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RESEARCH

Repurposing of semaglutide by targeting SIRT1 and TGF-β/Smad signaling in hepatic fibrosis

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Abstract

The transforming growth factor-β (TGF-β)/Suppressor of Mothers against Decapentaplegic (Smad) signaling pathway plays an important role in the pathogenesis and progression of liver fibrosis. This current study was conducted to investigate the effect of semaglutide (SEMA), a glucagon-like peptide-1 (GLP-1) receptor agonist, in a mouse model of liver fibrosis. The mice received thioacetamide (TAA) (150 mg/kg, biweekly) via intraperitoneal (i.p.) injection for nine consecutive weeks to induce liver fibrosis. SEMA was administered orally once daily at a dose of 0.12 mg/kg. Administration of SEMA improved liver function as demonstrated by the reduction in the plasma levels of aminotransferases and gamma-glutamyl transpeptidase (GGT) along with the rise in serum albumin level. Moreover, SEMA mitigated the TAA-induced histopathological changes and reduced the content of α-smooth muscle actin (α-SMA). SEMA ameliorated the TAA-induced oxidative stress by mitigating the derangement in nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, reducing glutathione (GSH) levels, and decreasing malondialdehyde (MDA) levels. The hepatic TGF-β/Smad signaling pathway was downregulated by SEMA treatment, while sirtuin 1 (SIRT1) content and phosphorylated AMP-activated protein kinase (p-AMPK) expression were upregulated. SEMA significantly reduced the severity of liver fibrosis induced by TAA through SIRT1 activation. And it holds promise as a therapeutic agent for liver fibrosis.

Keywords Liver fibrosis · Semaglutide · Thioacetamide · Oxidative stress · Hepatic stellate cells

Introduction

Liver fibrosis is a pathological repair process of the liver tissue, which can progress into irreversible cirrhosis and even hepatocellular carcinoma. It is one of the leading causes of morbidity and mortality worldwide. The hallmarks of liver fibrosis are the excessive deposition of extracellular matrix (ECM) and accumulation of collagen within the extracellular space (Wynn 2007). Upon liver injury, hepatic stellate cells (HSCs), the major players in liver fibrosis, are activated and transdifferentiated into myofibroblast-like cells. This leads to overproduction of collagen and deposition of ECM, which ultimately alters the normal liver architecture and negatively affects liver function (Huang et al. 2013).

Transforming growth factor-β (TGF-β) plays a pivotal role in the fibrotic process and is considered a key driver of fibrosis (Rahman et al. 2022; Meng et al. 2015; Chávez et al. 2008; Budi et al. 2021). TGF-β1 suppresses hepatocyte proliferation (Wang et al. 2009), while stimulating HSC proliferation and transformation into myofibroblasts (Ioannou et al. 2017);(Ioannou et al. 2013). HSCs further upregulate TGF-β1 expression in both autocrine and paracrine manners, thereby activating the membrane receptor, transforming growth factor-β receptor type I (TβRI). This activation promotes Suppressor of Mothers against Decapentaplegic 2 (Smad2) and Suppressor of Mothers against Decapentaplegic 3 (Smad3) phosphorylation (Yoshida and Matsuzaki 2012), leading to their complex formation and HSC activation (Dooley et al. 2003).

Oxidative stress plays a role in the pathogenesis of liver fibrosis. Reactive oxygen species (ROS) stimulate HSC activation and hepatocyte necrosis and apoptosis (Luangmonkong et al. 2018). Moreover, TGF-β is a redox-sensitive gene;

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therefore, ROS increase TGF-β expression in HSCs (Diesen and Kuo 2011). In addition, studies have indicated that TGF-β stimulates ROS production in fibroblasts by the activation of the membrane-bound enzyme nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (Urtasun et al. 2008; Paik et al. 2014). Consequently, targeting oxidative stress and the TGF-β signaling pathway has emerged as a promising therapeutic strategy for the treatment of fibrosis (Li et al. 2021; Györfi et al. 2018; Peng et al. 2022).

Sirtuin 1 (SIRT1) has a regulatory role in oxidative stress response, lipid homeostasis, and inflammatory reaction in liver tissues (Ding et al. 2017). Evidence has accumulated indicating that SIRT1 activation or overexpression may be detrimental to fibrogenesis in various organs. For example, it enhances survival rates and mitigates pulmonary fibrosis (Akgedik et al. 2012), and inhibits HSC activation and counteracts liver fibrosis in mice (Li et al. 2018). Hepatocyte-specific deletion of SIRT1 results in hepatic inflammation and steatosis (Purushotham et al. 2009). Recently, SIRT1 activation has been shown to ameliorate rhesus monkey liver fibrosis by inhibiting the TGF-β/Smad signaling pathway (Xia et al. 2024). Therefore, activating the SIRT1 signaling pathway can potentially alleviate liver fibrosis.

Semaglutide (SEMA) is a glucagon-like peptide-1 receptor agonist (GLP-1RA). It is currently utilized for weight loss and in the management of certain cases of type 2 diabetes mellitus (T2DM) (Chen et al. 2022). Recent research has shown that SEMA improves liver biomarkers in patients with T2DM (Chen et al. 2022) and reduces the severity of non-alcoholic fatty liver disease (NAFLD) and liver damage (Volpe et al. 2022). Moreover, GLP-1RAs have been found to enhance mitochondrial beta-oxidation of free fatty acids, promote the clearance of very low-density lipoproteins (VLDL), reduce de novo lipogenesis, and improve insulin resistance (Armstrong et al. 2016). Liraglutide, another GLP-1RA, has been shown to ameliorate the HSC phenotype, reduce cell proliferation, and possess antifibrotic activity (de Mesquita et al. 2017). Furthermore, exenatide, another GLP-1RA, has been found to ameliorate hepatic steatosis through SIRT1 activation (Xu et al. 2014). On the other hand, recent meta-analyses and systematic reviews have revealed that SEMA does not significantly improve the fibrosis stage compared to placebo (Zhu et al. 2023), indicating that further studies are needed. Therefore, the current study aims to evaluate the effects of SEMA in a mouse model of liver fibrosis, focusing on the role of SIRT1 in modulating oxidative stress and TGF-β/Smad signaling pathway.

Materials and methods

Animals

Adult male Swiss mice, each weighing 20–30 g, were obtained from the National Research Center for this study. The mice were kept in the MSA animal house under standard housing conditions, room temperature (25 ± 2 °C), humidity (60 ± 10%), and a constant light cycle for 1 week for adaptation before being subjected to the experiment. Normal laboratory water and chow will be provided ad libitum.

The investigation has been approved by the Ethical Committee of the Faculty of Pharmacy, Cairo University, approval number (PT3521), and the Ethical Committee for Animal Experimentation at the Faculty of Pharmacy, MSA University, approval number (PH16/REC16/2023MSc), based on the ethics guideline for the care and use of animals, and according to the 2014 Egyptian Constitution's article 45, the World Organization for Animal Health (WOAH), and the Guide for the Care and Use of Laboratory Animals, 8th Edition 2011.

Drugs and chemicals

Thioacetamide (TAA) was purchased from Sigma-Aldrich (Saint Louis, MO, USA). SEMA was purchased from Novo Nordisk (Denmark). All other chemicals were of analytical grade.

