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Ertugliflozin, a SGLT-2 Inhibitor, Guards Against Thioacetamide-induced Liver Fibrosis: The Nrf2/HO-1 and TLR4/ TGF-β1 Pathways

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ABSTRACT

Hepatic fibrogenesis is considered an epidemic health problem since it can lead to several insults that can be fatal. Ertugliflozin (Ertu), an inhibitor of the sodium-glucose cotransporter-2 (SGLT2), is one of the most recent anti-diabetic medications used to treat type 2 diabetes mellitus (T2DM). In a variety of human and animal models, SGLT2 inhibitors demonstrated anti-inflammatory, anti-fibrotic, and antioxidant qualities. Consequently, we designed the present investigation to clarify the preventive role of ertugliflozin in male rat liver fibrosis brought on by thioacetamide (TAA) as well as the anticipated mechanisms. 24 rats were divided into four groups: "a control group, "TAA group" (received intraperitoneal injections of 100 mg/kg b.wt. twice a week for six weeks), and "TAA + Ertu" groups (received oral Ertu at doses of 5 and 10 mg/kg b.wt. for four weeks in addition to TAA injections). Ertugliflozin promoted hepatic antioxidant effects by considerably increasing HO-1, Nrf2 protein and mRNA expression, GSH and SOD levels, and lowering hepatic MDA content. It also greatly reduced TAA-induced changes in liver function measures. Additionally, ertugliflozin suppressed the elevated levels of "PI3K, TGF-β1, α SMA, and caspase3" and enhanced the hepatic anti-inflammatory state by declining the pro-inflammatory cytokines "TNF-α, IL-6, and TLR4" levels. Histological examination showed that ertugliflozin significantly inhibited the liver alterations caused by TAA. Our findings imply that ertugliflozin's hepatoprophylactic effects may be mediated by improving antioxidant capacities and reducing inflammatory signals by modifying the Nrf2/HO-1 and TLR4/TGF-β1 pathways.

Keywords: Ertugliflozin, Liver fibrosis, Nrf2/HO-1 pathway, Thioacetamide, TLR4.

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INTRODUCTION

Hepatic fibrogenesis is regarded as a serious public health issue because of high rates of morbidity and death **(Kim *et al.*, 2024)**. Recurrent liver injury, which can be brought on by viral infections, medication toxicity, alcoholic and nonalcoholic steatohepatitis, can result in hepatic fibrogenesis **(Ramadan *et al.*, 2018)**. Liver fibrosis may develop into cirrhosis, liver cancer, or even liver failure if left untreated **(Lee *et al.*, 2019; Mohammed *et al.*, 2023)**. External stimuli that activate hepatic stellate cells (HSCs) cause them to secrete many pro-inflammatory cytokines and deposit extracellular matrix (ECM) proteins excessively **(Abd El-Rahman and Fayed 2019; Elbaset *et al.*, 2023).**

The defense mechanisms of the cells include a number of coordinated antioxidant enzymes that eliminate excess reactive oxygen species (ROS) **(Bhattacharyya *et al.*, 2014)**. Cellular antioxidant status is largely controlled by "nuclear factor erythroid 2-related factor 2 (Nrf2)" that provides defense against excessive ROS and modulates a number of cytodefensive proteins **(Satta *et al.*, 2017)**. A crucial transcription factor known as Nrf2 sustains cellular homeostasis by increasing the expression of antioxidant genes, which in turn inhibits the generation of ROS and the inflammation response **(Alsharif *et al.*, 2022)**. Normally, Kelch-like ECH-associated protein-1 (Keap1) keeps Nrf2 in the cytoplasm. Upon entering

the nucleus after oxidative stress, Nrf2 attaches itself to ARE, antioxidant response element, and initiates multiple antioxidants' transcription **(Raslan *et al.,* 2021)**. Prior research has demonstrated that SGLT2 inhibitors enhance the Nrf2/HO-1 pathway **(Tsai *et al.,* 2021).** Thus, we hypothesized that ertugliflozin might prevent experimental liver fibrosis by activating the "Nrf2 pathway".

Furthermore, the class of receptors known as toll-like receptors (TLRs) is distinguished by its capacity to identify patterns and is capable of precisely detecting infections and chemicals generated from bacteria **(Hassan *et al.,* 2025)**. A typical example of a TLR, TLR4 modulates both innate and adaptive immune responses and is crucial for triggering inflammation **(Mukherjee *et al.,* 2016)**. The inflammatory response may be moderated by TLR4, which triggers the NF-κB pathway and other downstream inflammatory components **(Caso *et al.,* 2007)**. Recent investigations explored that hyperglycemia increases the expression of TLR4 **(Wang *et al.,* 2020)**. Consequently, we postulated that ertugliflozin could inhibit the TLR4 pathway and thereby prevent experimental liver fibrosis.

