose and lipid profiles. However, histological examination showed that pancreas hemorrhage and intestine ulceration appeared after treatment. Immunohistochemistry and RT-PCR analysis revealed that insulin and glucagon expression were partially restored, CYP2E1 was over-expressed at a lower level after therapy. In conclusion, Semaglutide and Tirzepatide showed similar metabolic advantages; however, they caused significant changes in intestine and pancreatic structure, emphasizing the necessity for careful assessment over the long term.

Keywords: Semaglutide; Tirzepatide, Type 2 Diabetes, Swiss Albino Mice, Adverse Effects, GLP-1, GIP.

Introduction

Type 2 diabetes (T2D) is a major global public health concern, with its prevalence and complications continuing to rise worldwide. In 2019, the North African and Middle Eastern regions reported the highest global prevalence of diabetes at approximately 12.2%, accompanied by substantial morbidity and mortality rates [1]. Saudi Arabia ranks second in diabetes prevalence within the region and seventh globally [2]. Evidence and studies indicate that the genetic predisposition of the population, obesity, lack of physical activity, urbanization, and

poor dietary modifiable lifestyle behaviors have been associated with the increasing prevalence of diabetes [1].

T2D is a chronic metabolic disorder characterized by impaired glucose regulation [3]. The condition results from both insufficient insulin secretion by pancreatic β -cells and peripheral insulin resistance in skeletal muscle, adipose tissue, and the liver [4]. Individuals with T2D are at a higher risk of developing severe complications such as cardiovascular disease, nephropathy, neuropathy, and retinopathy [5].

*Corresponding authors: Ahmed Ateya, E-mail: dr_ahmedismail@mans.edu.eg Tel.: 01003541921

(Received 21 October 2025, accepted 09 November 2025)

DOI: 10.21608/ejvs.2025.434679.3213

©2025 National Information and Documentation Center (NIDOC)

Semaglutide (S) is a modified analog of human glucagon-like peptide-1 (GLP-1) with the molecular formula $C_{187}H_{291}N_{45}O_{59}$ [6,7]. It mimics the action of endogenous incretin hormones, stimulating insulin secretion in a glucose-dependent manner [7]. Semaglutide binds to GLP-1 receptors on pancreatic β -cells, enhancing insulin secretion while suppressing glucagon release, thereby improving glycemic control [8,7]. Additionally, it acts as an anti-obesity and appetite-suppressant agent by delaying gastric emptying [8,7]. Beyond metabolic regulation, Semaglutide exhibits neuroprotective effects by improving insulin sensitivity and signaling within the brain, potentially reducing the risk of neurodegenerative diseases such as Parkinson and Alzheimer's [9]. Moreover, it significantly lowers hemoglobin A1c (HbA1c) levels and systolic blood pressure [10].

Tirzepatide (T), also referred to as "twincretin," is the first dual agonist of both GLP-1 and glucose-dependent insulintropic polypeptide (GIP) receptors, with the molecular formula $C_{225}H_{348}N_{48}O_{68}$ [3,11]. Tirzepatide has demonstrated potent efficacy in improving glycemic control in T2D without increasing the risk of hypoglycemia [11,12]. It also promotes weight reduction and significantly lowers HbA1c levels [3].

Given the growing clinical use of both agents, the present study aimed to compare the antidiabetic efficacy and potential adverse effects of Semaglutide and Tirzepatide in a diabetic mouse model.

Material and Methods

Animals

A total of forty-two healthy male Swiss albino mice, aged 14 weeks and weighing approximately 30 ± 5 g, were used in this study. The animals were housed under standard laboratory conditions at a controlled temperature of 23 ± 5 °C and relative humidity, with a 12-hour light/dark cycle. All mice had ad libitum access to clean drinking water and a standard commercial rodent diet. They were maintained in polypropylene cages within a well-ventilated animal facility. Prior to the initiation of the experimental procedures, the animals were allowed an acclimatization period of one week to adapt to the laboratory environment. Body weight of the mice was recorded at the beginning of the experiment to determine the initial body weight and again at the end of the experimental period to determine the final body weight.

Experimental design and Experimental Groups

Type 2 diabetes was induced following a 20-hour fasting period, after which mice received intraperitoneal injections of streptozotocin (STZ) at a dose of 50 mg/kg body weight for three consecutive days [13]. The induction of diabetes was confirmed by measuring fasting blood glucose levels 72 hours

after the final injection. Mice with fasting glucose levels above the established diabetic threshold were considered diabetic and included in the subsequent experiments.

The animals were randomly divided into six groups, each comprising seven mice, as follows:

Group 1 (Control): Non-diabetic mice that received a cold citrate buffer solution (pH 4.5) and served as the untreated control group.

Group 2 (Semaglutide-treated control): Non-diabetic mice administered Semaglutide (0.25 mg/mouse) once weekly for four consecutive weeks[14].

Group 3 (Tirzepatide-treated control): Non-diabetic mice administered Tirzepatide (5 mg/mouse) once weekly for four consecutive weeks[15].

Group 4 (Diabetic control): Diabetic mice induced with STZ and left untreated for four weeks.

Group 5 (Semaglutide-treated diabetic): Diabetic mice treated with Semaglutide (0.25 mg/mouse) once weekly for four consecutive weeks[14].

Group 6 (Tirzepatide-treated diabetic): Diabetic mice treated with Tirzepatide (5 mg/mouse) once weekly for four consecutive weeks[15].

Sample Collection

At the end of the experimental period, the animals were anesthetized using controlled CO₂ inhalation and subsequently dissected. Liver tissues were carefully excised, homogenized, and stored at -80 °C for subsequent biochemical analyses. Pancreatic and intestinal tissues were also collected, sectioned into small fragments for histopathological examination.

Biochemical Analysis

Blood samples were collected from the tail vein and analyzed for glucose concentration using a digital glucometer [16]. Liver tissues were homogenized in cold phosphate-buffered saline (PBS) at a ratio of 1:5 (w/v). The homogenates were centrifuged. The resulting supernatants were collected and stored at -80 °C until further analysis.

The liver supernatants were used to determine the activities and concentrations of total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) using commercial diagnostic kits (Elabscience, USA). In addition, insulin (INS) and insulin-like growth factor-1 (IGF-1) levels were quantified using enzyme-linked immunosorbent assay (ELISA) kits provided by Elabscience (USA).

Histopathological Analysis

Following fixation, pancreatic and large intestinal tissues were processed using standard

histological procedures. Hematoxylin and eosin (H&E) staining was performed according to the method described by [17], while Periodic Acid–Schiff (PAS) staining was conducted following the protocol of [18].

Immunohistochemistry

The Avidin-Biotin complex (ABC) detection kits operate on the principle of avidin (or streptavidin) binding to biotin. The acronym ABC signifies Avidin/Biotin Complex. The specific kit utilized for rabbit detection is the HRP/DAB (ABC) IHC kit, Cat. No. ab6426, along with a universal antibody diluent, Cat. No. ab79995, from Abcam-UK. The primary antibodies used include anti-insulin (INS) Rabbit pAb (Cat. No. E-AB-70202), sourced from Elab Science Company in the USA, and anti-glucagon (GC) Rabbit pAb (Cat. No. AP03503), obtained from Bioassay Technology Laboratory Company in China.

Immunostained sections were analyzed using ImageJ and Fiji software to quantify the percentage of immunopositive area, cell count, and optical density (OD). The percentage and OD of immunoreactivity were calculated according to the formula:

$$OD = \log(\text{Max}/\text{Mean}) \times 1 \text{ [19].}$$

RNA extraction and Quantitative Real-Time PCR

Liver tissue samples were homogenized and subjected to total RNA extraction using the PureLink RNA Mini Kit (Thermo Fisher Scientific, MA, USA) according to the manufacturer's protocol. The quantity and purity of the extracted RNA were assessed spectrophotometrically. Complementary DNA (cDNA) was synthesized from the isolated RNA using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, USA), following the manufacturer's instructions.

