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Original Research Article

Semaglutide ameliorates diabetes-induced steatotic liver disease in rats: Role of AMPK, mTOR, ERK and ABHD6

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Abstract

Purpose: *To determine whether semaglutide protects rats with diabetes from metabolic dysfunction-associated steatotic liver disease (MASLD), and its underlying molecular mechanisms.*
Methods: *Fifty-three male Wistar rats were divided into five groups: control, streptozotocin (STZ, 45 mg/kg given intraperitoneally); STZ + low-dose semaglutide (12 µg/kg every 3 days), STZ + high-dose semaglutide (40 µg/kg every 3 days), and STZ + metformin (100 mg/kg/day orally). Rats (except control group) were given 10 % fructose in drinking water from the beginning of the experiment until the 12th week. Blood concentrations of glucose, insulin, liver biomarkers, and lipid profiles were measured. Oxidative stress and inflammatory markers in liver tissues were assessed, along with protein expressions of pAMPK, mTOR, ERK, and ABHD6.*
Results: *High-dose semaglutide enhanced blood glucose levels, reduced feed and water intake, decreased body weight, and enhanced liver function, when compared to the STZ group. Oxidative stress was reduced, and levels of inflammatory indices (TNF-α and IL-6) were supressed. Additionally, semaglutide decreased the expression levels of mTOR, ERK, and ABHD6, while activating the hepatic AMPK pathway. Reduced histopathological lesions were observed in liver tissues.*
Conclusion: *Semaglutide may be beneficial in preventing MASLD in T2DM, thereby providing new perspectives on its potential therapeutic role.*

Keywords: *Semaglutide, Streptozotocin, Metabolic dysfunction-associated steatotic liver disease, AMP-activated protein kinase, Adult-onset diabetes*

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INTRODUCTION

Diabetes is driven by a mix of genetic and environmental factors which include unregulated diet, lack of physical activity, and smoking. Currently, approximately 537 million people globally live with diabetes, a number which has been projected to rise to 643 million by 2030 [1].

Obesity and its complications pose a significant challenge to healthcare systems worldwide. It is known that MASLD is a major clinical complication of obesity. It impacts 30 % of the global population and approximately 70 – 80 % of individuals with obesity [2]. This disease involves liver conditions marked by lipid accumulation in hepatocytes, excluding factors like alcohol, viral hepatitis, and medications. It has the potential to progress from simple steatosis to metabolic dysfunction-associated steatohepatitis (MASH), and potentially advance to cirrhosis or hepatocellular carcinoma.

Semaglutide, a glucagon-like peptide-1 receptor agonist (GLP-1Ra), has shown promise in managing hepatic metabolic complications associated with obesity. It improves lipid degradation via activation of AMP-activated protein kinase (AMPK) and affects free fatty acids (FFAs) by enhancing insulin sensitivity, inhibiting lipolysis, and altering gastrointestinal motility [3]. The attenuation of oxidative stress (OS) could result from the overall metabolic improvements linked to GLP-1Ra. Additionally, the reduction in mammalian target of rapamycin (mTOR) level, and the subsequent decrease in hepatic injury caused by MASLD support the use of Semaglutide as a preventive treatment for the disease [4]. Furthermore, ERK, a component of the MAPK cascade, acts as a key integrator of diverse extracellular signals involving individual cytokines or hormones and cellular mechanical stress which affect lipid metabolism and other cellular processes [5]. Moreover, the integral membrane protein α/β hydrolase domain-6 (ABHD6) which hydrolyses monoacylglycerol, is believed to regulate brain endocannabinoid signaling. Recent findings also indicate the involvement of ABHD6 in the pathophysiology of metabolic syndrome (MS) [6].

Despite these promising effects, limited information exists regarding the effect of semaglutide on MASLD, particularly in relation to mTOR/AMPK, ERK, and ABHD6 pathways in diabetic rats. Therefore, the present research was carried out to investigate if Semaglutide protects diabetic rats from MASLD, and to identify potential underlying molecular mechanisms.

EXPERIMENTAL

Chemicals

Streptozotocin was acquired from Merck® (Cat. no. S0130-1G, USA). Dextrose was sourced from Merck® (Cat. no. PHR1000, USA), and fructose was obtained from I-Herb®). These were dissolved in distilled water. Sodium carboxymethylcellulose (Na-CMC) purchased from Merck® (Cat. no. 419273-1KG, USA) was prepared as a 0.5 % (w/v) solution which served as a vehicle. Semaglutide (Ozempic®), was acquired from Novo Nordisk, Denmark, and was prepared for administration by diluting it with 0.9 % NaCl, whereas metformin (Merck®, Cat. no. 317240, USA) was dissolved in 0.5 % Na-CMC.

Animals

The study was conducted at the Pharmacology Department of the Faculty of Medicine in Jeddah and Pharmacy Faculty, King Abdulaziz University, Saudi Arabia. The study was approved by the ethics committee of Faculty of Pharmacy (approval no. PH-1444-24), and the study procedure adhered to international ethical standards. Fifty-three male Wistar rats weighing 150-180 g were housed in groups of five and acclimatized to laboratory conditions for one week prior to the study. The rats were kept under controlled conditions (23 - 25 °C and 55 ± 10 % humidity) in an environment with a standard light-dark cycle. They were provided with a standard laboratory diet (20 % protein, 4 % fat and 5 % fiber), with *ad libitum* access to clean water.