Sodium glucose co-transporter 2 (SGLT2) inhibitors are more recent hypoglycemic treatments that reduce blood sugar levels by preventing the kidneys from reabsorbing glucose **(Vivian 2014).** The risk of hypoglycemia is minimal because this class of hypoglycemic medications operates entirely independently of the insulin hormone **(Chao 2014).** SGLT2 inhibitors are attractive anti-inflammatory drugs because they can directly alter inflammatory signaling pathways or indirectly improve metabolism and lower stress levels **(Elrakaybi *et al.,* 2022)**. The novel oral antidiabetic drug ertugliflozin has been shown to regulate changes linked to metabolism, lower the risk of heart failure, and slow the advancement of renal disorders **(Croteau *et al.,* 2021).**

Instead of stimulating insulin production, ertugliflozin blocks renal reabsorption of filtered glucose by suppressing SGLT2, which raises urine glucose excretion **(Derosa and Maffioli 2018)**. Therefore, unlike medications that encourage the release of insulin, ertugliflozin does not result in hypoglycemia **(Frias 2019)**. However, it is uncertain how ertugliflozin affects liver fibrosis. Therefore, our study's objective was to clarify the protective effects of Erut medication against TAA-induced hepatic fibrogenesis in a rat model and explore its mechanism related to Nrf2/HO-1 activation and TLR4 signaling pathway suppression. In clinical settings, because fatty liver and fibrosis are common in diabetes patients, the medication has a dual function.

MATERIALS AND METHODS

Experimental animals

We acquired adult male Wistar rats from the "Animal House Colony at the "National Research Centre" "(NRC, Egypt)" that were "six to eight weeks old and weighed 180 to 220 g." The animals were housed in standard laboratory cages (plastic cages with metal covers) with 6 animals per cage. They were provided with ad libitum access to a standard rodent chow (food pellets) and tap water. The animals were maintained under a 12-hour light/12-hour dark cycle with controlled temperature (22 ± 2°C) and humidity (55 ± 10%). The cages were cleaned regularly to maintain a hygienic environment. "National and international ethical standards" were adhered to when caring for every animal. The Cairo University Institutional Animal Care and Use Committee" (Vet CU13102024978) has permitted all experimental protocols.

Drug and Chemical

Thioacetamide was acquired from "Sigma-Aldrich in the United States". Germany's Merck was the source of ertugliflozin (STEGLATROTM).

Research design

Twenty-four rats were randomly assigned (six rats each to one of four groups) after a week of acclimatization: The control group (Group 1 rats) was given intraperitoneal (IP) saline twice a week for six weeks. Based on earlier research in our lab, our group determined the TAA dosage that can cause liver fibrosis **(Elbaset *et al.,* 2023; Elbaset *et al.,* 2024; Hassan *et al.,* 2025).** Group 2 rats (also known as the "TAA group") received intraperitoneal injections of TAA (100 mg/kg b.wt.) twice a week for six weeks in order to induce hepatic fibrogenesis **(Abd El-Rahman and Fayed 2019)**. Groups three and four (the treatment groups) were given two oral Erut dosages daily for four weeks at doses of 5 and 10 mg/kg b.wt. beginning two weeks after the TAA injections and continuing concurrently with TAA for 4 weeks **(Pang *et al.,* 2023).**

Preparation of blood and liver tissues

Blood was drawn from the tail vein twenty-four hours following the last injection while under anesthesia using intraperitoneal injections of ketamine (50 mg/kg) and xylazine (25 mg/kg) diluted in saline. Decapitation was performed after euthanasia in a CO_2 euthanasia chamber. Serum samples were frozen at -20°C for the biochemical assay. Immediately after removal, the livers were cleansed in ice-cold saline and allowed to dry. A section of the liver was stored for "molecular and biochemical analyses at -80°C." For immunohistochemistry and histology, a separate

portion was preserved in "10% buffered neutral formalin".

Assessment of liver injury indicators

The liver enzymes "alanine aminotransferase (ALT) and aspartate aminotransferase (AST)" and albumin were identified by enzymatic colorimetric methods using kits from "the Bio-diagnostic Company, Dokki, Giza, Egypt", "Catalog No AL 10 31 (45), AS 10 61 (45), and AB 10 10" respectively.

Evaluation of oxidation stress

As instructed by the manufacturer, "Bio-diagnostic Company, Dokki, Giza, Egypt" kits were used to measure the levels of "reduced glutathione (GSH), superoxide dismutase (SOD), and malondialdehyde (MDA)" in hepatic homogenate (Cat# GR2511, SD2521, MD2529).

Liver inflammation and pro-fibrosis biomarkers

"Toll-like receptor 4 (TLR4), tumor necrosis factor (TNF-α), interleukin-6 (IL-6), transforming growth factor-beta (TGF-β1), and phosphotidylinositol-3-kinase (PI3K)" were assayed in hepatic homogenate using specific "Rat ELISA kits (SunLong Biotech Co., LTD, China; Catalogs Number: SL0699Ra, SL0722Ra, SL0411Ra, SL0705Ra, and SL0571Ra respectively)" as directed by the manufacturer.