Experimental design

In this study, 20 mice were divided into four groups ($n = 5$ per group) and subjected to the following protocol: **Group I (Control group):** The mice received normal saline by intraperitoneal (i.p.) injection, administered twice weekly for 9 weeks. **Group II (TAA group):** The mice received TAA dissolved in normal saline, at a dose of 150 mg/kg by i.p. injection, administered twice weekly for 9 weeks (El-Tanbouly et al. 2017). **Group III (TAA + Sema group):** The mice were injected intraperitoneally with TAA at the same dose regimen given to Group II and were orally treated with SEMA at a dose of 0.12 mg/kg/day (Rakhat et al. 2023) through the last 3 weeks of the experimental period. **Group VI (Sema group):** The mice were injected intraperitoneally with normal saline at the same dose regimen given to Group I and were orally treated with SEMA at a dose of 0.12 mg/kg/day (Rakhat et al. 2023) through the last 3 weeks of the experimental period.

After the last dose of drugs/vehicle treatment, mice were anesthetized by sodium thiopental (20 mg/kg; i.p.) (Akter et al. 2022). Blood samples were collected from the retro-orbital sinus, and plasma samples were separated for the biochemical assessment of liver function tests. The mice were then euthanized, and their livers were quickly dissected. Portions of the livers from each group were preserved in 10% formalin, prepared in normal saline for histological examination. Whereas, tissues from the same portions of the liver

were collected and stored at − 80 °C for further biochemical analyses using western blot and enzyme-linked immunosorbent assay (ELISA) techniques.

Histopathological examination

Liver samples fixed in 10% formalin were used to prepare paraffin blocks. Sections of 5 µm were then obtained and stained with hematoxylin & eosin (H&E) stain (Grizlle et al. 2008). The examination was done using an electric light microscope, and Adobe Photoshop (version 8) was used to capture and process the images. Moreover, other sections were stained with Masson's Trichrome (Sigma, USA) to identify collagen fibers in liver tissues, and to assess the degree of fibrosis (El-Tanbouly et al. 2017). Six distinct, non-overlapping fields were randomly chosen and analyzed per tissue section in each sample to determine the area percentage of reactive collagen fibers in Masson's trichrome-stained sections. Data were collected using a full HD microscopic imaging system and Leica Application software for tissue section analysis (Leica Microsystems GmbH, Germany), operated by the examining histologist. All standard procedures for sample fixation and staining protocols were performed per Culling, C.F.A. (2013).

Biochemical measurements

Determination of liver function tests

Plasma samples were used to estimate the activity of alkaline phosphatase (ALP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT), as well as the level of gamma-glutamyl transferase (GGT) calorimetrically using the commercially available kits (BioMed Diagnostics, Egy-Chem for lab technology, Badr City, Egypt; Cat. # ALP101090, GOT111060, GPT113100, and GGT124030, respectively). The procedures were done according to the manufacturer's instructions. Moreover, the bilirubin level was estimated in the plasma using a colorimetric kit obtained from BioMed-Diagnostic (Egy-Chem for lab technology, Badr City, Egypt; Cat. # ALB100250).

Determination of oxidative stress biomarkers

Oxidative stress status was evaluated in liver homogenate by assessing the levels of reduced glutathione (GSH), an essential non-enzymatic antioxidant in mammalian cells, and malondialdehyde (MDA), a final product of lipid peroxidation, using corresponding ELISA kit (Biodiagnostics Co. Giza, Egypt; Cat. # GR 25 10 and MD 25 29, respectively). Moreover, NADPH oxidase content was measured using a specific ELISA kit obtained from ELK Biotechnology (Wuhan, China; Cat. # ELK2600) according to the manufacturer's instructions.

Determination of α-SMA and TGF-β content

The levels of the pro-fibrotic cytokine TGF-β, as well as the HSC activation marker α-smooth muscle actin (α-SMA), were measured in the liver homogenate (10% w/v) using the corresponding mouse-specific ELISA kits (MyBiosource, San Diego, USA; Cat. # MBS267551 and MBS160136, respectively). The procedures were performed according to the manufacturer's instructions, and the results were expressed as ng/mg protein and pg/mg protein, respectively.

Determination of p-Smad3 and p-AMPK expression

The protein expression levels of the p-Smad3 and phosphorylated AMP-activated protein kinase (p-AMPK) were analyzed using the western blot technique. Firstly, the liver tissue proteins were extracted, and the protein concentrations were determined using the Bradford protein assay kit (BIO-BASIC, Markham, Ontario, Canada; Cat. # SK3041). Protein extracts were heat-denatured in boiling water, and the lysates containing equal protein amounts from each group were separated by SDS–polyacrylamide gel electrophoresis (SDS-PAGE) using 10% polyacrylamide gels and transferred onto polyvinylidene difluoride membranes (Pierce, Rockford, IL, USA). After that, the membranes were subsequently incubated for 2 h at room temperature in a blocking solution containing 0.05% Tween 20 and 5% Bovine Serum Albumin (BSA) in Tris-buffered saline (Sigma, St. Louis, MO, USA). The membranes were incubated overnight at 4 °C with p-Smad3 or p-AMPK alpha-1 monoclonal antibody (Thermo Fisher Scientific, Waltham, USA). The membranes were then incubated with secondary antibodies conjugated to horse-radish peroxidase (Rockland Immunochemicals, Gilbertsville, PA) at 37 °C for 1 h. Band intensity was analyzed by Molecular Imaging Software, Kodak version 4.0 (Rochester, NY). The results were expressed as arbitrary units after normalization for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) protein expression.

Determination of SIRT1 level

The level of SIRT1 was measured in liver homogenate using a mouse-specific ELISA kit (MyBiosource, San Diego, USA; Cat. #MBS2601957) following the manufacturer's instructions, and the results were expressed as ng/mg protein.

Determination of protein content

Protein content was measured using Bradford protein assay kit (BIO-BASIC, Markham, Ontario, Canada; Cat. # SK3041).

Statistical analysis

All statistical tests were carried out using GraphPad Prism® software, version 8 (GraphPad Software, Inc., USA). The data were expressed as means ± standard error (SEM). The one-way analysis of variance (ANOVA) test was used to analyze the results, followed by the Tukey–Kramer multiple comparison test. The level of significance was set at $p < 0.05$ for all statistical tests.

Result

Effect of SEMA on liver histopathological changes in mice with TAA-induced liver fibrosis

Liver fibrosis induced by TAA in mice was estimated by H&E staining. As shown in Fig. 1, the control group samples exhibited intact, normal, and organized morphological structures of hepatic parenchyma and hepatocytes, with intact vasculature, as illustrated in Fig. 1a. However, TAA group samples showed marked disorganization of histological features of hepatic parenchyma with extensive hepatocytomegaly, hyperchromatic binucleated cells, and hepatocellular degenerative changes with multiple apoptotic figures. In addition, significant dilatation of hepatic blood vessels, severe mononuclear inflammatory cells infiltrate all over hepatic lobules and perivascular areas, as well as substantial perivascular and interlobular fibroblastic activity, were observed along with an obvious bridging of hepatic lobules by newly formed collagen fibers, as shown in Fig. 1b. TAA + SEMA samples showed significant hepatocellular protective efficacy with abundant figures of apparent intact hepatocytes, fewer nucleomegally, and a marked reduction in fibroblastic activity. However, moderate focal mononuclear cell infiltrates were shown with moderate dilatation and congested hepatic vasculatures, as shown in Fig. 1c. SEMA samples showed apparent intact, well-organized histological features of hepatic parenchyma, as shown in Fig. 1d. Scale bars were added to the figure.