Design

Fifty-three male Wistar rats weighing 150 - 180 g were used, with 8 in the control group and 45 administered STZ. Fasting blood glucose (FBG) was measured 72 h post-STZ injection, and five rats were excluded for failing to develop T2DM. The remaining 40 rats were randomly assigned to four experimental groups (10 rats/group). Group 1 (control) received 0.5 % Na-CMC orally daily and 0.9 % NaCl subcutaneously twice weekly. Group 2 (STZ) received STZ at a dose of 45 mg/kg intraperitoneally, to induce diabetes. Groups 3 and 4 received (STZ + Semaglutide at doses of 12 and 40 µg/kg, respectively) subcutaneously every three days. The dosing regimen was close to the weekly human doses (0.25 and 1.0 mg/week), based on Paget's scale for interconversion between animal and human doses. However, because the half-life of Semaglutide is shorter in rodents than in humans, Semaglutide's weekly exposure level was halved, and one dose was given every three days. As a reference group, group 5 (STZ + Metformin) received oral Metformin (100 mg/kg/day) along with 10 % fructose water. The control group received tap water, while the other groups received 10 % fructose for 12 weeks. From week 4, groups 3, 4, and 5 received Semaglutide and Metformin for 9 weeks. Blood was obtained via the retro-orbital plexus, and the serum sample was kept frozen at -20 °C. Liver tissues were excised, rinsed, weighed, and preserved in 10 % neutral buffered formalin for histopathological examination.

Determination of diabetic parameters

Fasting blood glucose (FBG) levels were assessed with a Contour-next® glucometer. Weekly water consumption was monitored, and feed intake was measured over 24 hours using an ADAM® AQT–2600 scale. Body weight changes (W) were calculated as percentages using Eq 1.

$W (\%) = (w_f\text{-}w_i/w_i)100$ …… (1)

where w_f and w_i are the final and initial weights, respectively.

Insulin levels were assessed using ELISA (E-lab Science, USA, Cat. no. E-EL-R3034), and the Homeostatic model assessment of insulin resistance (HOMA-IR) index was calculated using Eq 2.

HOMA-IR = (Gf*If)/405 …… (2)

where Gf and If are fasting glucose in mg/dL and If is fasting insulin in mIU/L

Assessment of hepatic biomarkers

Serum levels of ALT and AST and total bilirubin (TB) were measured using ELISA kits from Bio Diagnostic (Giza, Egypt; catalogue numbers AL 1031, AS 1061, and BR 1111, respectively).

Histopathological studies

Liver samples were fixed in 10% neutral buffered formalin for 24 h, followed by H&E staining for histological examination. Tissue processing was performed according to the procedures of Bancroft & Gamble [7]. The slides were examined at ×200 magnification using an Olympus BX-50 microscope and photographed at the Histology Technical Unit of our institution.

DETERMINATION OF LIPID PROFILES

Serum lipid profiles were determined using ELISA kits from Bio Diagnostic (Giza, Egypt), with catalogue numbers CH 1220, TR 2030, CH 1230 and CH 1231, for TC, TG, HDL and LDL, respectively.

Assessment of oxidative status

Levels of MDA and GSH, and catalase (CAT) activity were measured using biochemical kits from Bio Diagnostic® (Giza, Egypt), with catalogue numbers GR 2511, MD 2529, and CA 2517, respectively.

Assay of proinflammatory factor levels

The levels of TNF-α and IL-6 in liver tissue homogenates were measured with ELISA, with kits from Cloud-Clony Corp (Texas, USA), using catalogue numbers SEA133ra and SEA079ra, respectively.

Assessment of associated molecular pathways

Rat expression levels of pAMPK, mTOR, ERK and ABHD6 were determined with MyBioSource ELISA (San Diego, CA, USA), with kit catalog numbers MBS164089, MBS744326, MBS034880 and MBS9323855, respectively.

Determination of protein content in hepatic homogenate

Protein content was determined using BCA assay kit (Thermo Scientific™, Rockford, IL, USA) with catalog number 23227.

Statistical analysis

Data are expressed as mean ± standard deviation (SD; n = 8). Graphics were prepared with GraphPad Prism® version 10. Parametric data from five groups were analyzed with 1-way ANOVA and Tukey's post-*hoc* test, with repeated measures ANOVA where applicable. Values of $p < 0.05$ were taken as indicative of significant differences.

RESULTS

Effect of semaglutide on serum fasting blood glucose (FBG), water intake, and feed intake

Figure 1 shows that at weeks 4, 8, and 12, the STZ group exhibited significant increases ($p < 0.05$) in FBG levels, water intake, and feed consumption, relative to the control group. In the last week of the experiment (week 12), semaglutide (40 µg/kg) and Metformin groups showed significant ($p < 0.05$) declines in FBG concentrations (59 and 48 % respectively), relative to the corresponding STZ cohort. Furthermore, high-dose semaglutide was significantly more effective than its low dose (12 µg/kg every 3 days) as well as metformin, in reducing FBG levels. Additionally, treatment with semaglutide (12 & 40 µg/kg) and metformin resulted in significant ($p < 0.05$) reductions in water intake by approximately 25, 47, and 24 %, respectively, when compared to the STZ-exposed group. Furthermore, treatment with semaglutide doses (12 and 40 µg/kg) and metformin significantly ($p < 0.05$) reduced feed intake by about 61, 69, and 47 %, respectively, on week twelve, when compared to the corresponding STZ-exposed group.