ELISA measurement of Nrf2 level

The ELISA method was used to test the Nrf2 level in liver tissues "(Cat# SL0985Ra)" using a "Sunlong Biotech Co., Ltd., China kit" in compliance with the manufacturer's instructions.

Evaluation of the expression of the "Nrf2 and HO-1" genes in the liver

Amplification sizes, primer sequences, target genes, and cycle parameters for SYBR green rt-PCR are listed in Table 1 outlined by **Yuan *et al.*, (2006)**.

Table 1: Amplification sizes, primer sequences, target genes, and cycle parameters for SYBR green rt-PCR:

		"Sequence (5′-3′)"
Nrf2 **(Yamashita *et al.*, 2014)**	"F"	"CACATCCAGACAGACACCAGT"
	"R"	"CTACAAATGGGAATGTCTCTGC"
HO-1 **(Chu *et al.*, 2020)**	"F"	"GGCTTTAAGCTGGTGATGGC"
	"R"	"GGGTTCTGCTTGTTTCGCTC"
Rat ß. Actin **(Banni *et al.*, 2010)**	"F"	"TCCTCCTGAGCGCAAGTACTCT"
	"R"	"GCTCAGTAACAGTCCGCCTAGAA"

Liver histology

Liver tissues were collected, cleaned, dried, preserved in 10% neutral buffered formalin. Haematoxylin and eosin staining was subsequently applied to the 5-micron-thick slices for histological examination **(Bancroft and Gamble 2008)**. Masson's trichrome stain (MTC) was used to further stain liver slices in order to evaluate hepatic fibroplasia. A Japanese light microscope, the Olympus BX50, was used to examine all stained sections.

Scores for histopathological lesions

The liver's histological alterations were identified and categorized as following: mild (1), moderate (2), severe (3), and no change (0). The grading was done by percentage, with mild changes being those that were less than 30%, moderate changes being those that were between 30% and 50%, and severe changes being those that were greater than 50% **(El-Maksoud *et al.*, 2020)**. "Image J 1.52 p software" "(Wayne Rasband, National Institutes of Health (U.S.A.))" was used to assess and quantify liver fibrosis as an area percentage **(Baraka *et al.*, 2023)**. High-power (x 400) microscopes in ten microscopical regions were analyzed.

Immuno-histochemical evaluation

Immunohistochemistry was performed using the procedures outlined by **Shamseldean *et al.*, (2022)**. Graded alcohol was used to rehydrate liver tissue sections after they had been deparaffinized in xylene. In order to inhibit endogenous peroxidase activity, Hydrogen Peroxide Block (Thermo Scientific, USA) was introduced. Tissue slices were pretreated with 10 mM citrate and then heated in a microwave oven for 10 minutes in order to retrieve the antigen.

Caspases-3 and α-SMA immunostaining evaluation in hepatic tissue

According to **Deabes *et al.*, (2025)**, the immune-reactivity of "caspase-3 and α-SMA" was evaluated in five liver sections. A high-power (x400) microscope was used to analyze the immune-reactivity of each segment in 10 microscopical regions. The percentage of positively stained cells (%) was estimated by color deconvolution image J 1.52 p

software (Wayne Rasband, National Institutes of Health (U.S.A.)).

Statistics of the experiment

Statistics were done according to **Elbaset *et al.*, (2023)** "Using the Shapiro test, values were guaranteed for normality. Means ± S.E. are used to represent the results. The Tukey–Kramer Post hoc test was performed after one-way analysis of variance to process the data. The figures were created and the statistical analysis was carried out using GraphPad Prism program (version 10, California, USA). The significance level was set to $p < 0.05$ for all statistical tests".

RESULTS

Ertugliflozin's efficacy on hepatic function indicators in rats given TAA

TAA significantly impairs liver function, as seen by elevation in ALT and AST of 568.18% and 527.08%, respectively, and a decrease in albumin of 56.51% in comparison with the control group. Ertu (5 mg/kg) considerably improved these outcomes when compared to the TAA group, increasing albumin by 72.96% and decreasing "ALT and AST" by 65.15% and 62.26%, respectively. When compared to the TAA group, the higher dosage (10 mg/kg) resulted in superior results and brought these parameters closer to normal control levels, boosting albumin by 117.47% and decreasing "ALT and AST" by 76.06% and 74.57%, respectively **(Fig. 1A,B,C)**.