Quantitative real-time polymerase chain reaction (qRT-PCR) was performed using iTaq™ Universal SYBR® Green Supermix (Bio-Rad, USA; Cat. No. 1725120) according to the manufacturer's protocol. The specific primer sequences for the target gene CYP2E1 are listed in Table 2.1, with GAPDH used as the endogenous reference gene. Each reaction was conducted in triplicate to ensure reproducibility. Relative gene expression levels were calculated using the $2^{-\Delta\Delta C_t}$ method.

Statistical analysis

Data were analyzed using the Statistical Package for the Social Sciences (SPSS), version 16.0 (IBM Corp., Armonk, NY, USA). Results are presented as the mean $\pm$ standard error of the mean (SEM). Statistical comparisons among groups were performed using one-way analysis of variance (ANOVA), followed by appropriate post hoc tests

when necessary. Differences were considered statistically significant at $p < 0.05$.

Results

Difference in Body Weight

Throughout the experimental period, the control mice exhibited an average body weight gain of approximately +5 g. In contrast, non-diabetic groups treated with Semaglutide (S) and Tirzepatide (T) showed a mean body weight reduction of about –7 g. The untreated diabetic group demonstrated an average body weight loss of approximately –5 g during the same period. Similarly, diabetic groups treated with Semaglutide (S) and Tirzepatide (T) exhibited body weight losses of approximately –4 g and –5 g, respectively (Fig. 1).

Biochemical analysis

The non-diabetic groups treated with Semaglutide (S) and Tirzepatide (T) exhibited no significant changes in blood glucose levels compared to the control group. In contrast, the diabetic group showed a significant elevation in blood glucose levels relative to the control. Treatment of diabetic mice resulted in a significant reduction in blood glucose levels compared to the untreated diabetic group (Fig. 2).

Similarly, the non-diabetic groups treated with S or T displayed no significant alterations in lipid profile parameters (triglycerides [TG], total cholesterol [TC], high-density lipoprotein [HDL], and low-density lipoprotein [LDL]) compared with the control group. Conversely, the diabetic group exhibited a significant increase in TG, TC, and LDL levels, accompanied by a notable decrease in HDL concentration relative to the control group. Treatment of diabetic mice with S or T led to a significant improvement in the lipid profile, characterized by decreased TG, TC, and LDL levels and a concomitant increase in HDL concentration compared with the untreated diabetic group (Fig. 3).

Regarding hormonal parameters, non-diabetic groups treated with S or T showed no significant differences in insulin (INS) and insulin-like growth factor-1 (IGF-1) levels compared with the control group. In contrast, the diabetic group demonstrated a significant reduction in both INS and IGF-1 concentrations. Treatment of diabetic mice with S or T produced no significant change in INS levels but resulted in a notable increase in IGF-1 concentrations compared with the untreated diabetic group (Fig. 4).

Histopathological Analysis

The pancreatic tissue of the control group exhibited a normal histological structure with well-defined and intact islets of Langerhans (Fig. 5A). The non-diabetic group treated with Semaglutide (S) showed a noticeable reduction in islet size, accompanied by hypocellularity and mild

intercellular hemorrhage between pancreatic acini (Fig. 5B). In contrast, the non-diabetic group treated with Tirzepatide (T) demonstrated an apparent increase in islet size, associated with cellular infiltration, mild vascular dilatation, and focal hemorrhage between the acinar cells (Fig. 5C). The diabetic group displayed a marked reduction in the size of islets and a decrease in cellular density (Fig. 5D).

Diabetic animals treated with S or T exhibited clusters of inflammatory cell infiltration extending into the islets, along with evident hemorrhage between pancreatic acini (Fig. 5E,F).

The large intestine of the control group revealed normal histological features, with no detectable pathological alterations (Fig. 6A, H&E) and a normal mucin content within the goblet cells (Figure 7A, PAS). In contrast, the non-diabetic group treated with S exhibited mucosal degeneration with focal ulceration (Figure 6B, H&E) and a marked reduction in mucin content within goblet cells (Figure 7B, PAS). Quantitative analysis showed a significant decrease ($p < 0.05$) in both area percentage (Area%) and optical density (OD%) compared with the control group (Fig. 8).

Similarly, the non-diabetic group treated with T demonstrated goblet cell degeneration, cellular infiltration, and mucosal hemorrhage associated with ulcer formation (Fig. 6C, H&E), along with reduced mucin content in goblet cells (Figure 7C, PAS) and a significant decline in Area% and OD% relative to the control (Fig. 8).

The diabetic group exhibited mucosal degeneration, inflammatory cell infiltration, and goblet cell hypertrophy (Fig. 6D, H&E), coupled with a weak mucin content within goblet cells (Fig. 7D, PAS) and a significant reduction ($p < 0.05$) in Area% and OD% compared to the control group (Fig. 8). In contrast, diabetic groups treated with S or T showed mucosal degeneration and hemorrhage associated with ulceration (Fig. 6E,F, H&E). The mucin content in goblet cells appeared relatively comparable to that of the untreated diabetic group (Fig. 7E,F, PAS), with no significant differences in Area% or OD% between the treated and untreated diabetic groups (Fig. 8).

Immunohistochemical Analysis

The control group pancreas exhibited an intense immunoreactive response for insulin (INS) localized within the islets of Langerhans (Fig. 9A). In contrast, the non-diabetic group treated with Semaglutide (S) showed a noticeably weaker immunoreactivity (Fig. 9B), with a significant reduction ($p < 0.05$) in both the immunopositive area percentage (Area%) and optical density (OD%) compared with the control group (Figure 10). Similarly, the non-diabetic group treated with Tirzepatide (T) demonstrated diminished

immunoreactivity for INS (Fig. 9C), accompanied by a significant decrease ($p < 0.05$) in Area% and OD% relative to the control (Fig. 10).

The diabetic group exhibited markedly reduced insulin immunoreactivity within the islets (Fig. 9D), with a significant decrease ($p < 0.05$) in both Area% and OD% compared to the control group (Fig. 10). However, the diabetic groups treated with S or T displayed a mild enhancement in INS immunoreactivity (Fig. 9E,F), which was associated with a slight but significant increase ($p < 0.05$) in Area% and OD% when compared with the untreated diabetic group (Fig. 10).

The control group pancreas exhibited intense immunoreactivity for glucagon (GC), with α -cells distinctly distributed around the periphery of the islets of Langerhans (Fig. 11A). In the non-diabetic group treated with Semaglutide (S), glucagon immunoreactivity appeared slightly increased, characterized by the accumulation of α -cells predominantly on one side of the islet (Figure 11B), showing no significant change ($p > 0.05$) in the immunopositive area percentage (Area%) or optical density (OD%) compared with the control group (Fig. 12). Conversely, the non-diabetic group treated with Tirzepatide (T) exhibited reduced glucagon immunoreactivity (Fig. 11C), accompanied by early migration of α -cells toward the central region of the islets and a significant decrease ($p < 0.05$) in both Area% and OD% relative to the control group (Fig. 12).

The diabetic group demonstrated weak glucagon immunoreactivity with marked α -cell migration from the periphery toward the islet core (Fig. 11D), along with a significant reduction ($p < 0.05$) in Area% and OD% compared to the control (Figure 12). However, the diabetic groups treated with S or T showed enhanced glucagon immunoreactivity (Fig. 11E,F) and evident α -cell migration into the islet core, with a significant increase ($p < 0.05$) in Area% and OD% compared with the untreated diabetic group (Fig. 12).