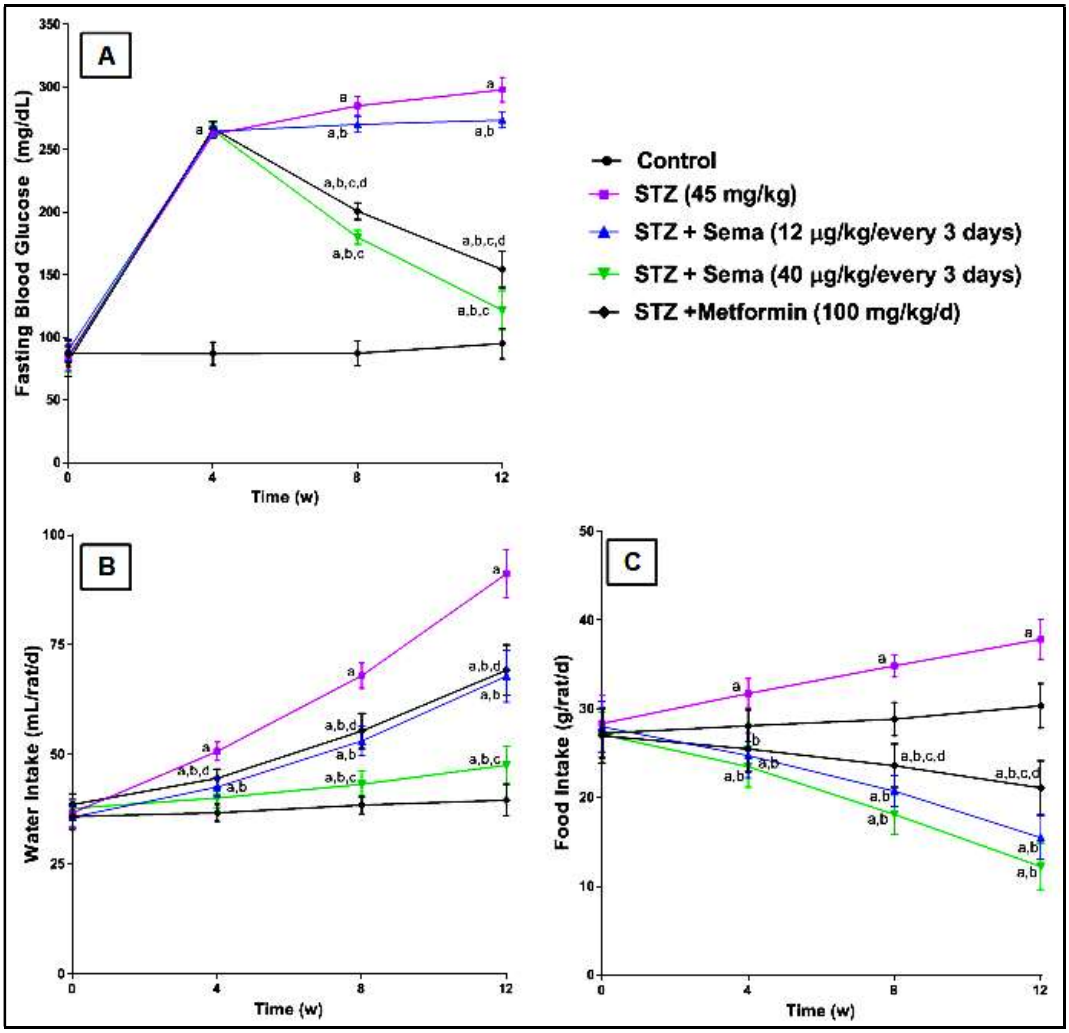

Figure 1: Effect of Semaglutide on (A) FBG, (B) water intake, and (C) feed intake in rats with T2DM-induced MASLD. Values are presented as mean ± SD. Statistics were done using repeated measures ANOVA. a: vs control; b: vs. STZ cohort; c: vs. Semaglutide (12 µg/kg)-treated group; d: vs. Semaglutide (40 µg/kg)-treated cohort. (FBG: Fasting blood glucose; i.p.: intraperitoneally; STZ: Streptozotocin)

Effect of semaglutide in rats with STZ-induced MASLD

As shown in Table 1, the STZ-exposed rats had a significant weight decline ($p < 0.05$), relative to the control at week 12 of treatment. In the STZ-exposed groups, both semaglutide doses produced no changes in body weight, while only Metformin led to a marked rise in body weight ($p < 0.05$). Serum insulin level was significantly increased by 69 % in STZ-exposed group when compared to the control ($p < 0.05$). In addition, treatment of STZ rat groups with semaglutide (12 and 40 µg /kg) or metformin led to insignificant decreases in serum insulin levels. Insulin resistance measured with HOMA-IR showed a five-fold increase in STZ-treated cohort, relative to control. In STZ rat groups, semaglutide (12 µg/kg) significantly ($p < 0.05$) alleviated the increase in HOMA- IR by about 42 %. Then again, high doses of semaglutide and metformin significantly ($p < 0.05$) mitigated the rise in HOMA-IR by 59 and 57 %, respectively.

Effect of semaglutide on hepatic biomarkers and lipid profile

As illustrated in Table 2, STZ exposure resulted in significant rises in serum ALT (> 4-fold), AST (> 5-fold) and TB (> 2-fold), relative to the control cohort, but semaglutide (12 and 40 µg/kg) significantly reversed the increases in hepatic parameters in a dose-related manner. A similar response was seen in the serum lipid profile markers (TC, TG, and LDL). In contrast, serum HDL level was significantly ($p < 0.05$) decreased by about 38 % in STZ-exposed group, when compared to the control.