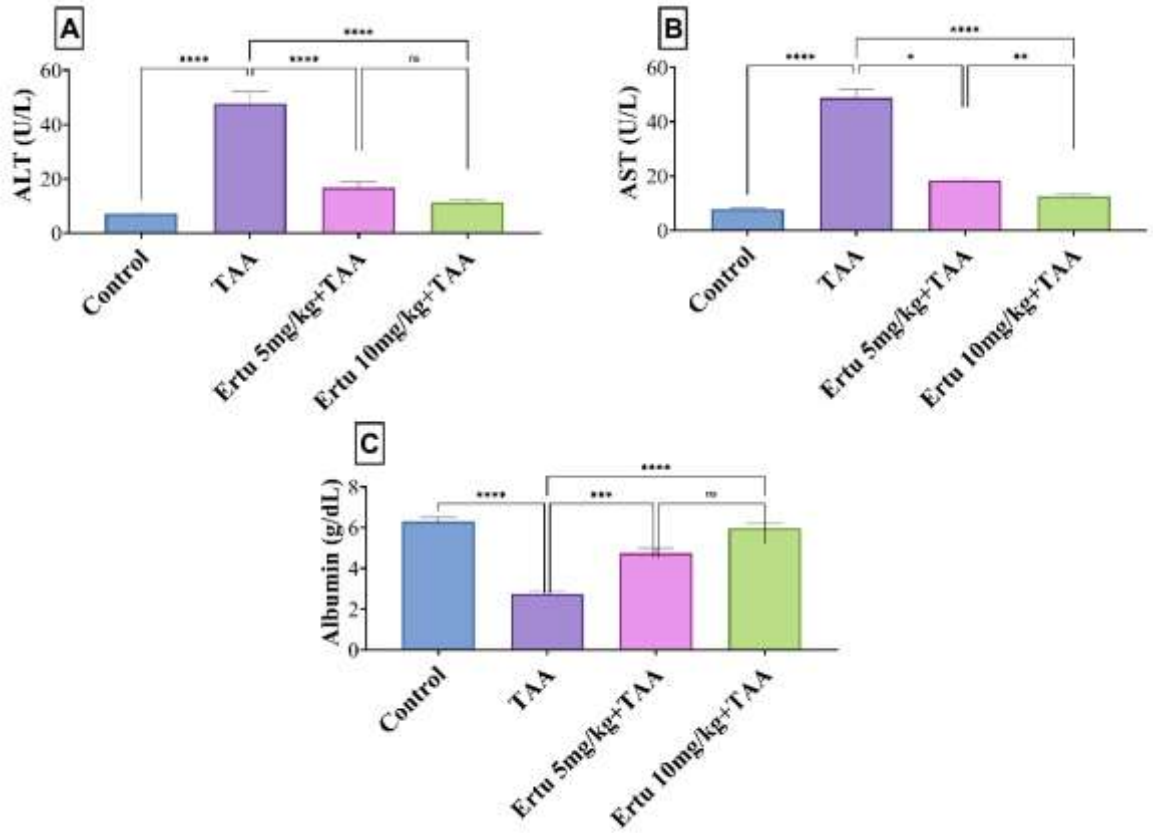

Fig. 1: Assessment of Ertugliflozin impact on hepatic function: AST, ALT, albumin in TAA intoxicated rats. (A) "Serum ALT" (U/L). (B) "Serum AST" (U/L). (C) "Serum albumin" (mg/dL). Data are displayed as mean ± SEM of six rats, with p-values displayed on the bars. TAA: Thioacetamide; Ertu: Ertugliflozin.

Ertugliflozin's efficacy on oxidation stress indicators in rats given TAA

TAA caused significant oxidative stress, increasing MDA by 672.02% and lowering GSH and SOD by 85.35% and 73.88%, respectively, in comparison to the control. These indicators were improved in a dose-dependent manner following Erut therapy. The dose of 5 mg/kg raised "GSH and SOD" in comparison to the TAA group by 442.33% and 220.39%, respectively, and decreased "MDA" by 71.77%. The advantages of the 10 mg/kg dose were more pronounced than in the TAA group, with "MDA" being reduced by 76.35% and "GSH and SOD" being raised by 595.76% and 342.82%, respectively, almost normalizing these levels (**Fig 2 A, B, C**).