The non-diabetic groups treated with Semaglutide (S) and Tirzepatide (T) demonstrated a significant decrease ($p < 0.05$) in the total number of islets of Langerhans and the percentage of β -cells, while no significant changes ($p > 0.05$) were observed in α -cell percentage, islet area ($\mu\text{m}^2 \times 10^6$), or islet volume ($\mu\text{m}^3 \times 10^8$) compared with the control group. In contrast, the diabetic group exhibited a significant reduction ($p < 0.05$) in both the total islet count and β -cell percentage, accompanied by a significant increase ($p < 0.05$) in α -cell percentage, with no significant differences ($p > 0.05$) in islet area or volume relative to the control group. However, the diabetic groups treated with S or T showed a significant increase ($p < 0.05$) in the total islet count compared with the untreated diabetic group, while β -

cell percentage, α -cell percentage, islet area, and volume remained statistically unchanged ($p > 0.05$) (Fig.13).

Effect Of Semaglutide (S) and Tirzepatide (T) on The Expression Level Of The CYP2E1 Gene in Liver

The non-diabetic groups treated with Semaglutide (S) and Tirzepatide (T) exhibited no significant change ($p > 0.05$) in hepatic CYP2E1 gene expression compared with the control group. In contrast, the diabetic group showed a significant upregulation ($p < 0.05$) of CYP2E1 expression in the liver relative to the control group. However, treatment of diabetic animals with S or T resulted in a significant downregulation ($p < 0.05$) of hepatic CYP2E1 expression compared with the untreated diabetic group (Fig. 14).

Discussion

Streptozotocin (STZ) is a compound selectively toxic to insulin-secreting β -cells, leading to their degeneration. STZ enters pancreatic cells through glucose transporter 2 (GLUT2), inducing DNA alkylation and subsequent cell death. Therefore, STZ is widely used to experimentally induce diabetes in animal models [20,21,22]. In the present study, diabetes was induced using a single intraperitoneal dose of STZ (50 mg/kg body weight), which successfully produced a type 2 diabetes mellitus (T2DM) model in mice.

Previous studies have demonstrated that diabetic mice exhibit a reduction in total body weight, which aligns with the findings of the current work. This weight loss may result from β -cell destruction and impaired insulin secretion, leading to metabolic dysregulation and increased tissue protein catabolism [23]. In the present study, treatment with Semaglutide (S) and Tirzepatide (T) resulted in significant weight reduction in both non-diabetic and diabetic animals, consistent with recent reports [24,25]. This weight loss is attributed to the actions of dual incretin agonists, which act centrally to modulate appetite regulation by stimulating receptors on hypothalamic neurons that promote satiety, reduce food intake, and increase energy expenditure [24]. Notably, previous research has shown that Tirzepatide possesses a greater weight-reducing capacity than Semaglutide [26], which corroborates the present findings.

The current study revealed STZ-induced diabetic mice exhibited a significant elevation in blood glucose levels compared with the control group, consistent with previous studies demonstrating partial pancreatic β -cell destruction following STZ administration [23]. Treatment with S significantly decreased blood glucose levels in diabetic animals, in agreement with earlier reports highlighting the anti-diabetic effects of GLP-1 receptor agonists [27,28]. Semaglutide acts as a GLP-1 analog that binds to

specific receptors on pancreatic β -cells, enhancing glucose-dependent insulin secretion and suppressing glucagon release, thereby maintaining glucose homeostasis. Similarly, T exerted a glucose-lowering effect through its dual agonism of GIP and GLP-1 receptors (GIPR and GLP-1R) on β -cells, promoting insulin secretion and improving glycemic control [27,29]. Previous reports have also demonstrated the superior glucose-lowering efficacy of T compared to S [30], which aligns with the results of the current study.

Regarding lipid metabolism, diabetic animals exhibited marked dyslipidemia, manifested by elevated TC, TG, and LDL levels and reduced HDL levels, which can be attributed to insulin deficiency and diminished lipoprotein lipase activity [23]. These results were confirmed in the current study. Treatment with S or T in diabetic animals resulted in a significant reduction in TC, TG, and LDL levels and a concomitant increase in HDL levels. This improvement may be attributed to the ability of S to modulate hepatic lipid metabolism and enhance the production of omega-3 polyunsaturated fatty acids, exerting hepatoprotective and anti-inflammatory effects [25]. Similarly, T improved lipid metabolism and protected hepatic tissue, consistent with its role in regulating fat oxidation [24].

The current research indicated diabetic subjects exhibited a marked reduction in both INS and IGF-1 levels, likely attributable to dysfunction of β -cells and liver impairment related to diabetes [23,31]. Prior investigations have indicated that S promotes insulin secretion and increases IGF-1 levels in diabetic models by functioning as a GLP-1 receptor agonist, facilitating glucose-dependent insulin release while inhibiting glucagon secretion [8,7]. However, the findings from this study revealed that S and T did not significantly affect INS but did increase IGF-1 levels in diabetic mice. This implies that these treatments may primarily reduce glucose levels through mechanisms such as appetite suppression, weight loss, and modifications in metabolic energy use rather than directly stimulating β -cell activity.

Histopathological examination of the pancreas revealed significant structural changes induced by both semaglutide (S) and tirzepatide (T), even in non-diabetic mice. The administration of S was linked to a decrease in islet size, cellular degeneration, and mild hemorrhage. In contrast, T treatment resulted in enlarged islets accompanied by infiltration of inflammatory cells and vascular dilation, suggesting potential pancreatic stress[7]. These observations imply that incretin-based therapies could lead to dose-dependent or adaptive responses in tissue that extend beyond their effects on glycemia. In diabetic subjects, the typical characteristics of β -cell depletion and inflammatory infiltration were observed, aligning with earlier

studies [32]. Nevertheless, neither S or T treatment restored normal islet morphology.

Histopathological analysis of the large intestine indicated that treatment with semaglutide (S) in non-diabetic mice resulted in mucosal degeneration and a reduction in goblet cell mucin. Similarly, administration of tirzepatide (T) caused mucosal damage characterized by epithelial deterioration, decreased mucin production, inflammatory cell infiltration, and localized hemorrhage. These observations align with earlier research indicating vacuolar degeneration and glandular disruption within diabetic intestinal tissues [33]. In this study, diabetic mice displayed significant mucosal degeneration, hypertrophy of goblet cells, and diminished mucin levels. Importantly, treatment with S or T intensified these changes, resulting in further mucosal damage and additional loss of mucin. These findings imply that although these medications enhance metabolic outcomes [34], they may impair the integrity of the intestinal barrier, possibly through receptor-mediated impacts on mucosal turnover or local inflammation.

Immunohistochemical analysis revealed that S-treated non-diabetic mice exhibited reduced insulin (INS) immunoreactivity and increased glucagon (GC) expression compared with controls. Similarly, T-treated mice showed reduced INS and GC reactivity. Previous reports have indicated that reduced INS and GC immunoreactivity in diabetic pancreas is associated with inflammatory activation involving NF- κ B, IL-1 β , and IL-6 pathways, leading to β -cell destruction and impaired insulin synthesis [34]. The present study corroborates these findings, demonstrating diminished INS and GC responses in diabetic animals. Treatment of diabetic mice with S and T led to mild restoration of GC expression, accompanied by migration of α -cells toward the islet core, while INS immunoreactivity showed only slight improvement.

Finally, the expression of hepatic CYP2E1 remained unchanged in non-diabetic mice treated with S or T. However, diabetic mice exhibited significant upregulation of CYP2E1 expression, consistent with previous findings attributing this elevation to reduced insulin-mediated suppression of CYP2E1 transcription, increased stabilization by ketone bodies, and enhanced gluconeogenic activity [35]. Elevated CYP2E1 levels promote oxidative stress, hepatocyte apoptosis, and liver injury. In contrast, treatment of diabetic mice with S and T significantly downregulated CYP2E1 expression, indicating that both agents may mitigate hepatic oxidative stress and contribute to the protection against diabetes-associated hepatic dysfunction.