Table 1: Body weight change, serum insulin level, and homeostatic model assessment of insulin resistance (HOMA IR) in rats with T2DM-induced MASLD at week 12 following Semaglutide treatment

Group	% Body weight change at week 12	Serum insulin level (pg/mL)	HOMA IR
Control	87.36±21.912	582.7±69.33	0.52±0.06
STZ (45 mg/kg)	-20.93±15.79[a]	985.4±74.58[a]	2.65±0.23[a]
STZ + Semaglutide (12 µg/kg /every 3 days)	-7.14±15.78[a]	815.4±138.98[a]	1.533± 0.38[a,b]
STZ + Semaglutide (40 µg/kg /every 3 days)	-8.04±15.88[a]	899.8±222.68[a]	1.065±0.29 [a,b,c]
STZ + Metformin (100 mg/kg/day)	16.39±13.59 [a,b,d]	767.2±94.67	1.125±0.19 [a,b]

Values are expressed as mean ± SD; n = 8. [a]$P < 0.05$ vs control, [b]$P < 0.05$ vs STZ cohort. [c]$P < 0.05$ Semaglutide (12 µg/kg)-treated cohort. [d]$P < 0.05$ semaglutide (40 µg /kg)-treated cohort (STZ:streptozotocin)

Histopathological features

The microscopic assessment of hepatic tissue of rats in the control cohort showed healthy hepatic lobular pattern in which the polyhedral hepatic lobules were separated by a scanty connective tissue having portal spaces. The hepatocytes had eosinophilic cytoplasm and large vesicular nuclei (Figure 2 A). On the other hand, diabetic rats showed noticeable pathological changes manifested by the presence of dilated and irregular central veins with disruption of their endothelial lining, dilated sinusoids, and inflammatory cell infiltration. Moreover, many hepatocytes exhibited vacuolated cytoplasm with indistinct boundaries and pyknotic nuclei, while others displayed cloudy swelling and ballooning of the cytoplasm (Figure 2 B). Treatment of diabetic rats with a low dose of Semaglutide resulted in mild improvement of the hepatic lobular architecture when compared to the untreated diabetic rats (Figure 2 C). Interestingly, diabetic rats treated with a high dose of Semaglutide exhibited significant reversal of the pathological changes. Most hepatocytes displayed their normal appearance, like those of control rats (Figure 2 D). The liver sections from diabetic rats treated with Metformin (Figure 2 E) displayed mild dilatation and congestion of the central veins, and little inflammatory cell infiltration, while some hepatocytes showed vacuolated cytoplasm.

Effect of semaglutide on liver inflammatory and oxidative markers

Oxidative status was assessed in hepatic homogenates by measuring GSH, MDA and CAT. As shown in (Table 3), STZ group had significantly ($p < 0.05$) reduced GSH content (87 % reduction), relative to control cohort. Remarkably, high-dose semaglutide (40 µg/kg) and metformin treatments were more efficacious than low-dose semaglutide, causing significant ($p < 0.05$) increases in GSH content (613 and 571 %, respectively), relative to STZ-exposed cohort. This was confirmed by measuring MDA: STZ produced significant ($p < 0.05$) elevation in MDA concentration by more than four folds (Table 3). On the other hand, Semaglutide treatment at doses of 12 and 40 µg/kg resulted in dose-related declines in MDA concentration by 34 and 70 %, respectively, when compared to STZ-exposed group. The effect of Metformin was comparable to that of high-dose Semaglutide. Furthermore, the activity of CAT was significantly ($p < 0.05$) reduced by 56 % in the STZ-exposed group, when compared to control group (Table 3). Conversely, semaglutide treatment (12 and 40 µg /kg) triggered significant ($p < 0.05$) and dose-dependent rises in CAT activity (68 and 120 %, respectively), relative to STZ-exposed cohort.

Regarding inflammatory status, STZ exposure induced a significant ($p < 0.05$) increase in TNF-α concentration (46 %), when compared to the control group (Table 3). Again, STZ animals treated with semaglutide at both doses (12 and 40 µg/kg) showed marked ($p < 0.05$) but dose-independent reductions in TNF-α levels (29 and 27 %, respectively). The same profile was seen in IL-6 concentration in hepatic homogenate, as indicated in (Table 3). semaglutide treatment (12 and 40 µg/kg) resulted in marked but dose-independent ($p < 0.05$) reductions in IL-6 concentration (32 and 41 %, respectively), when compared to STZ-exposed group. Furthermore, the impact of metformin treatment was comparable to the effect of low-dose semaglutide.

Effect of semaglutide on p-AMPK /mTOR/ERK/ABHD6 signaling cascade

The phosphorylated (active) form of AMPK was assayed in rat hepatic homogenate. As shown in Figure 3 A, STZ-exposed cohort had marked ($p < 0.05$) reduction in AMPK concentration (42 %), relative to the control cohort.

Table 2: Impact of semaglutide on hepatic biomarkers and lipid profile in rats with T2DM-induced MASLD

Group	ALT (U/L)	AST (U/L)	TB (mg/dL)	TC (mg/dL)	TG (mg/dL)	HDL (mg/dL)	LDL (mg/dL)
Control	16.57±2.73	19.32±1.73	0.69±0.05	123.87±6.78	73.28±1.34	42.32±3.57	79.43±2.40
STZ (45 mg/kg)	70.02±8.88[a]	105.60±9.75[a]	1.44±0.15[a]	259.18±19.95[a]	180.35±3.48[a]	25.95±2.85[a]	239.52±7.89[a]
STZ + Semaglutide (12 µg /kg /every 3 days)	36.22±4.18[b,c]	37.52±5.41 [a,b]	0.63±0.06[b]	140.17±13.34[b]	76.10±1.73[b]	40.32±1.95[b]	118.85±4.43[a,b]
STZ + Semaglutide (40 µg /kg /every 3 days)	21.78±1.20[b,c]	29.4±3.92[a,b]	0.61±0.06[b]	116.82±4.59[b,c]	75.07±1.24[b]	44.97±3.84[b]	75.30±3.73[b,c]
STZ + Metformin (100 mg/kg/d**ay**)	19.42±1.99[b,c]	24.53±3.06[b,c]	0.59±0.06[b]	126.85±4.60[b]	74.30±1.44[b]	47.02±4.19[b,c]	73.32±2.05 [b,c]