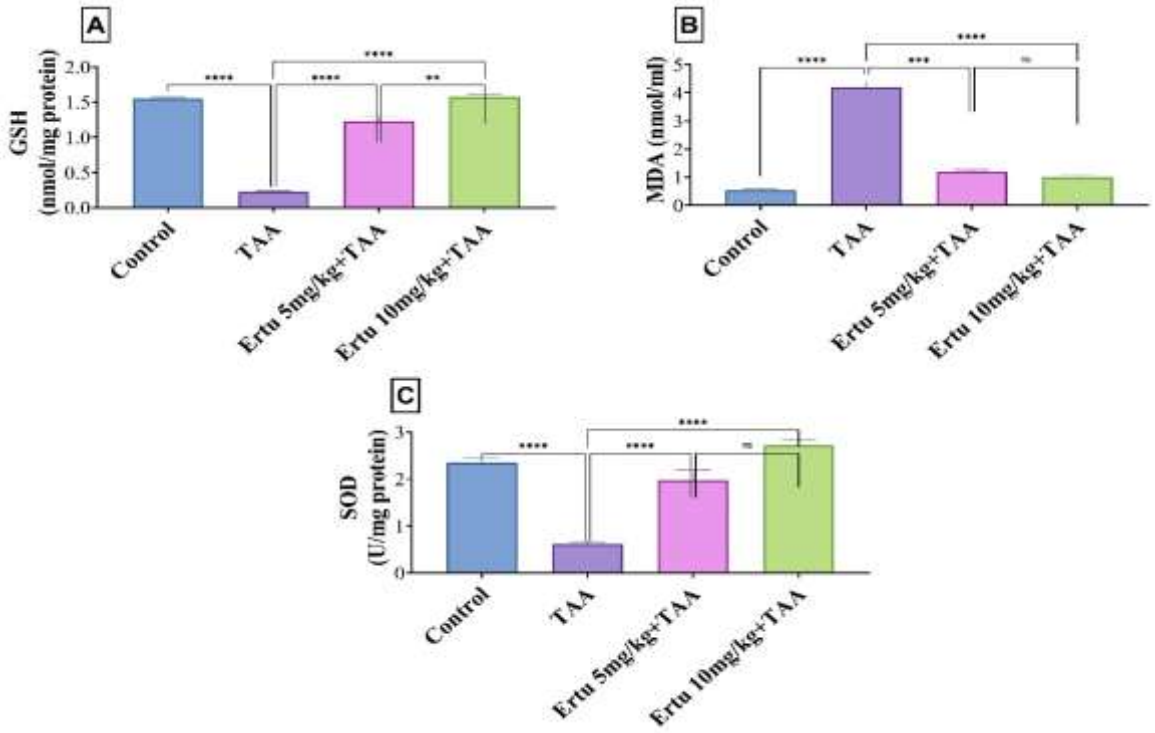

Fig.2: Ertugliflozin impact on oxidative stress markers: GSH, MDA, SOD in TAA-intoxicated rats. (A) "GSH (nmol/mg protein)". (B) "MDA (nmol/ml)". (C) "SOD (U/mg protein) activity". The data are displayed as mean ± SEM of six rats, with p-values displayed on the bars. TAA: Thioacetamide; Ertu: Ertugliflozin.

Ertugliflozin's efficacy on inflammatory indicators in rats given TAA

Comparing to the control, TAA markedly amplified inflammation, raising TLR4, IL-6, and TNF-α level by 611.23%, 402.71%, and 365.36%, respectively. These inflammatory indicators were lowered in a dose-dependent manner by Ertu therapy. Comparing the 5 mg/kg dose of Ertu to the TAA group, these values decreased by 51.91%, 62.48%, and 54.99%, respectively. The 10 mg/kg dose, on the other hand, showed more noticeable effects, reducing them by 56.65%, 69.58% **(Fig.3).**