Regarding Masson's trichrome staining, as shown in Fig. 2, the livers from the control group had clear central veins and intact lobular architecture with no collagen deposition, as shown in Fig. 2a, an effect which is almost the same as that of the SEMA-only treated group, as shown in Fig. 2d. However, the livers of the TAA-treated group showed major collagen deposition in the liver, as shown in Fig. 2b. SEMA treatment after TAA treatment significantly reduced the accumulation and deposition of collagen fibers, as shown in Fig. 2c. Scale bars were added to the figure. All the original data generated

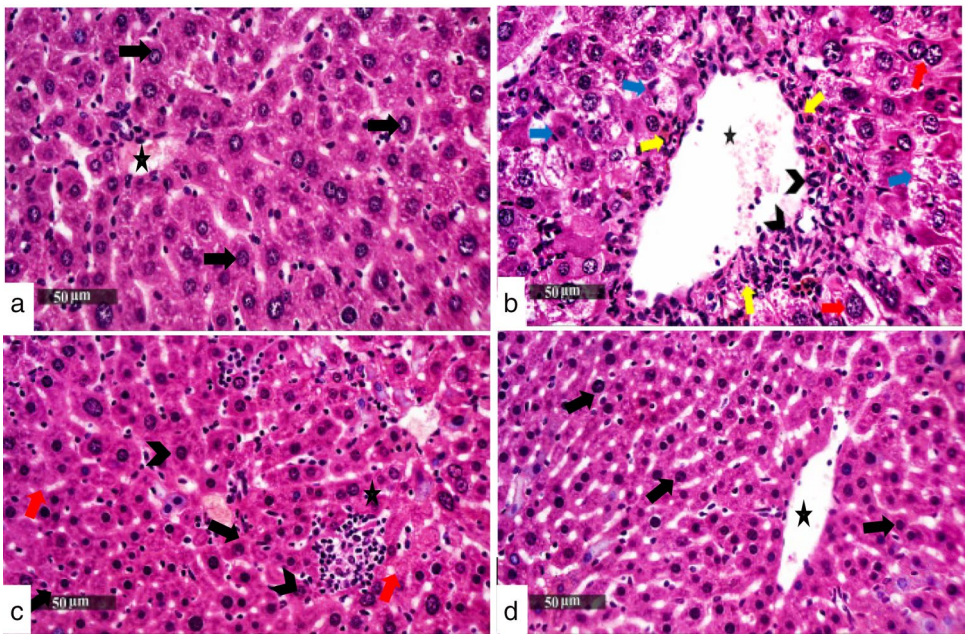

Fig. 1 Effects of SEMA on histopathological examination in mice with TAA-induced liver fibrosis. Representative panels demonstrating H&E staining in liver tissues (50×original magnification) from **a** control group showing intact hepatocytes and subcellular details (***arrow***), intact vasculatures (***star***), **b** TAA group showing hyperchromatic binucleated cells (***red arrow***), multiple apoptotic figures (***blue arrow***), fibroblastic activity (***yellow arrow***), significant dilatation of hepatic blood vessels (***star***),inflammatory cell infiltrates (***arrow head***), **c** TAA + SEMA group showing intact hepatocytes (***black arrow***), nucleomegally (***red arrow***), mononuclear cells infiltrates (***arrow head***), moderate dilatation and congested hepatic vasculatures (***star***), **d** SEMA group showing intact hepatocytes and subcellular details (***arrow***), intact vasculatures (***star***)

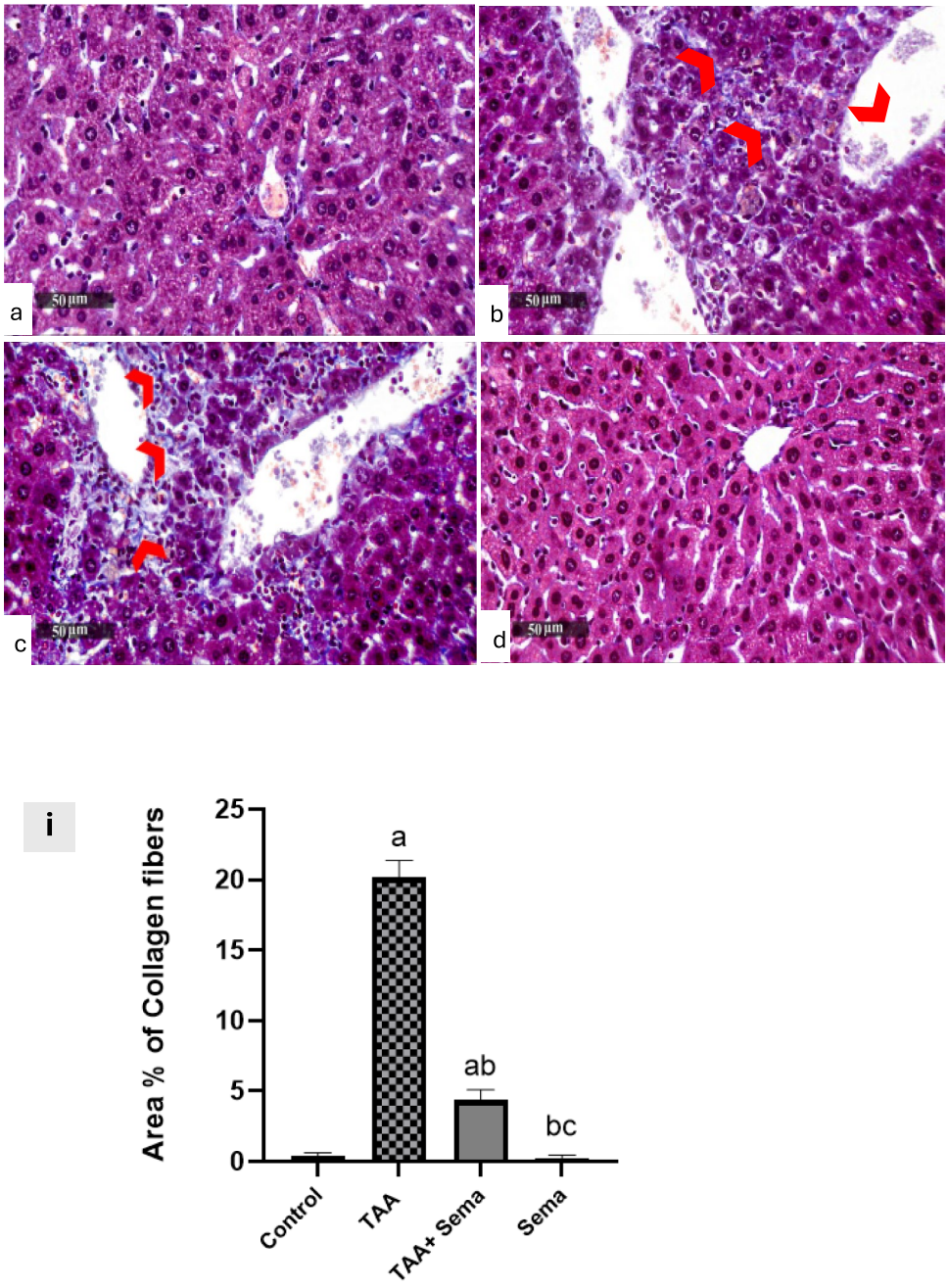

Fig. 2 Representative panels demonstrating Masson's trichrome staining in liver tissues (50× original magnification) from **a** control group, **b** TAA group showing major collagen deposition (***arrow head***), **c** TAA + SEMA group showing reduced collagen deposition (***arrow head***), **d** SEMA group. Panel (i): Area % of collagen fibers in mice with TAA-induced liver fibrosis. Values were expressed as the mean of 5 experiments ± SEM. For statistical analysis of the data, a one-way ANOVA followed by a Tukey–Kramer post hoc test was used. a: significantly different from the control group at $p < 0.05$. b: significantly different from the TAA-treated group at $p < 0.05$. c: significantly different from the TAA + SEMA-treated group at $p < 0.05$. H&E hematoxylin and eosin stain, TAA thioacetamide, SEMA semaglutide