Values are presented as mean ± S.D. (n = 8; [a]*P* < 0.05 vs control; [b]*P* < 0.05 vs STZ cohort; [c]*P* < 0.05 vs semaglutide (12 µg /kg)-treated cohort. STZ: streptozotocin; ALT: alanine transaminase; AST: aspartate transaminase; TB: total bilirubin; TC: total cholesterol; TG: triglycerides; HDL: high density lipoprotein cholesterol; LDL: low density lipoprotein cholesterol; T2DM: type 2 diabetes mellitus; MASLD: metabolic dysfunction-associated steatotic liver disease

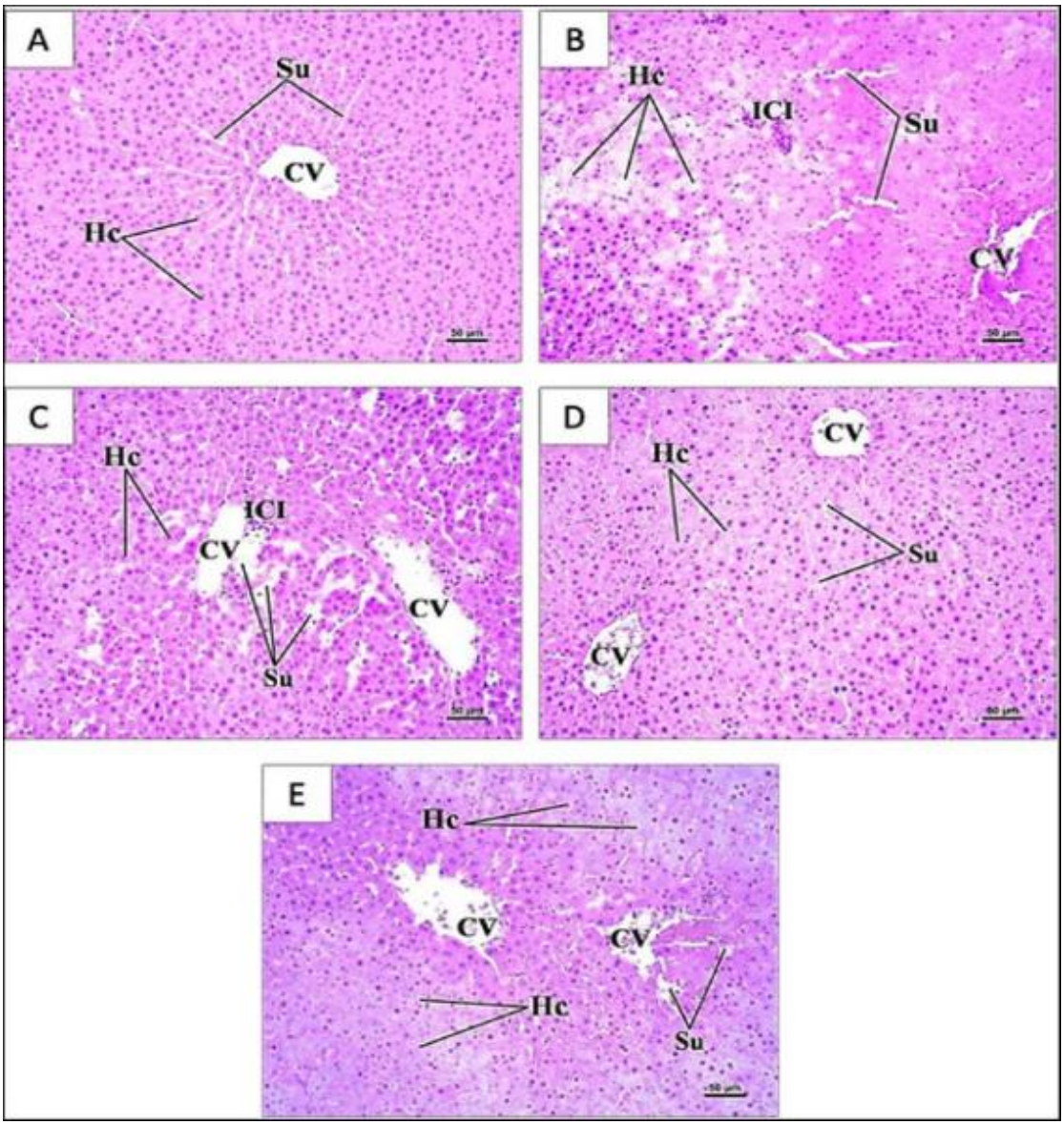

Figure 2: Representative photomicrographs of rat liver sections stained with H&E (x200). (A) control rats showing normal architecture of centrilobular region. Cords of hepatocytes (Hc) are observed radiating from the central vein (CV) and separated by slit-like sinusoids (Su). The hepatocytes have eosinophilic cytoplasm with central nuclei. (B) Diabetic untreated rats showing dilated and irregular CV, dilated Su and inflammatory cell infiltration (ICI) with vacuolated cytoplasm and indistinct boundaries between many Hc. (C) Diabetic rats treated with semaglutide (12 µg/kg/every 3 days) showing slightly dilated CV and Su, with less ICI and vacuolated cytoplasm in some Hc. (D) Diabetic rats treated with semaglutide (40 µg/kg/every 3 days) showed marked improvement, with normal CV, Su and Hc. (E) metformin (100 mg/kg/day)-treated rats showing mild improvement due to slightly dilated CV and Su, with vacuolated cytoplasm in some Hc.