From a global health standpoint, this research aligns with the United Nations Sustainable Development Goal 3 (Good Health and Well-being)

by focusing on diabetes mellitus, a significant non-communicable disease that is becoming more prevalent globally. By investigating the effectiveness and safety of these treatments, the study bolsters international initiatives aimed at decreasing diabetes-related illness and death while fostering sustainable metabolic health results. These results highlight the importance of focusing on metabolic pathways for effective and sustainable disease management in both clinical and public health contexts.

Conclusion

The present study concludes that both Semaglutide (S) and Tirzepatide (T) effectively induce weight reduction, improve glycemic control, and modulate the dysregulated lipid profile associated with diabetes. However, both agents demonstrated notable adverse effects on pancreatic and intestinal tissues, indicating potential organ-specific toxicity. Therefore, further investigations are warranted to elucidate the underlying mechanisms of these histopathological alterations and to assess the long-term safety of S and T, particularly regarding their impact on the pancreas and intestine. Moreover, the current findings emphasize a critical warning against the non-therapeutic use of these agents for weight reduction in non-diabetic individuals.

Acknowledgments

The authors are grateful for the Researchers supporting program from King Saud University, Saudi Arabia.

Funding statement

The authors are grateful for the Researchers supporting program (ORF-2025-214) from King Saud University, Saudi Arabia.

Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

Author Contributions:

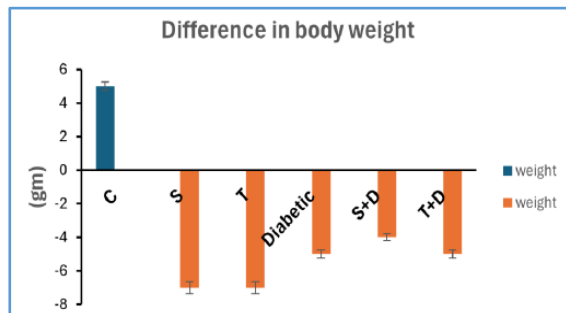
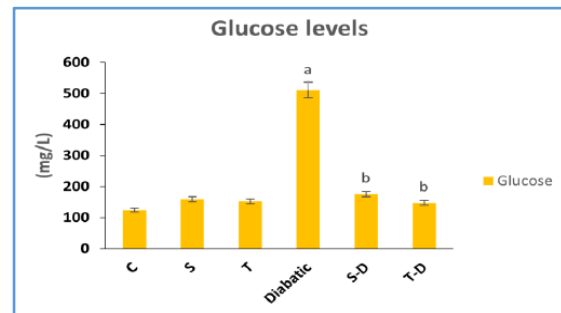
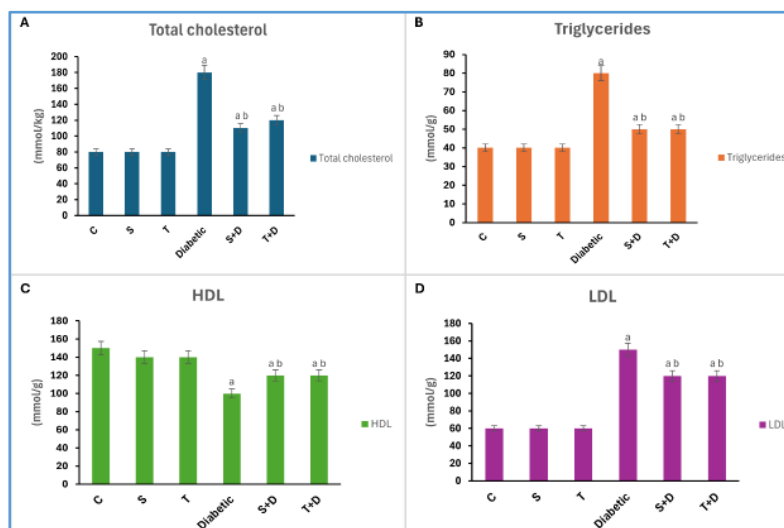
Elham M Alzahrani, Badr A Aldhmash, Doaa M Elnagar, Mohammed Batis, Mukhallad Almutairi, Aisha H Alqarni, Wajdan S Alqahtani, Mansour Almansour, Ahmed Rady, Amjad Khan and Ahmed Ateya: Conceptualization, Investigation, Methodology, Writing – original draft, Writing – review & editing, Formal analysis, Resources. Elham M Alzahrani, Badr A Aldhmash, Doaa M Elnagar, Mohammed Batis, Mukhallad Almutairi, Aisha H Alqarni and Ahmed Ateya: Supervision, Writing – review & editing, Resources.

Ethical of approval

The present work was ethically approved by the Institutional Review Board (IRB), Committee of Ethics, King Saud University, Riyadh, Saudi Arabia. Ethics Reference No (KSU-SE-23-88).

TABLE 1. Real-time PCR primers made of oligonucleotides that are forward and reverse genes under study

Investigated marker	Primer	Product size (bp)	Annealing Temperature (°C)
CYP2E1	F5'- TCCCTAAGTATCCTCCGTGA -3'	529	60
	R5'- GTAATCGAAGCGTTTGTGA- 3'		
GAPDH	F5'- CCTCGTCCCGTAGACAAAATG-3'	152	60
	R5'- TGAAGGGGTCGTTGATGGC - 3'		

**Fig.1.** Bar chart showing body weight difference during the period of experiment Data = Mean $\pm$ SEM, $P \leq 0.05$ considered sig a sig. against control b sig. against Diabetic**Fig.2.** Bar chart showing blood glucose levels. Data = Mean $\pm$ SEM, $P \leq 0.05$ considered sig a sig. against control b sig. against Diabetic**Fig. 3.** Bar chart showing the effect of Semaglutide (S) and Tirzepatide (T) ameliorated lipid profile, A. Triglycerides, B. Cholesterol, C. HDL, and D. LDL. Data = Mean $\pm$ SEM, $P \leq 0.05$ considered sig a sig. against control b sig. against Diabetic

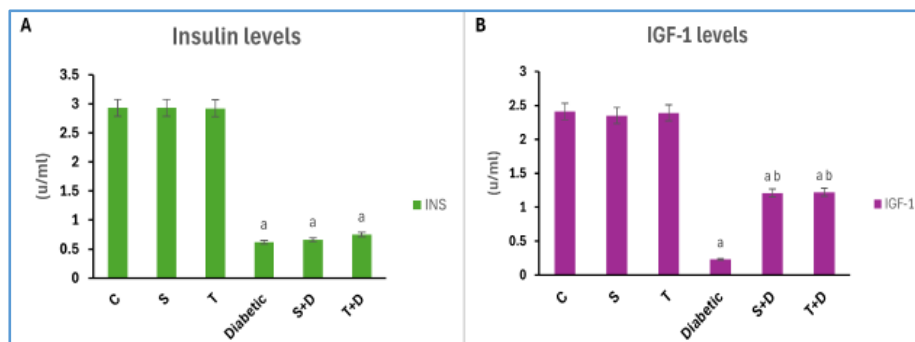


Fig. 4. Bar chart showing the effect of Semaglutide (S) and Tirzepatide (T) on, A. Insulin hormone (INS) levels, B. Insulin-like growth factor 1 hormone (IGF-1) levels. Data = Mean ± SEM, $P \leq 0.05$ considered sig a sig. against control b sig. against Diabetic