or analyzed in this section are included in the supplementary file titled (Original data).

Effect of SEMA on liver function tests in mice with TAA-induced liver fibrosis

TAA injection for 9 weeks resulted in a significant elevation in plasma ALP, AST, and ALT activities compared to the control group, as shown in Fig. 3a, b, c, respectively. Treatment with SEMA was effective in normalizing the ALT activity and reducing the raised activities of both ALP and AST in mice with TAA-induced liver fibrosis. Regarding the SEMA-only treated group, it is almost the same as the control group (Fig. 3). All the original data generated or analyzed in this section are included in the supplementary file titled (Original data).

Compared to the control group, TAA significantly elevated the plasma GGT level while attenuating the plasma albumin levels, as shown in Fig. 4a, b, respectively. SEMA treatment, along with TAA, normalized the plasma levels of both GGT and albumin. Animals that received SEMA alone showed almost no change when compared to the control group (Fig. 4).

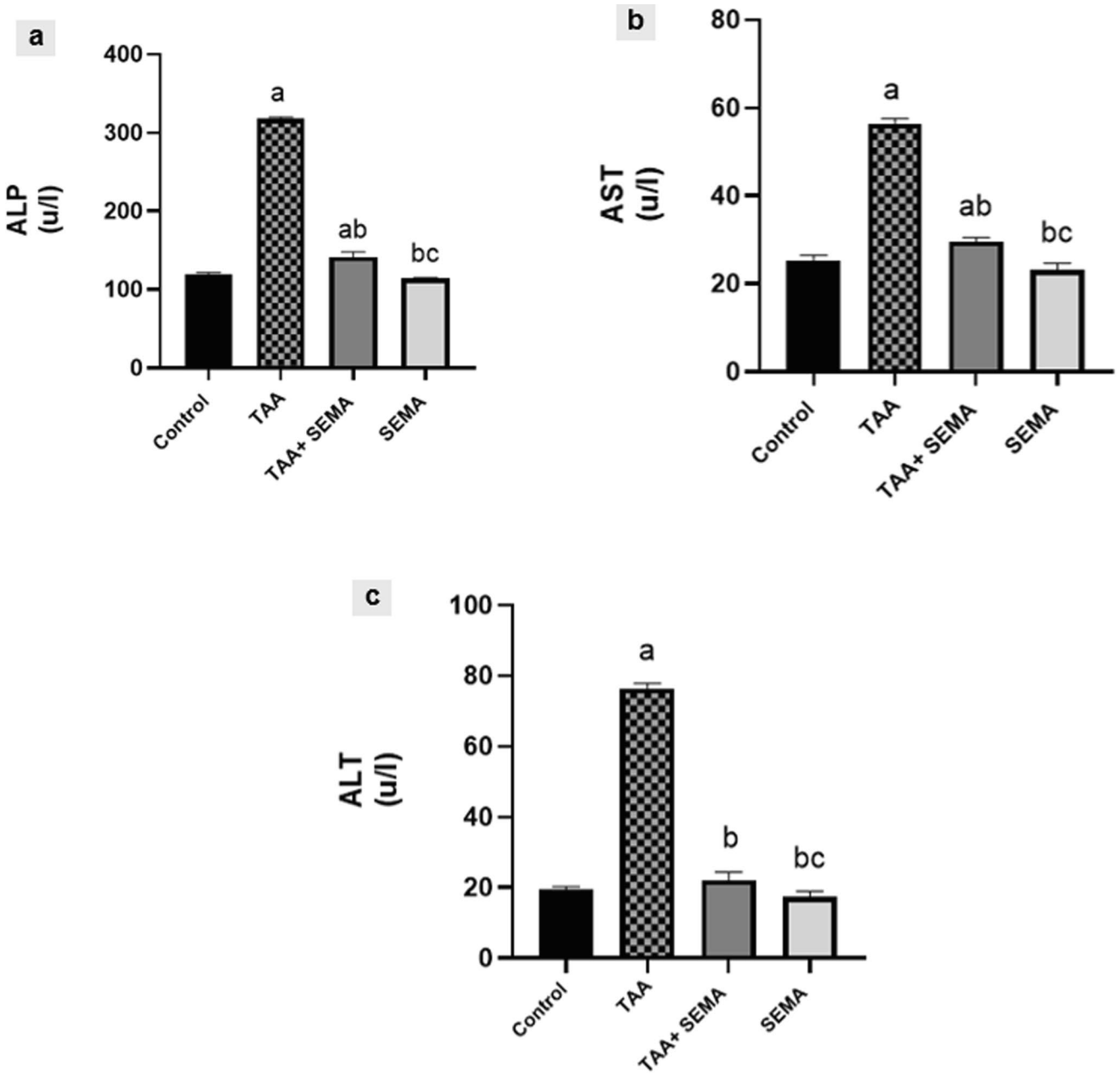

Fig. 3 Effects of SEMA on plasma **a** ALP, **b** AST, and **c** ALT activities in mice with TAA-induced liver fibrosis. Values were expressed as the mean of 5 experiments ± SEM. For statistical analysis of the data, a one-way ANOVA followed by a Tukey–Kramer post hoc test was used. a: significantly different from the control group at $p < 0.05$. b: significantly different from the TAA-treated group at $p < 0.05$. c: significantly different from the TAA + SEMA-treated group at $p < 0.05$. TAA thioacetamide, SEMA semaglutide, ALP Alkaline phosphatase, AST aspartate aminotransferase, ALT Alanine aminotransferase

All the original data generated or analyzed in this section are included in the supplementary file titled (Original data).