Treatment with semaglutide (12 and 40 µg/kg) and Metformin significantly ($p < 0.05$) increased the concentration of p-AMPK by about 88, 137, and 239 %, respectively. It is worth noting that the concentration of p-AMPK following metformin treatment was more pronounced than that due to semaglutide. Besides, the expression of mTOR was markedly ($p < 0.05$) raised in STZ-exposed cohort by about 124 %, when compared to control cohort (Figure 3 B). Once again, semaglutide treatment (12 and 40 µg/kg) markedly ($p < 0.05$) and dose-dependently decreased mTOR expression (37 and 53 %, respectively), relative to STZ-exposed cohort. Moreover, metformin treatment led to marked ($p < 0.05$) reduction in mTOR expression by nearly 45 %, when compared to STZ-exposed group.

Table 3: Effect of semaglutide on hepatic oxidative stress indices, TNF-α and IL-6 in rats with T2DM-induced MASLD

Group	GSH (µmol/mg protein)	MDA (nmol/mg protein)	CAT (U/mg protein)	TNF-α (pg/mg protein)	IL-6 (pg/mg protein)
Control	0.357±0.062	19.84±3.62	90.99±8.82	205.8±22.69	14.14±2.31
STZ (45 mg/kg)	0.046±0.008[a]	93.74±4.22[a]	39.34±8.72[a]	300.9±33.18[a]	31.27±3.84[a]
STZ + Semaglutide (12 µg/kg/every 3 days)	0.153±0.023[a,b]	61.55±10.89[a,b]	66.15±7.38[a,b]	213.2±25.67[b]	21.09±3.61[a,b]
STZ + Semaglutide (40 µg/kg/every 3 days)	0.328±0.038[b,c]	27.41±2.65[b,c]	86.60±5.78[b,c]	216.9±28.85[b]	18.31±2.73[b]
STZ + Metformin (100 mg/kg/day)	0.309±0.022[b,c]	30.75±3.49[a,b,c]	81.24±5.78[b, c]	268.7±26.11[a,b,c]	21.23±3.23[a,b]

Values are mean ± SD. (n = 8). [a]*P* < 0.05 vs control. [b]*P* < 0.05 vs STZ cohort. [c]*P* < 0.05 vs semaglutide (12 µg/kg)-treated cohort. (STZ: Streptozotocin; GSH: reduced glutathione; MDA: malondialdehyde; CAT: catalase enzyme)

As shown in Figure 3 C, ERK expression was markedly ($p < 0.05$) increased upon STZ exposure by about 59 %, when compared to control group. Interestingly, treatment with only the high-dose semaglutide (40 µg/kg) significantly ($p < 0.05$) deceased the expression of ERK (approximately 35 % reduction), relative to the STZ-exposed cohort. Additionally, metformin caused markedly ($p < 0.05$) higher ERK expression (30 % increase), relative to the semaglutide (40 µg/kg) cohort. Regarding ABHD6, its expression was significantly ($p < 0.05$) augmented in STZ-exposed cohort (77 % increase), when compared to its control (Figure 3 D). Once more, treatment with only the high dose semaglutide (40 µg/kg) significantly ($p < 0.05$) decreased the expression of ABHD6 by about 37 %, when compared to the STZ-exposed group. However, treatment with low-dose semaglutide (12 µg/kg) and metformin (100 mg/kg) did not induce any significant decline of ABHD6 expression. The beneficial effects of Semaglutide are summarized in Figure 4.