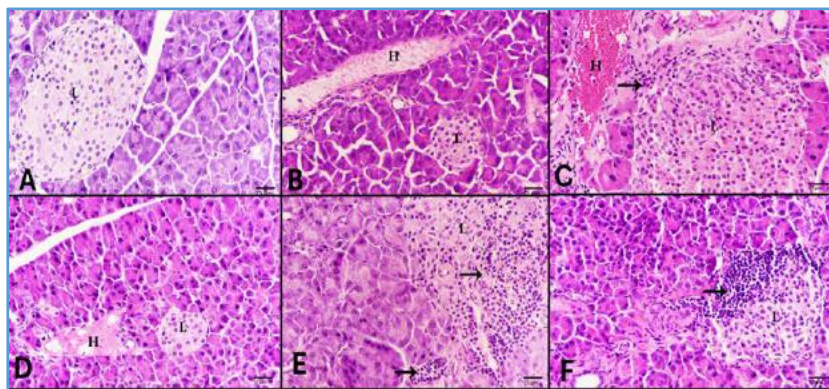


Fig. 5. Photomicrograph of mice Pancreases. (A) control group showing normal appearance, Islet of Langerhans (L), (B) non-diabetic animal group treated with single dose/week of S (0.25mg/kg) displaying reduction in size Islets of Langerhans (L) and hemorrhage between pancreatic cells (H), (C) non-diabetic animal's groups treated with single dose/week of T (5mg/kg) displaying increase in size Islet of Langerhans (L), hemorrhage between pancreatic acini (H) and inflammatory cells (black arrow), (D) diabetic group reduction in size Islet of Langerhans (L) and hemorrhage between pancreatic acini (H), (E) diabetic animal group treated with single dose/week of S (0.25mg/kg) showing islets of Langerhans appeared disorganized (L) and inflammatory cells (black arrow), (F) diabetic animal group treated with single dose/week of T (5mg/kg) showing islets of Langerhans appeared disorganized (L) and inflammatory cells (black arrow) (H & E – 400 X).

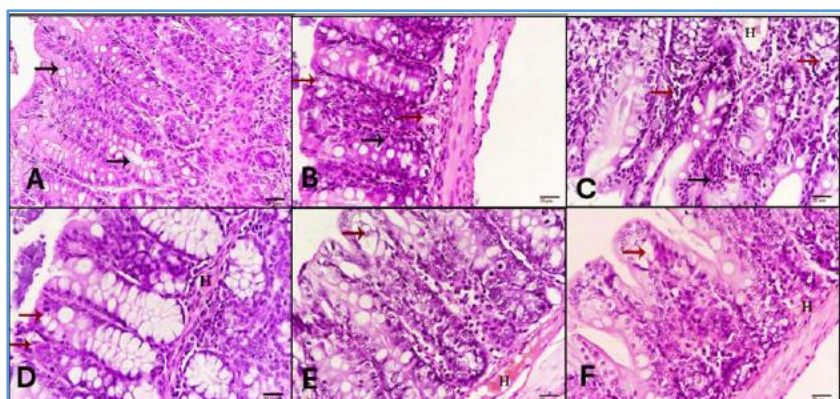


Fig. 6. Photomicrograph of the mice large intestinal (A) control group showing normal appearance, glands and goblet cells (green arrow), (B) non-diabetic animal group treated with single dose/week of S (0.25mg/kg) displaying degeneration (red arrow) and ulcers (orange arrow), (C) non-diabetic animal group treated with single dose/week of T (5mg/kg) displaying degeneration (red arrow), inflammatory cells (black arrow) and hemorrhage (H), (D) diabetic group revealing enlargement cells (brown arrow) and hemorrhage (H), (E) diabetic animal group treated with single dose/week of S (0.25mg/kg) showing degeneration of gland (red arrow) and hemorrhage (H), (F) diabetic animal group treated with single dose/week of T (5mg/kg) showing large intestinal degeneration of gland (red arrow) and hemorrhage (H) (H & E – 400 X).

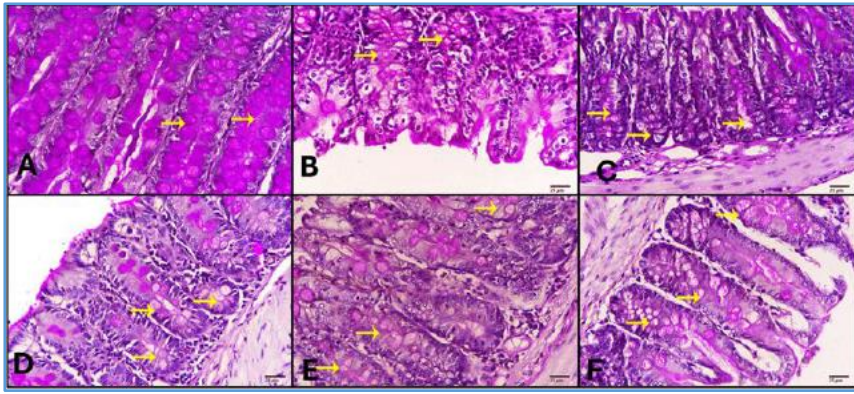


Fig 7. Photomicrograph of the mice large intestinal stained by Periodic Acid Schiff (PAS) (A) control group showing intense stain of mucous in goblet cells (yellow arrow), (B) non-diabetic animal group treated with single dose/week of S (0.25mg/kg) displaying more less content of mucous in goblet cells (yellow arrow), (C) non-diabetic animal' group treated with single dose/week of T (5mg/kg) displaying more less content of mucous in goblet cells (yellow arrow), (D) diabetic group showing weak content of mucous in goblet cells (yellow arrow), (E) diabetic animal group treated with single dose /week of S (0.25mg/kg) weak content of mucous in goblet cells (yellow arrow), (F) diabetic animal group treated with single dose/week of T (5mg/kg) showing weak content of mucous in goblet cells (yellow arrow) (PAS – 400 X).

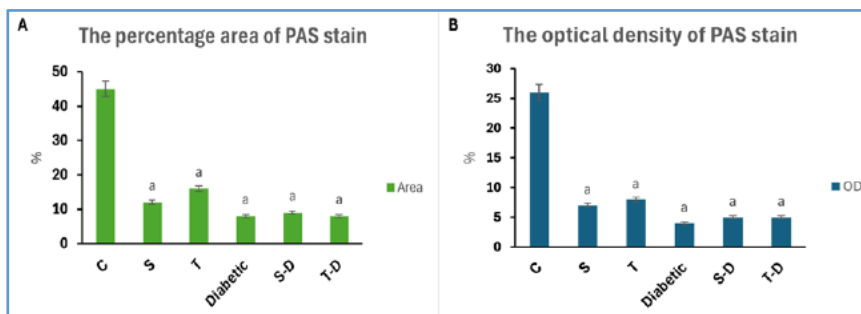


Fig. 8. Bar chart showing the Area % and Optical density of PAS stain in large intestine.

Data = Mean ± SEM, $P \leq 0.05$ considered sig a sig. against control b sig. against Diabetic

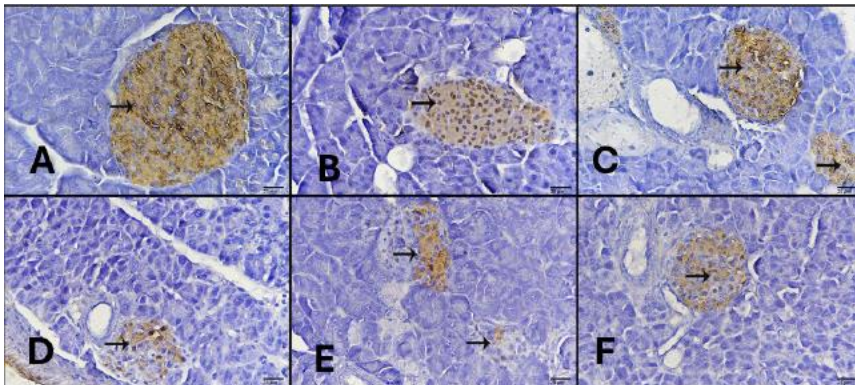


Fig. 9. Photomicrograph of the Pancreas Langerhans Islands staining immunohistochemically against INS(β cells):

(A) control group showing intense immunoreactivity (black arrow), (B) non-diabetic animal group treated with single dose /week of S (0.25mg/kg) displaying more less immunoreactivity (black arrow), (C) non-diabetic animal group treated with single dose/week of T(5mg/kg) displaying more less immunoreactivity (black arrow), (D) diabetic group displaying weak immunoreactivity (black arrow), (E) diabetic animal group treated with single dose/week of S(0.25mg/kg) showing weak immunoreactivity (black arrow), (F) diabetic animal group treated with single dose/week of T showing slight increase immunoreactivity (black arrow) (ABC-400X).