Effect of SEMA on oxidative stress biomarkers in mice with TAA-induced liver fibrosis

TAA induced a state of oxidative stress compared to the control group, as evidenced by a marked rise in NADPH oxidase levels, a significant decrease in GSH content, and an increase in MDA levels in the livers of the TAA group. However, SEMA treatment modulated the oxidative stress status by attenuating NADPH oxidase levels, restoring GSH content, and reducing MDA accumulation in liver tissues. Almost no or minimal change was observed in mice treated only with SEMA compared to the control group, as shown in Fig. 5a, b, c, respectively. All the original data

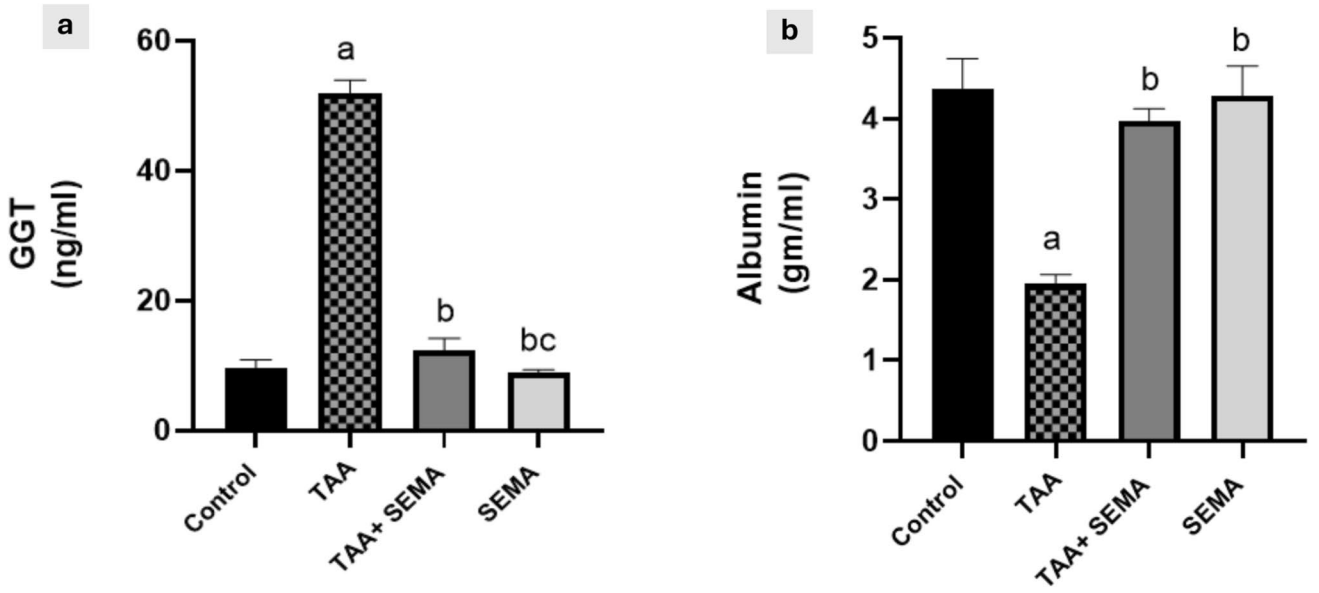

Fig. 4 Effect of SEMA on plasma levels of **a** GGT and **b** albumin in mice with TAA-induced liver fibrosis. Values were expressed as the mean of 5 experiments ± SEM. For statistical analysis of the data, a one-way ANOVA followed by a Tukey–Kramer post hoc test was used. a: significantly different from the control group at $p < 0.05$. b: significantly different from the TAA-treated group at $p < 0.05$. c: significantly different from the TAA + SEMA-treated group at $p < 0.05$. TAA thioacetamide, SEMA semaglutide, GGT gamma-glutamyl transpeptidase

generated or analyzed in this section are included in the supplementary file titled (Original data).

Effect of SEMA on HSCs activation and TGF-β/Smad signaling pathways in mice with TAA-induced liver fibrosis

The hepatic content of α-SMA, an activation marker for HSCs, was significantly elevated in the livers of TAA-treated mice compared to the control group. However, SEMA treatment normalized α-SMA levels in TAA-treated mice. No statistically significant change was observed in mice treated with SEMA alone, compared to the control group, as shown in Fig. 6a. Similarly, TGF-β levels were significantly elevated in TAA-treated mice relative to the control values. Consequently, the western blotting assay revealed that p-Smad3 expression in the TAA-treated group was higher than in the control group, as shown in Fig. 6b, c, respectively. The uncropped figures of western blotting were shown in the supplementary materials file under the title Fig. 1. On the other hand, these observed increases were significantly reduced in mice treated with SEMA along with TAA. The mice treated only with SEMA showed almost the same as the control group (Fig. 6). All the original data generated or analyzed in this section are included in the supplementary file titled (Original data).

Effect of SEMA on SIRT1/AMPK signaling pathway in mice with TAA-induced liver fibrosis

The hepatic SIRT1 levels as well as the p-AMPK protein expression were remarkably reduced in the TAA-treated group compared to the control group values, as shown in Fig. 7a, b, respectively. The uncropped figures of western blotting of AMPK were shown in the supplementary materials file under the title Fig. 2. Treatment with SEMA markedly upregulated the hepatic SIRT1 content and the protein expression of p-AMPK in TAA-treated mice. However, the SEMA-alone treated group showed almost the same findings as the control group (Fig. 7). All the original data generated or analyzed in this section are included in the supplementary file titled (Original data).

Discussion

Liver fibrosis is a progressive condition that can lead to severe complications such as cirrhosis and hepatocellular carcinoma (HCC) if left untreated. Therapeutic strategies that prevent fibrosis progression or repair hepatic damage are of critical importance. The current study demonstrates that SEMA, GLP-1 RA, effectively attenuates liver fibrosis induced by TAA through a multifaceted mechanism involving anti-inflammatory, antioxidative, and antifibrotic effects.