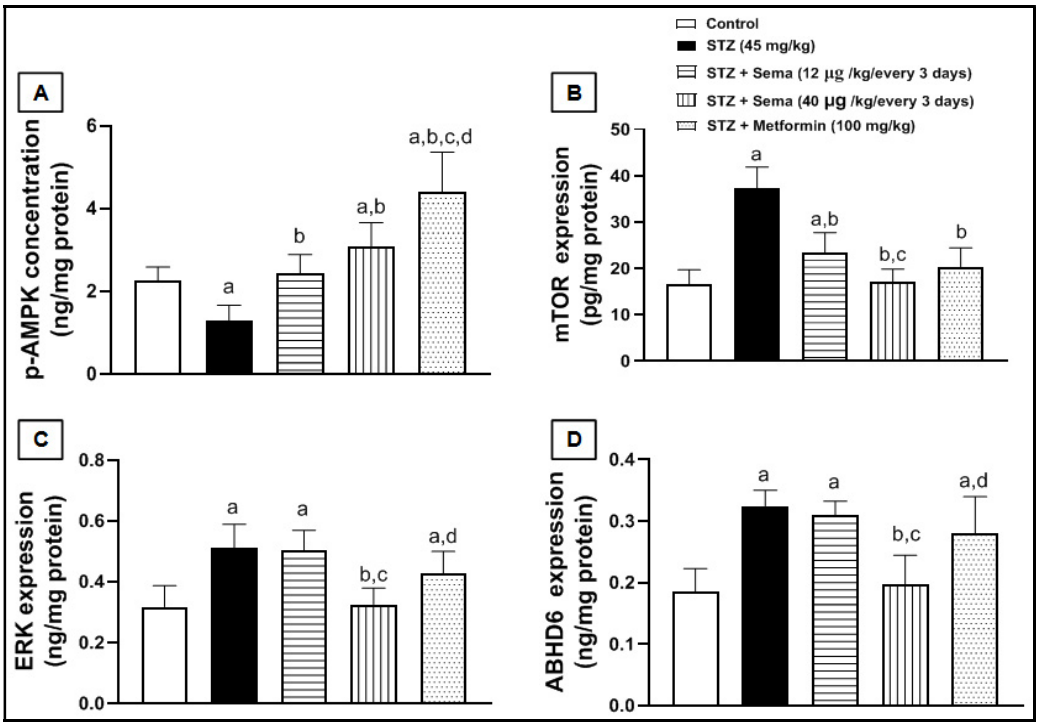

Figure 3: Impact of semaglutide on (A) p-AMPK concentration, (B) mTOR, (C) ERK and (D) ABHD6 expressions in rats with T2DM-induced MASLD. Values are presented as mean ± S.D. (n = 8). [a]*P* < 0.05 vs control. [b]*P* < 0.05 vs STZ cohort. [c]*P* < 0.05 vs semaglutide (12 µg/kg)-treated cohort. [d]*P* < 0.05 vs semaglutide (40 µg/kg)-treated cohort. STZ: Streptozotocin; p-AMPK: phosphorylated adenosine monophosphate-activated protein kinase; mTOR: mammalian target of rapamycin; ERK: extracellular signal regulated kinase; ABHD6: α/β hydrolase domain-6

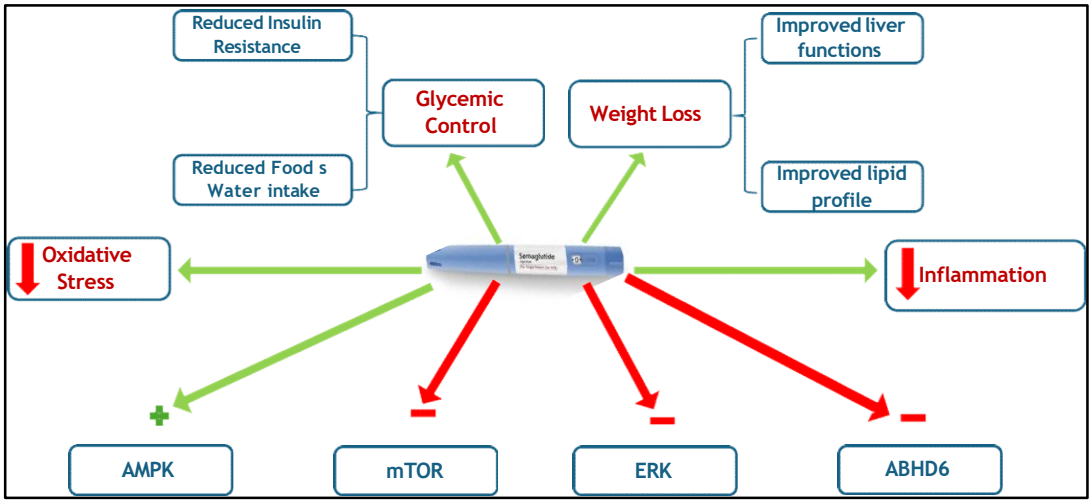

Figure 4: Representative scheme of the potential beneficial effects of memaglutide in diabetic rats with steatotic liver disease

DISCUSSION

In T2DM, the liver is primarily affected by metabolic abnormalities and insulin resistance. However, optimal treatment for MASLD is not well defined, highlighting the need for new therapies. In this regard, semaglutide is recognized for its positive effects on metabolic parameters that are directly associated with MASLD. This research was carried out to study the likely benefits of semaglutide in rats with experimentally-induced T2DM and MASLD, as well as the fundamental mechanisms of action involved. This study combined a low-dose STZ and fructose, for better replication of the clinical features of T2DM and MASLD in humans, when compared to a single high dose of STZ. Similarly, fructose alone does not achieve desirable levels of hyperglycaemia. This model has proven effective in inducing the pathogenesis of both diseases, and it accurately replicates their development. Streptozotocin (STZ) is commonly used to induce diabetes in animal models due to its harmful effects on pancreatic beta cells and its capacity to induce liver steatosis. It causes hyperglycaemia, impaired glucose tolerance, and insulin resistance, and it worsens blood lipid profiles, leading to fat accumulation in the liver and hepatotoxicity, with both diabetogenic and hepatotoxic effects. In this study, two rats from each group died post-STZ administration, possibly due to infection, malnutrition, or STZ toxicity.

The findings from the current study indicate that high-dose semaglutide significantly reduced FBG, thereby enhancing glycaemic control through mechanisms such as enhanced insulin secretion, glucagon suppression, and delayed gastric emptying. However, the low-dose semaglutide was not effective in achieving glycaemic control, as found in previous studies. It serves primarily as a starting dose to help patients tolerate the medication and minimize side effects. In addition, serum insulin and HOMA-IR were significantly reduced, when compared to the STZ group, as semaglutide helps control insulin resistance, partly by inhibiting glucagon secretion [8].

Semaglutide significantly reduced feed intake, when compared to the STZ group. This finding is consistent with results obtained in a previous study [9]. This effect is likely due to the capacity of semaglutide to decrease appetite and delay gastric emptying. While semaglutide does not directly cause dehydration, its side effect, e.g., nausea, may reduce appetite and limit fluid consumption, indirectly leading to dehydration. In addition, this study showed that semaglutide effectively reduced body weight, likely due to its appetite-suppressing and gastric-emptying delay effects.