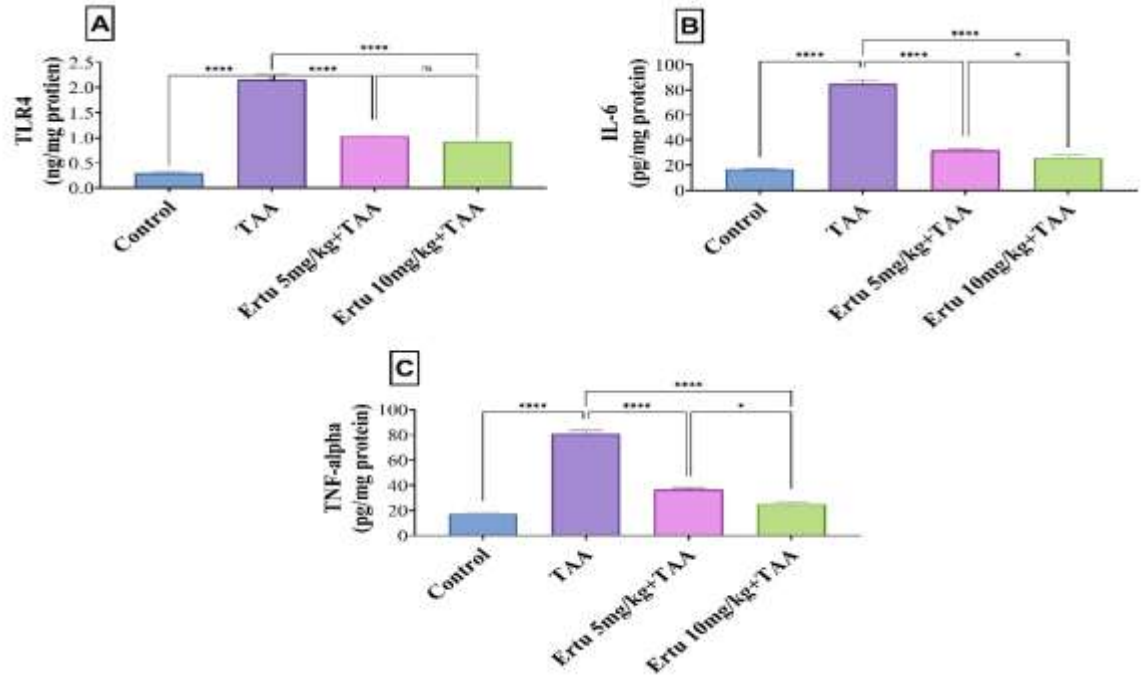

Fig. 3: Ertugliflozin effect on inflammatory mediators: TLR4, IL-6, and TNF-α level in TAA intoxicated rats. (A) "TLR4 (ng/mg protein)". (B) "IL-6 (pg/mg protein)". (C) "TNF-α (pg/mg protein)". The data are displayed as mean ± SEM of six rats, with p-values displayed on the bars. TAA: Thioacetamide; Ertu: Ertugliflozin.

Ertugliflozin's efficacy on the antioxidant pathway in rats given TAA

TAA significantly reduced HO-1 expression and Nrf2 (protein and gene expression) by 80.67%, 49.91%, and 69.38%, respectively, when compared to control. Ertu's treatment caused a dose-dependent increase in the antioxidant pathway. Efficacy of the 5 mg/kg dose was more noticeable than those of the TAA group; Nrf2 (protein and gene expression) and HO-1 expression increased by 354.63%, 160.54%, and 183.15%, respectively, while the 10 mg/kg dose had more pronounced effects, increasing them to normal values by 468.29%, 234.39%, and 241.42%, respectively (**Fig. 4 A, B, C**).

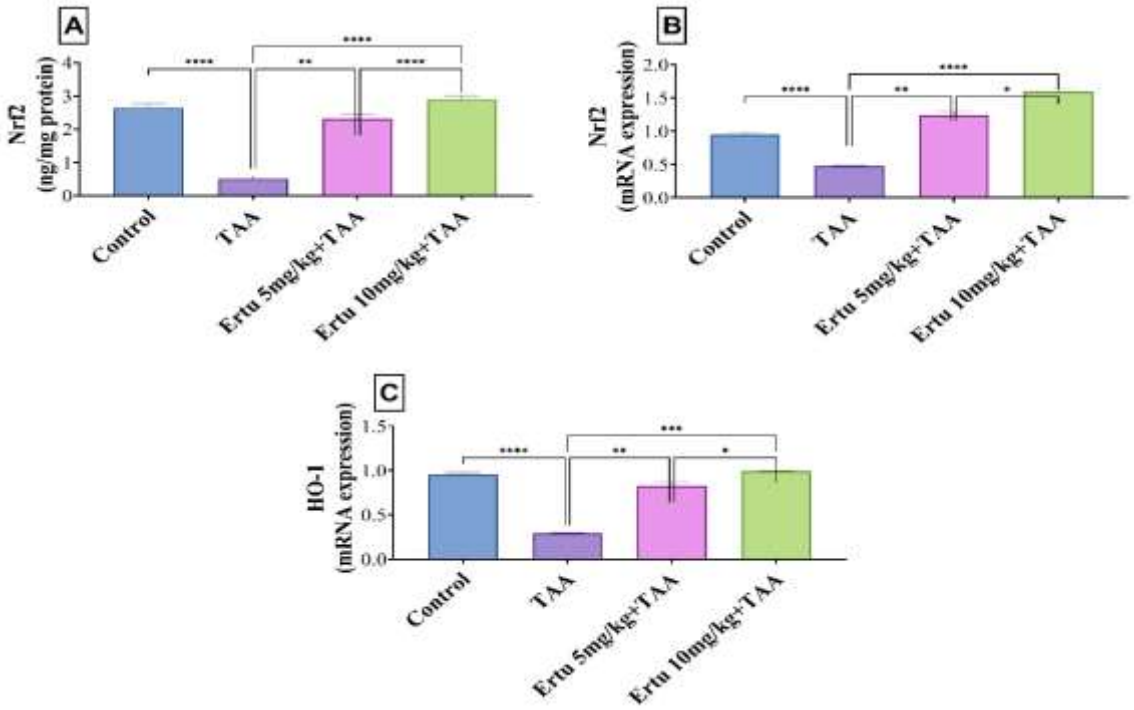

Fig. 4: Ertugliflozin's effect on Nrf2 and HO-1gene expression and level in TAA intoxicated rats. (A) "Nrf2 (ng/mg protein)". (B) "Nrf2 gene expression". (C) "HO-1 gene expression". The data are displayed as mean ± SEM of six rats, with p-values displayed on the bars. TAA: Thioacetamide; Ertu: Ertugliflozin.