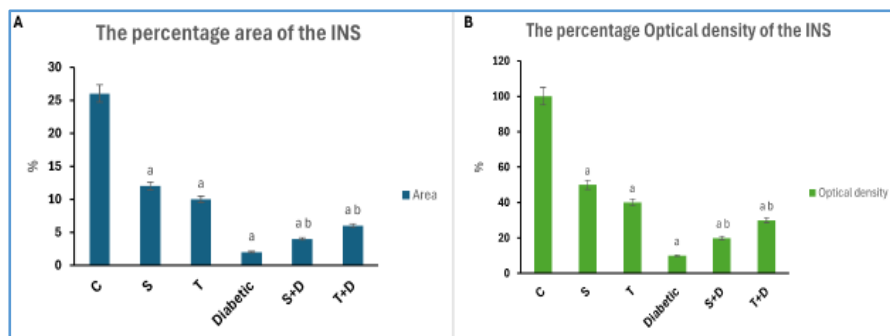


Fig. 10. Bar chart showing the effect of Semaglutide (S) and Tirzepatide (T) on immune response in the pancreas against (INS), represented by A. area% of the pancreas, B. optical density% of the pancreas. Data = Mean $\pm$ SEM, $P \leq 0.05$ considered sig a sig. against control b sig. against Diabetic

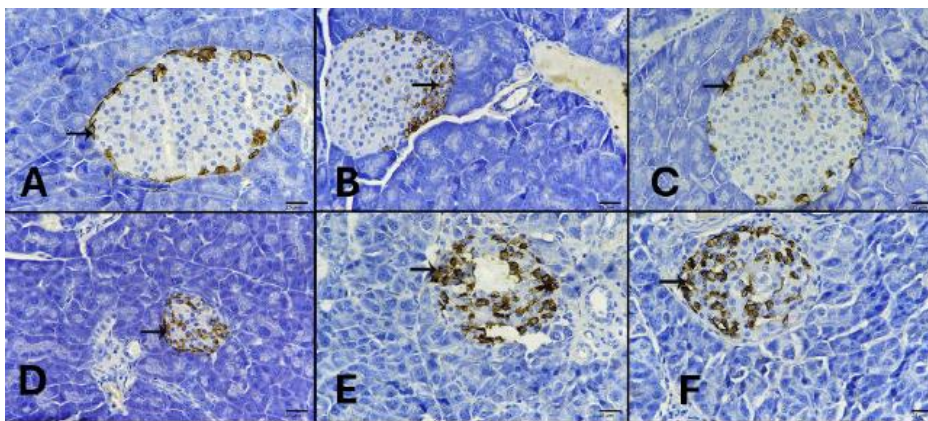


Fig. 11. Photomicrograph of the Pancreas Langerhans Islands staining immunohistochemically against GC:

(A)control group showing intense immunoreactivity (black arrow), (B) non-diabetic animal group treated with single dose/week of S (0.25mg/kg) displaying increase immunoreactivity with the accumulation of α cells on one side of the islet (black arrow), (C) non-diabetic animal group treated with single dose/week of T (5mg/kg) showing less immunoreactivity with started to migration α cells (black arrow),(D) diabetic group displaying less immunoreactivity with migrated α cells (black arrow),(E) diabetic animal group treated with single dose /week of S (0.25mg/kg) showing more immunoreactivity with migrated α cells into the core of islets (black arrow),(F) diabetic animal group treated with single dose /week of T showing more immunoreactivity with migrated α cells into the core of islets (black arrow) (ABC-400X).

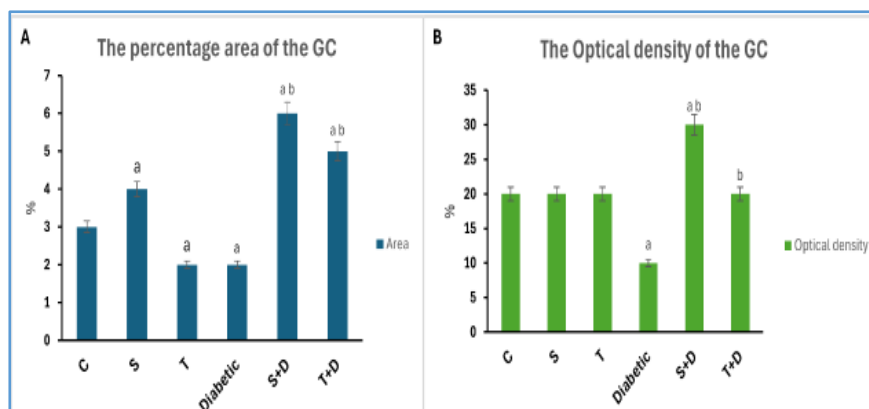


Fig. 12. Bar chart showing the effect of Semaglutide (S) and Tirzepatide (T) on immune response in the pancreas against (GC), represented by A. area% of the pancreas, B. optical density% of the pancreas. Data = Mean $\pm$ SEM, $P \leq 0.05$ considered sig a sig. against control b sig. against Diabetic

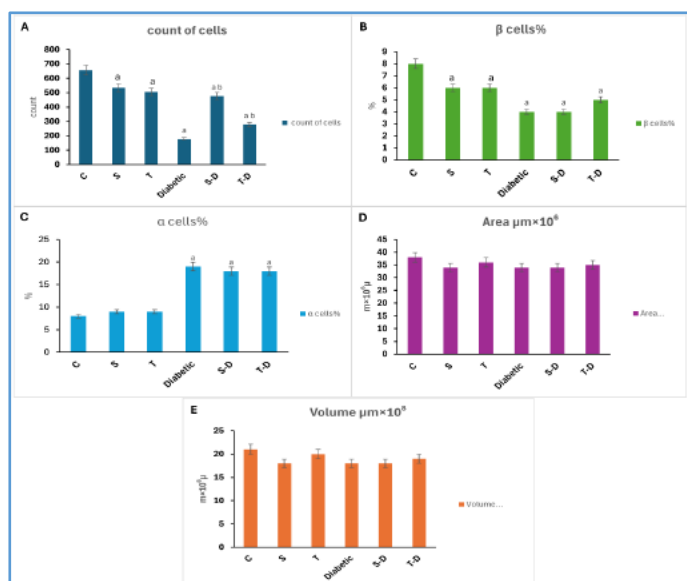


Fig. 13. Bar chart showing the effect of Semaglutide (S) and Tirzepatide (T) on Langerhans cells., A. count of cells., B. β cells%, C. α cells%, D. Area $\mu\text{m}^2 \times 10^6$., E. Volume $\mu\text{m}^3 \times 10^8$. Data = Mean $\pm$ SEM, $P \leq 0.05$ considered sig a sig. against control b sig. against Diabetic