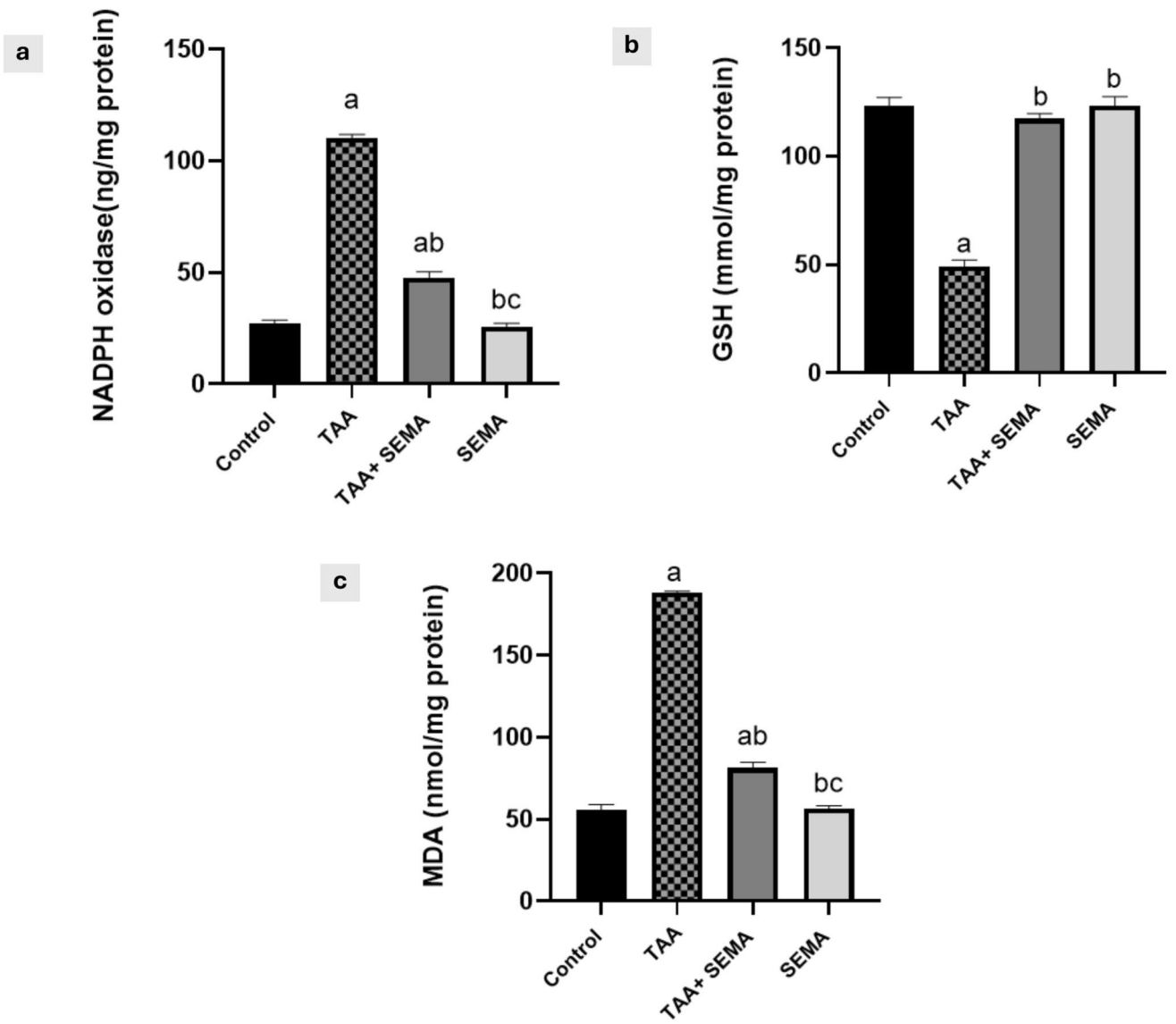

Fig. 5 Effect of SEMA on **a** NADPH oxidase, **b** GSH, **c** MDA levels in hepatic tissues of mice with TAA-induced liver fibrosis. Values were expressed as the mean of 5 experiments ± SEM. For statistical analysis of the data, a one-way ANOVA followed by a Tukey–Kramer post hoc test was used. a: significantly different from the control group at $p < 0.05$. b: significantly different from the TAA-treated group at $p < 0.05$. c: significantly different from the TAA + SEMA-treated group at $p < 0.05$. TAA thioacetamide, SEMA semaglutide, NADPH oxidase nicotinamide adenine dinucleotide phosphate oxidase, GSH glutathione, MDA malondialdehyde

In the present study, TAA administration for 9 weeks resulted in liver histopathological alteration, degenerative changes, inflammation, and apoptosis. This is consistent with findings from previous studies on TAA-induced liver fibrosis (Abdelhamid et al. 2021). Conversely, SEMA treatment demonstrated significant hepatoprotective effects against TAA-induced liver fibrosis, maintaining intact hepatocytes. Similar results were reported in a NAFLD mouse model treated with SEMA, where it alleviated lesions such as steatosis, lymphoid foci, and ballooning degeneration (Niu et al. 2022).

The histopathological changes were reflected in the biochemical measurements, as TAA injection induced marked elevation in the plasma levels of aminotransferases (ALP, AST, and ALT) as well as GGT, all of which are indicative of hepatocellular damage. Consistently, TAA was found to impair ribonucleic acid (RNA) transport from the nucleus to the cytoplasm, leading to cellular damage and increased biomarker levels in the blood (Thilagavathi et al. 2023). Although the plasma levels of ALP, AST, and ALT in the TAA-treated mice remained within the published normal ranges for healthy mice, their levels were significantly

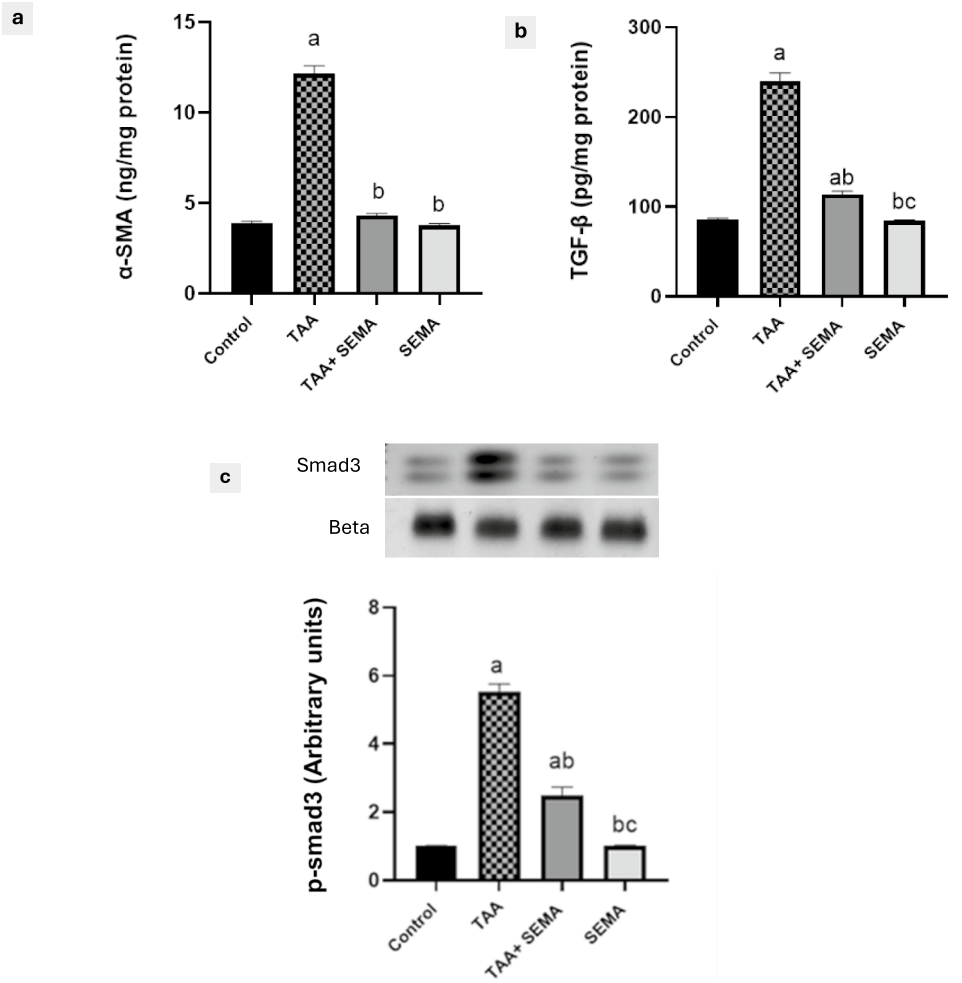

Fig. 6 Effect of SEMA on **a** hepatic α-SMA levels, **b** TGF-β levels, and **c** Smad3 protein expression in mice with TAA-induced liver fibrosis. Values were expressed as the mean of 5 experiments ± SEM. For statistical analysis of the data, a one-way ANOVA followed by a Tukey–Kramer post hoc test was used. a: significantly different from the control group at $p < 0.05$. b: significantly different from the TAA-treated group at $p < 0.05$. c: significantly different from the TAA + SEMA-treated group at $p < 0.05$. TAA thioacetamide, SEMA semaglutide, α-SMA alpha smooth muscle actin, TGF-β transforming growth factor beta, Smad3 Suppressor of Mothers against Decapentaplegic homolog 3

elevated compared to our internal control group, as there is a significant difference between the two groups as shown in Fig. 3. However, our study was designed to induce moderate liver injury to model chronic hepatic damage, without the need to cause acute liver failure or high mortality. Importantly, the liver histology in the TAA-treated group showed fibrosis and hepatocellular damage. This supports our TAA-fibrosis model and the biochemical results. Treatment with SEMA markedly decreased the plasma levels of these enzymes in line with a recent study on NAFLD, where SEMA reduced aminotransferase levels (Ghusn et al. 2023). SEMA also reversed the TAA-induced reduction in serum albumin levels. Chronic inflammation in patients with liver cirrhosis has been shown to decrease albumin mRNA and suppress albumin production, primarily through interleukin-1 signaling (Wake et al. 2020).