Ertugliflozin's efficacy on PI3K and TGF-β1 levels in rats given TAA

TAA injections significantly raised PI3K and TGF-β1 levels by 517.13% and 502.75%, respectively, in comparison to control. These markers decreased in a dose-dependent way with Ertu therapy. These indices were reduced by the 5 mg/kg dose in comparison to the TAA group by 74.82% and 75.47%, respectively; however, the effects of the 10 mg/kg dose were more noticeable, by lowering them by 78.41% and 76.23%, respectively **(Figs 5 A, B)**.

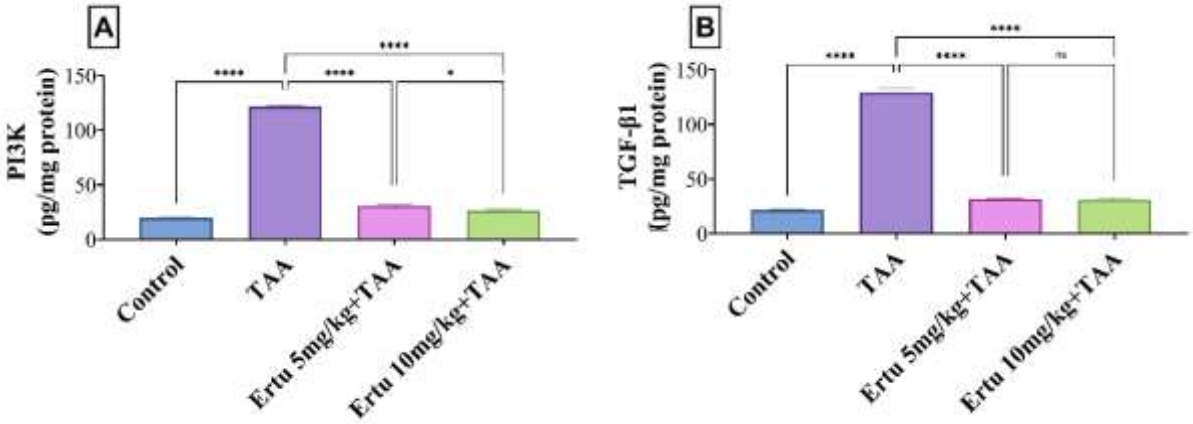

Fig. 5: Ertugliflozin effect on PI3K and TGF-β1 levels in TAA intoxicated rats. (A) "PI3K (pg/mg protein)". (B) "TGF-β1 (pg/mg protein)". The data are displayed as mean ± SEM of six rats, with p-values displayed on the bars. TAA: Thioacetamide; Ertu: Ertugliflozin.

Results of histopathology

The liver of the control group showed normal structure **(Fig. 6 a).** In addition to portal fibrosis, bile duct hyperplasia, and the development of newly formed bile ductules **(Fig. 6c),** the TAA group displayed bridging fibroplasia and pseudolobulation **(Fig. 6b),** vacuolar degeneration of certain hepatocytes, karyocytomegaly of others, and mitotic figures (**Fig. 6d).** In the group treated with Ertu 5 mg, the lesions were less than those of control positive and hepatocytes displayed minor vacuolar degeneration and bridging and portal fibrosis were moderate **(Figs. 6e & f).** The group that received Ertu 10 mg showed a notable decrease in the aforementioned lesions, with fibrous tissue between hepatocytes being sparse and the hepatic architecture normal **(Fig. 6 g & h).**