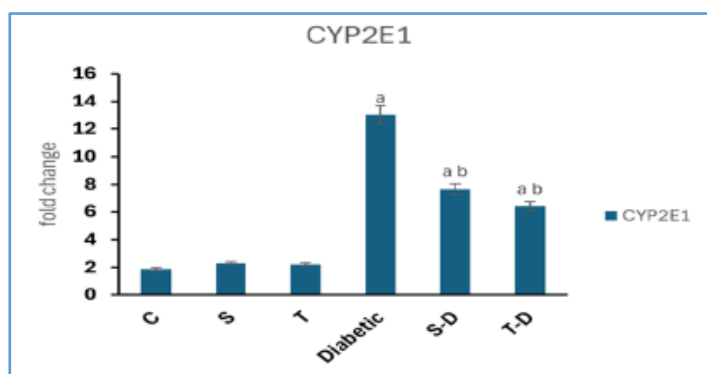


Fig. 14. Bar chart showing the expression level of the CYP2E1 gene in Liver induced by Semaglutide (S) and Tirzepatide (T). Data = Mean $\pm$ SEM, $P \leq 0.05$ considered sig a sig. against control b sig. against Diabetic

References

- El-Kebbi, I.M., Bidikian, N.H., Hneiny, L. and Nasrallah, M.P. Epidemiology of type 2 diabetes in the Middle East and North Africa: Challenges and call for action. *World J. Diabetes*, **12**, 1401–1425 (2021). 10.4239/wjd.v12.i9.1401.
- Robert, A.A., Al Dawish, M.A., Braham, R., Musallam, M.A., Al Hayek, A. and Al Kahtany, N.H. Type 2 diabetes mellitus in Saudi Arabia: Major challenges and possible solutions. *Curr. Diabetes Rev.*, **13**, 59–64 (2017). 10.2174/1573399812666160215152601.
- Chavda, V.P., Ajabiya, J., Teli, D., Bojarska, J. and Apostolopoulos, V. Tirzepatide, a new era of dual-targeted treatment for diabetes and obesity: A mini-review. *Molecules*, **27**, 4315 (2022). 10.3390/molecules27134315.
- Razzaki, T.S., Weiner, A. and Shukla, A.P. Tirzepatide: Does the evidence to date show potential for the treatment of early-stage type 2 diabetes? *Ther. Clin. Risk Manag.*, **18**, 955–964 (2022). 10.2147/TCRM.S369948.
- Asif, M. The prevention and control of type 2 diabetes by changing lifestyle and dietary pattern. *J. Educ. Health Promot.*, **3**, 1 (2014). 10.4103/2277-9531.127541.
- Dhillon, S. Semaglutide: First global approval. *Drugs*, **78**, 275–284 (2018). 10.1007/s40265-018-0866-7
- National Institutes of Health (NIH). *Semaglutide*. National Center for Biotechnology Information. Available online: <https://pubchem.ncbi.nlm.nih.gov/compound/Semaglutide> (accessed on 12 October 2025).
- Liu, Y. and Luo, X. New practice in semaglutide on type 2 diabetes and obesity: Clinical evidence and expectation. *Front. Med.*, **16**, 17–24 (2022). 10.1007/s11684-021-0895-5.
- Mahapatra, M.K., Karuppasamy, M. and Sahoo, B.M. Semaglutide, a glucagon-like peptide-1 receptor agonist with cardiovascular benefits for management of

- type 2 diabetes. *Rev. Endocr. Metab. Disord.*, **23**, 521–539 (2022). 10.1007/s11154-021-09692-4.
10. Andreadis, P., Karagiannis, T., Malandris, K., Avgerinos, I., Liakos, A., Manolopoulos, A., Bekiari, E., Matthews, D.R. and Tsapas, A. Semaglutide for type 2 diabetes mellitus: A systematic review and meta-analysis. *Diabetes Obes. Metab.*, **20**, 2255–2263 (2018). 10.1111/dom.13360.
 11. Farzam, K. and Patel, P. Tirzepatide. In *StatPearls [Internet]*. StatPearls Publishing, Treasure Island, FL, USA (2023).
 12. Heise, T., Mari, A., DeVries, J.H., Urva, S., Li, J., Pratt, E.J., Coskun, T., Thomas, M.K., Mather, K.J., Haupt, A. and Milicevic, Z. Effects of subcutaneous tirzepatide versus placebo or semaglutide on pancreatic islet function and insulin sensitivity in adults with type 2 diabetes: A multicentre, randomized, double-blind, parallel-arm, phase 1 clinical trial. *Lancet Diabetes Endocrinol.*, **10**, 418–429 (2022). 10.1016/S2213-8587(22)00089-7
 13. Araya, T.Y., Karim, A., Hailu, G.S., Periasamy, G and Kahsay, G. Antihyperglycemic Activity of TLC Isolates from the Leaves of Aloe megalacantha Baker in Streptozotocin-Induced Diabetic Mice. *Diabetes Metab Syndr Obes.*, **15**, 14, 1153–1166 (2021). 10.2147/DMSO.S288313.
 14. Inia, J.A., Stokman, G., Morrison, M.C., Worms, N., Verschuren, L., Caspers, M.P.M., Menke, A.L, Petitjean, L., Chen, L., Petitjean, M., Jukema, J.W., Princen, H.M.G., van den Hoek, A.M. Semaglutide Has Beneficial Effects on Non-Alcoholic Steatohepatitis in Ldlr^{-/-} Leiden Mice. *Int J Mol Sci.*, **9**, 24(10):8494 (2023). 10.3390/ijms24108494.
 15. Hegedüs, Z.I., Jakab, M.E., Gergely, T.G., Sayour, N.V., Kovács, A., Antal, S., Kovács, T., Ferdinandy, P., Varga, Z.V and Tóth, V.E. Tirzepatide, a dual GIP/GLP1-receptor co-agonist preserves cardiac function and improves survival in angiotensin II-induced heart failure model in mice: comparison to liraglutide. *Cardiovasc Diabetol.*, **14**, 24(1):253 (2025). 10.1186/s12933-025-02806-5.
 16. Togashi, Y., Shirakawa, J., Okuyama, T., Yamazaki, S., Kyohara, M., Miyazawa, A., Suzuki, T., Hamada, M. and Terauchi, Y. Evaluation of the appropriateness of using glucometers for measuring the blood glucose levels in mice. *Sci. Rep.*, **6**, 25465 (2016). 10.1038/srep25465.
 17. Bancroft, J.D. and Gamble, M. *Theory and Practice of Histological Techniques*. Elsevier Health Sciences, London, UK (2019).
 18. Steinke, H., Wiersbicki, D., Speckert, M.L., Merkwitz, C., Wolfskämpf, T., Wolf, B. Periodic acid-Schiff (PAS) reaction and plastination in whole body slices. A novel technique to identify fascial tissue structures. *Ann. Anat. Mar.*, **216**, 29–35 (2018). 10.1016/j.aanat.2017.10.001.
 19. Hartig, S.M. Basic image analysis and manipulation in ImageJ. *Curr. Protoc. Mol. Biol.*, **14**, Unit14.15 (2013). 10.1002/0471142727.mb1415s102.
 20. Damasceno, D.C., Netto, A.O., Iessi, I.L., Gallego, F.Q., Corvino, D., Sinzato, Y.K., Bueno, A.I., Calderon, M.P. and Rudge, M.V.C. Streptozotocin-induced diabetes models: Pathophysiological mechanisms and fetal outcomes. *Biomed. Res. Int.*, **2014**, 819065 (2014). 10.1155/2014/819065.
 21. Furman, B.L. Streptozotocin-induced diabetic models in mice and rats. *Curr. Protoc.*, **1**, e78 (2021). 10.1002/cpz1.78.
 22. Kamli-Salino, S.E.J., Brown, P.A.J., Haschle, T.N., Liang, L., Feliars, D., Wilson, H.M. and Delibegovic, M. Induction of experimental diabetes and diabetic nephropathy using anomer-equilibrated streptozotocin in male C57Bl/6J mice. *Biochem. Biophys. Res. Commun.*, **634**, 109–116 (2022). 10.1016/j.bbrc.2022.10.039
 23. Al-Attar, A.M. and Alsalmi, F.A. Effect of *Olea europaea* leaves extract on streptozotocin-induced diabetes in male albino rats. *Saudi J. Biol. Sci.*, **26**, 118–128 (2019). 10.1016/j.sjbs.2018.09.001
 24. Liang, J., Liu, H., Lv, G., Chen, X., Yang, Z., Hu, K. and Sun, H. Exploring the molecular mechanisms of tirzepatide in alleviating metabolic dysfunction-associated fatty liver in mice through integration of metabolomics, lipidomics, and proteomics. *Lipids Health Dis.*, **24**, 8 (2025). 10.1186/s12944-025-02023-y
 25. Soto-Catalán, M., Opazo-Ríos, L., Quiceno, H., Lázaro, I., Moreno, J.A., Gómez-Guerrero, C., Egido, J. and Mas-Fontao, S. Semaglutide improves liver steatosis and de novo lipogenesis markers in obese and type 2 diabetic mice with metabolic-dysfunction-associated steatotic liver disease. *Int. J. Mol. Sci.*, **25**, 2961 (2025). 10.3390/ijms25062961.
 26. Rodriguez, P.J., Goodwin Cartwright, B.M., Gratzl, S., Brar, R., Baker, C., Gluckman, T.J. and Stucky, N.L. Semaglutide vs tirzepatide for weight loss in adults with overweight or obesity. *JAMA Intern. Med.*, **184**, 1056–1064 (2024). 10.1001/jamainternmed.2024.2334
 27. Harada, N. and Inagaki, N. Role of GIP receptor signaling in β -cell survival. *Diabetol. Int.*, **8**, 137–138 (2017). 10.1007/s13340-017-0317-7.
 28. Steinke, H., Wiersbicki, D., Speckert, M.L., Merkwitz, C., Wolfskämpf, T., Wolf, B. Periodic acid-Schiff (PAS) reaction and plastination in whole body slices. A novel technique to identify fascial tissue structures. *Ann. Anat. Mar.*, **216**, 29–35 (2018). 10.1016/j.aanat.2017.10.001.
 29. Kong, W., Deng, B., Shen, X., John, C., Haag, J., Sinha, N., Lee, D., Sun, W., Chen, S. and Zhang, H. *et al.* Tirzepatide as an innovative treatment strategy in a pre-clinical model of obesity-driven endometrial cancer. *Gynecol. Oncol.*, **191**, 116–123 (2023). 10.1016/j.ygyno.2023.05.030.
 30. Mather, K.J., Mari, A., Heise, T., DeVries, J.H., Hua, M., Urva, S., Coskun, T., Haupt, A., Heine, R.J. and Pratt, E. *et al.* Effects of tirzepatide vs semaglutide on β -cell function, insulin sensitivity, and glucose control during a meal test. *J. Clin. Endocrinol. Metab.*, **109**, 3046–3054 (2024). 10.1210/clinem/dgae105.
 31. Iwai, S., Kaji, K., Nishimura, N., Kubo, T., Tomooka, F., Shibamoto, A., Suzuki, J., Tsuji, Y., Fujinaga, Y. and Kitagawa, K. *et al.* Glucagon-like peptide-1