Diabetes-induced MASLD results in droplets of lipids in over 5% of hepatocytes, elevated LDL and TG, and reduced HDL levels. Liver transaminases are increased in only about 20% of MASLD cases [10]. In this study, the STZ group showed significantly elevated serum AST, ALT, TB, TC, TG, and LDL, with a decrease in HDL. However, semaglutide treatment enhanced these parameters by reducing lipid accumulation and mitigating liver damage. Histological analysis revealed that while the 12 µg/kg dose offered limited protection, the 40 µg/kg dose significantly reduced hepatic injury and prevented further damage, thereby reinforcing the potential of

semaglutide as a hepatoprotective agent in diabetic rats. These results are consistent with those reported in a previous study [4].

Oxidative stress (OS) notably plays a key role in the pathogenesis of various chronic diseases. It results from an imbalance between reactive oxygen species (ROS) production and the capacity of *in vivo* antioxidant defence systems. In this regard, the liver is particularly susceptible to oxidative lesions, due to its high oxidative activity. The consequences of OS are cellular dysfunction, injury, and eventual cell death. Moreover, the compromised antioxidant status in the liver has a substantial function in the pathogenesis and advancement of chronic hepatic diseases, including MASLD [11]. In the current study, OS marker levels were evaluated in order to determine the hepatoprotective effects of semaglutide against MASLD. Rats in the STZ group exhibited compromised antioxidant defence system in the liver. This was evident through substantial elevation in lipid peroxidation, depletion of GSH content, and diminished CAT in hepatocytes. However, the administration of semaglutide alleviated OS in hepatic tissues and mitigated oxidative damage in the liver. In a clinical setting, the administration of injectable Semaglutide therapy resulted in amelioration of OS [12].

In association with OS, inflammation plays a crucial role in the pathophysiology of T2DM, thereby contributing to insulin resistance, impaired insulin function, and liver dysfunction [13]. In this study, Semaglutide reduced the concentrations of proinflammatory indices. This supports its anti-inflammatory effects seen in other models, including MASLD [14]. Low-grade inflammation in obesity promotes MASLD progression by increasing energy intake and impairing the AMPK pathway. Inflammation hinders AMPK activation, leading to cellular damage. In MASLD, steatosis increases OS and proinflammatory cytokines, thereby altering the balance between levels of proinflammatory and anti-inflammatory factors. Therefore, the AMPK pathway is downregulated due to factors such as inflammation, steatosis, lipogenesis, and DNL. The current study has demonstrated a significant decrease in the expression of pAMPK in the liver homogenate of the STZ group. In contrast, Semaglutide, at a dose of 40 µg/kg, effectively enhanced the activation of AMPK, as was evident in the raised expression of pAMPK in the group treated with high-dose Semaglutide. The observed results are in tandem with findings in a previous study where hypericin was found to activate AMPK signalling in mice with high-sucrose, high-fat-mediated MASLD and dyslipidaemia [15].

The hepatic mTOR pathway regulates processes such as lipogenesis, proliferation, cell growth, and protein synthesis in the liver. In MASLD, mTOR influences lipid metabolism, IR, OS, and inflammation [4]. The current study found that STZ-induced T2DM rats had increased mTOR expression which was reduced by semaglutide treatment. This aligns with prior research data showing that semaglutide attenuated mTOR in mice on a high-fat diet (HFD) [4].

The MAPK family comprises widely preserved serine/threonine kinases. Extensive research has been done on ERK, JNK, and p38. These kinases respond to stress signals and regulate various cellular processes. As far as we know, the present research is the first to explore the effect of semaglutide on ERK signalling in an MASLD model. Although treatment with high-dose semaglutide was effective in reducing ERK expression, metformin failed to produce any changes. Therefore, semaglutide, independent of AMPK activation, alleviated MASLD through direct inhibition of the ERK pathway. Given these findings, semaglutide may be a promising drug for future studies into the treatment of MASLD through the ERK pathway. The current findings align with findings from previous studies (including those where herbal Chinese medicine was used), indicating that the attenuation of MAPK signalling pathway has potential to alleviate MASLD [16].

The α/β hydrolase domain-6 is a transmembrane protein expressed in several tissues such as the brain, liver, kidney, and adipose tissue. It plays a role in pancreatic β-cells where its inhibition enhances glucose-stimulated insulin secretion [17]. In mice, ABHD6 deficiency decreased weight gain, improved glucose tolerance and insulin sensitivity, reduced liver steatosis, and increased locomotor activity [18]. In addition, it has been demonstrated that HFD-induced increase in ABHD6 expression in the liver of mice with T2DM and MASLD was reduced following semaglutide treatment [6]. This is in agreement with the results obtained in the present investigation.

CONCLUSION

Semaglutide has a potential hepatoprotective effect on T2DM-induced MASLD in rats. This could be due to its potential to attenuate oxidative damage and inflammation, in addition to promoting hepatic AMPK pathway while reducing the protein expressions of mTOR, ERK, and ABHD6.

DECLARATIONS

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Ethical approval

The study protocol was approved from the research ethics committee at the Faculty of Pharmacy, King Abdulaziz University, Jeddah (approval no. PH-1444-24).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the author(s) named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. SA Alfawaz contributed to study design, animal models, data acquisition study design, data analysis, and manuscript drafting. AS Burzangi contributed to the development of study idea, study design, data analysis, and revision of the first draft of the manuscript. A Esmat contributed to the development of study idea, study design, study supervision, data analysis, and critical revision and submission of the manuscript. All the authors read and approved the final draft of the manuscript for publication.

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