Oxidative stress plays a role in the pathogenesis of liver fibrosis. ROS stimulate HSC activation, a key driver of liver fibrosis (Luangmonkong et al. 2018; Ibrahim et al. 2023). TAA is activated in the liver by the Cytochrome P450 2E1 (CYP450 2E1) enzyme, leading to the production of both TAA-S-dioxide and TAA-S-oxide, which induce oxidative stress and lipid peroxidation of the hepatic cell membrane (Ezhilarasan 2023). Consistently, TAA injection, in the present study, induced oxidative stress as evidenced by the rise in the level of NADPH oxidase, a generator of ROS, and the drop in the content of the antioxidant GSH in the hepatic tissues, with subsequent increase in the hepatic level of MDA, an end product of lipid peroxidation. However, SEMA treatment modulated the oxidative stress status and mitigated the derangement in the hepatic levels of NADPH oxidase, GSH, and MDA. In the same context, SEMA was recently found to impact hepatic oxidative stress in obese rats (Alzubaidy and Al-Isawi 2024). The antioxidant properties of SEMA were previously recorded in several experimental and clinical studies (Yaribeygi et al. 2024).

As a redox-sensitive gene, the expression of the potent fibrogenic cytokine TGF-β is upregulated by ROS in HSCs (Diesen and Kuo 2011). In addition, studies have indicated that TGF-β stimulates ROS production (Urtasun et al. 2008; Paik et al. 2014). TGF-β accelerates the activation of HSCs, which in turn secrete additional TGF-β, creating a feedback

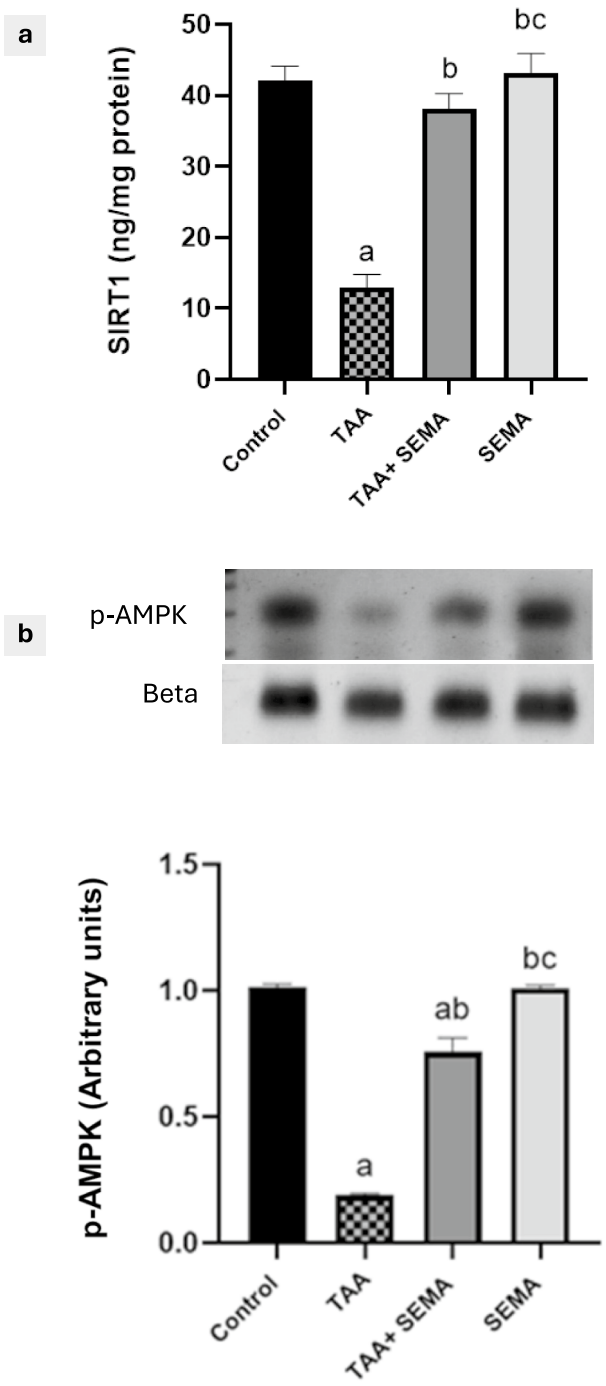

Fig. 7 Effect of SEMA on **a** SIRT1 levels and **b** p-AMPK protein expression in liver tissues of mice with TAA-induced liver fibrosis. Values were expressed as the mean of 5 experiments ± SEM. For statistical analysis of the data, a one-way ANOVA followed by a Tukey–Kramer post hoc test was used. a: significantly different from the control group at $p < 0.05$. b: significantly different from the TAA-treated group at $p < 0.05$. c: significantly different from the TAA + SEMA-treated group at $p < 0.05$. TAA thioacetamide, SEMA semaglutide, SIRT1 sirtuin 1, AMPK AMP-activated protein kinase

loop that exacerbates the fibrotic process (Hellerbrand et al. 1999; Tsuchida and Friedman 2017). Activated TGF-β binds to transforming growth factor-β receptor type II (TβRII) and phosphorylates TβRI, which in turn phosphorylates Smad proteins. The phosphorylated Smad forms complexes that translocate into the nucleus, where they bind to deoxyribonucleic Acid (DNA), and regulate the expression of profibrotic molecules, including collagen I and α-SMA (Abdelhamid et al. 2021). The TAA administration was found to induce oxidative stress and upregulate the TGF-β signaling pathway (Ibrahim et al. 2023). Consistently, the present findings showed that TAA injection increased the hepatic TGF-β levels, Smad protein expression, and α-SMA content, while the treatment with SEMA modulated the TGF-β/Smad/α-SMA signaling pathway, an effect that could be attributed to its antioxidant effect. Reduced α-SMA expression impairs cytoskeletal signaling in HSCs, thereby attenuating liver fibrosis (Rockey et al. 2019). GLP-1RAs have been found to inhibit fibrosis in various solid organs. For example, the lungs, heart, liver, and kidneys (Yang et al. 2022). Liraglutide, one of the GLP-1RAs, was recently revealed to suppress TGF-β/Smad signaling pathway in a rat model of diabetes-induced testicular dysfunction (Fathy et al. 2023).