- receptor agonist semaglutide attenuates chronic liver disease-induced skeletal muscle atrophy in diabetic mice. *Biochim. Biophys. Acta Mol. Basis Dis.*, **1869**, 166770 (2023). 10.1016/j.bbdis.2023.166770.
32. Ibrahim, H.A.E., Hashem, M.A., Mohamed, N.E. and El-Rahman, A.A.A. Assessment of ameliorative effects of *Zingiber officinale* and *Nigella sativa* in streptozotocin-induced diabetic rats. *Adv. Anim. Vet. Sci.*, **8**, 1211–1219 (2020). 10.17582/journal.aavs/2020/8.12.1211.1219.
33. Cai, C., Cheng, W., Shi, T., Liao, Y., Zhou, M. and Liao, Z. Rutin alleviates colon lesions and regulates gut microbiota in diabetic mice. *Sci. Rep.*, **13**, 4897 (2025). 10.1038/s41598-025-07108-9.
34. Heise, T., Mari, A., DeVries, J.H., Urva, S., Li, J., Pratt, E.J., Coskun, T., Thomas, M.K., Mather, K.J., Haupt, A. and Milicevic, Z. Effects of subcutaneous tirzepatide versus placebo or semaglutide on pancreatic islet function and insulin sensitivity in adults with type 2 diabetes: A multicentre, randomized, double-blind, parallel-arm, phase 1 clinical trial. *Lancet Diabetes Endocrinol.*, **10**, 418–429 (2022). 10.1016/S2213-8587(22)00089-7.
35. Maksymchuk, O., Shysh, A. and Story, D. Treatment with omega-3 PUFAs does not increase the risk of CYP2E1-dependent oxidative stress and diabetic liver pathology. *Front. Endocrinol.*, **13**, 1004564 (2023). 10.3389/fendo.2022.1004564.

التقييم المزدوج لفعالية سيماجلوتيد وتيرزيباتيد كمضادات للسكري والآثار الجانبية لهما في ذكور فئران سويسرية ألبينو مصابة بداء السكري من النوع الثاني

إلهام الزهراني^{1,2}، بدر الدهمش¹، دعاء النجار¹، محمد باتيس³، مخلد المطيري¹، عائشة القرني¹،
وجدان القحطاني¹، منصور المنصور¹، أحمد راضي¹، أمجد خان⁴ وأحمد عطية^{5*}

¹ قسم علم الحيوان، كلية العلوم، جامعة الملك سعود، الرياض، المملكة العربية السعودية.
² قسم الأحياء، كلية العلوم، جامعة الأميرة نورة بنت عبد الرحمن، الرياض، المملكة العربية السعودية.
³ قسم طب الأسرة والمجتمع، كلية الطب، جامعة الملك سعود، الرياض، المملكة العربية السعودية.
⁴ مستشفى كانبوك سامسونج، مركز سامسونج الطبي، كلية الطب، جامعة سونغ كيون كوان، سيول، كوريا
⁵ قسم تنمية الثروة الحيوانية، كلية الطب البيطري، جامعة المنصورة، المنصورة 35516، مصر.

المخلص

إيماجلوتيد (S) هو ناهض لمستقبلات GLP-1 ؛ بينما يتميز تيرزيباتيد (T) بناهض مزدوج لمستقبلات GLP-1/GIP. قارن هذا البحث آثارهما العلاجية وآثارهما الجانبية في نموذج فأر مصاب بداء السكري من النوع الثاني (T2DM). تم تقدير وزن الجسم، ومستوى السكر في الدم، ومستوى الدهون، والأنسولين، وعامل النمو الشبيه بالأنسولين (IGF1)، بالإضافة إلى الفحص النسيجي المرضي والتعبير الجيني. أدى كل من S و T بشكل مستقل إلى انخفاض ملحوظ في وزن الجسم لدى الفئران المصابة بداء السكري، بالإضافة إلى تحسينات في مستويات الجلوكوز والدهون. ومع ذلك، أظهر الفحص النسيجي ظهور نزيف في البنكرياس وتقرحات معوية بعد العلاج. وكشفت الكيمياء المناعية وتحليل تفاعل البوليميراز المتسلسل العكسي (RT-PCR) عن استعادة جزئية لمستوى الأنسولين والجلوكاجون، بينما انخفض مستوى التعبير المفرط لإنزيم CYP2E1 بعد العلاج. في الختام، أظهر كل من سيماجلوتيد وتيرزيباتيد مزايا أيضاً متشابهة؛ إلا أنهما تسببا في تغييرات كبيرة في وظائف الجهاز الهضمي والبنكرياس، مما يؤكد ضرورة إجراء تقييم دقيق على المدى الطويل.

الكلمات الدالة: سيماجلوتيد؛ تيرزيباتيد؛ داء السكري من النوع الثاني؛ فئران ألبينو سويسرية؛ الآثار الجانبية؛ GLP-1 ؛ GIP.