Growing evidence suggests that SIRT1 functions as a key regulatory sensor in the cellular response to oxidative stress. Its protective effects against oxidative stress involve multiple mechanisms. SIRT1 inhibits NADPH oxidase to trigger an antioxidant protective response. Moreover, SIRT1 directly or cooperatively with p-AMPK activates various downstream effectors, including peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1α), forkhead box O (FOXOs), and peroxisome proliferator-activated receptor alpha (PPARα) (Meng et al. 2020). AMPK regulates lipid metabolism through the intracellular adenosine monophosphate (AMP) phosphorylation to adenosine triphosphate (ATP) in the liver, and exhibits an antifibrotic effect by reducing inflammation and oxidative stress (Liang et al. 2017). Therefore, SIRT1 serves as a crucial metabolic sensor in various tissues and has been extensively documented for its vital role in alcoholic liver disease, NAFLD, and HCC (Singh et al. 2018; Finkel et al. 2009; Gao et al. 2016; Wu et al. 2014). The SIRT1 expression is reduced in NAFLD, but its overexpression alleviates the disease (Tian et al. 2024). Recently, SIRT1 was found to inhibit the TGF-β/Smad signaling pathway in the monkey model of liver fibrosis and was suggested to serve as a crucial protective factor against liver fibrosis (Xia et al. 2024). In the current study, TAA-induced liver fibrosis reduced SIRT1 levels and p-AMPK protein expression compared to the normal control group, while the treatment with SEMA elevated SIRT1 levels and upregulated p-AMPK. SEMA alleviates early brain injury via the SIRT1 pathway in a recent study

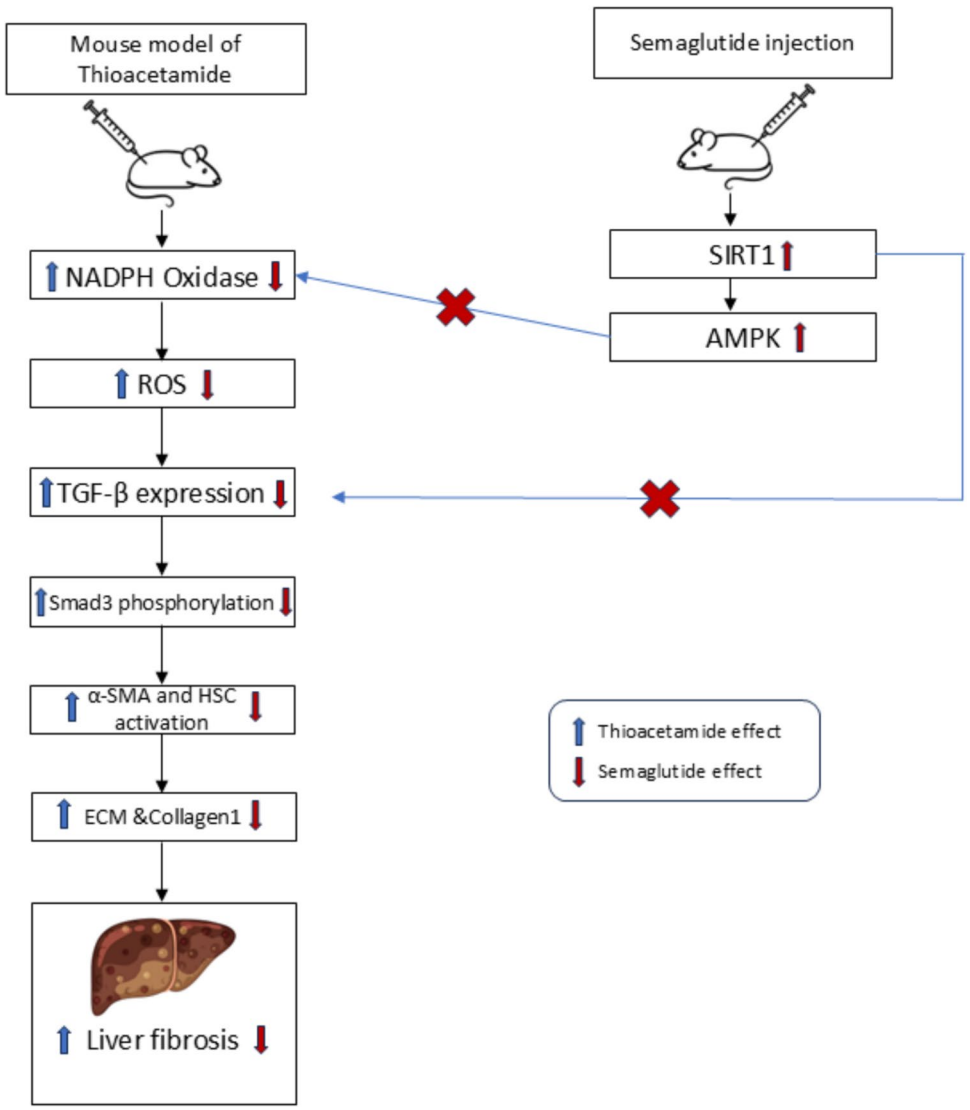

Fig. 8 Mechanistic figure of SEMA mechanism in attenuating TAA-induced hepatic fibrosis via SIRT1/AMPK activation and inhibition of TGF-β/Smad signaling. TAA thioacetamide, SEMA semaglutide, SIRT1 sirtuin 1, AMPK AMP-activated protein kinase, TGF-β transforming growth factor beta, Smad3 Suppressor of Mothers against Decapentaplegic homolog 3

(Chen et al. 2024). In addition, detailed antioxidant mechanisms of SEMA via modulating the activity of p-AMPK and SIRT1 were recently reviewed (Papakonstantinou et al. 2024).

Conclusion

In conclusion, SEMA significantly reduced the severity of liver fibrosis induced by TAA by modulation of oxidative stress, TGF-β/Smad signaling pathway, and HSC activation. These effects are mediated by the upregulation SIRT1/p-AMPK signaling, as shown in (Fig. 8). The potential reduction of liver fibrosis severity of SEMA holds promise as a therapeutic agent for liver fibrosis. However, additional clinical studies are necessary to evaluate whether this promising antifibrotic effect will translate into actual therapies against liver fibrosis.

Limitations

Some limitations should be acknowledged. First, the study was conducted in a mouse model only, which may not mimic the complexity of human liver pathology. Second, this study only used adult male Swiss mice and didn't use any female mice. Future studies could use both sexes to enhance the translational relevance of the findings. Third, the sample size was relatively small, which could increase the possibility of errors and affect the statistics of certain parameters. Finally, the used TAA dose and duration in this study caused relatively normal plasma levels of ALP, AST,

and ALT, as plasma levels remained within the reported normal ranges. Longer duration or higher TAA could provide a more comprehensive picture of liver damage.

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Data availability The datasets and all the original data generated during and/or analysed during the current study are included in the supplementary file titled (Original data). And the original uncropped images of western blotting are included in the supplementary file titled (supplementary data for western blotting).

Declarations

Ethics approval The investigation has been approved by the Ethical Committee of the Faculty of Pharmacy, Cairo University; Approval number (PT3521, Cairo, Egypt), and the Ethical Committee for Animal Experimentation at the Faculty of Pharmacy, MSA University, Approval number (PH16/REC16/2023MSc, Giza, Egypt), based on the ethics guideline for the care and use of animals, and according to the 2014 Egyptian constitution article 45, the World Organization for Animal Health (WOAH), and the, 2011.

Competing interests The authors declare no competing interests.

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