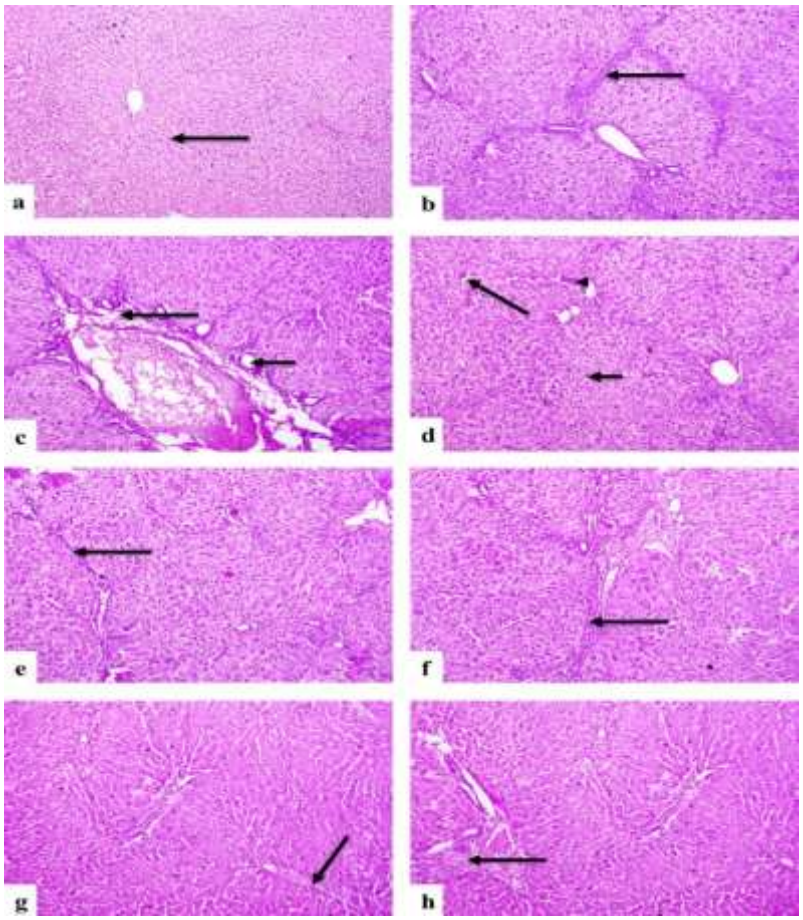

Fig. 6: Photomicrograph, liver rat. (a) Control group showing normal histological structure of hepatocytes (arrow). **(b)** TAA group showing bridging fibrosis and pseudolobulation of liver parenchyma (arrow). **(c)** Portal fibrosis (long arrow) and newly developed bile ductules (short arrow) are visible in the TAA group. **(d)** TAA group showing vacuolation of hepatocytes (short arrow), karyocytomegaly (long arrow) and mitotic figures (arrowhead). **(e)** Ertu 5 mg treated group showing moderate bridging fibrosis (arrow). **(f)** Ertu 5 mg group showing moderate portal fibrosis (arrow). **(g)** There is scanty fibrosis between the hepatocytes and normal hepatocytes in the Ertu 10 mg group (arrow). **(h)** Ertu 10 mg group showing mild portal fibrosis (arrow). **(H&EX100)**.

Hepatic lesions were identified and ranked according to their severity, as shown in **table (2).**

Table (2): Changes in the liver histopathologically:

Lesions	Contro	TAA	Ertu 5 mg	Ertu 10 mg
“Bridging fibroplasia”	0	3	2	1
“Pseudolobulation of hepatic parenchyma”	0	3	2	1
“Vacuolar degeneration of hepatocytes”	0	3	1	0
“karyocytomegaly of hepatocytes”	0	3	1	0
“Mitotic figures”	0	2	1	0
“Portal fibrosis”	0	3	2	1
“Hyperplasia of bile ducts”	0	3	1	0
“Formation of newly-formed bile ductules”	0	3	1	0

Histochemical findings

For fibroplasia, slices stained with MTC were analyzed. The area percentage of collagen deposition was depicted in **Fig. 7e**, while **Fig. 7a** demonstrated weakly stained collagen in the control group. TAA group revealed portal and bridging fibrosis **(Fig. 7 b),** The collagen deposition in the Ertu 5 mg treated group decreased noticeably (**Fig. 7 c),** whereas the group treated with Ertu 10 mg showed a considerable drop in collagen deposition **(Fig. 7 d).**

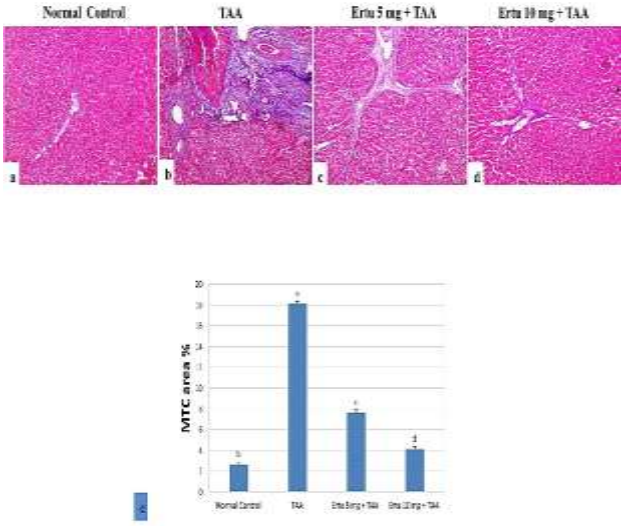

Fig. 7: Photomicrograph, MTC stained rat liver. **(a)** Weakly stained collagen is seen in the control group. **(b)** The TAA group exhibits bridging and portal fibrosis. **(c)** The group that received Ertu 5 mg showed less collagen fiber deposition. **(d)** The group treated with Ertu 10 mg had minimal collagen fiber deposition **(MTC X100)**. **(e)** Area percentage of collagen deposition in each group (mean ±SE was used to express the data, with different letters denoting significant differences at $p < 0.05$).

Immuno-histochemical findings of caspase-3 and *α*-SMA

Fig. 8e showed the immuno-expression of caspase-3 and α-SMA area percentage in liver tissue. Caspases-3 immunostaining revealed that the control group had few immune-reactive cells **(Fig. 8a).** Hepatocyte immune-expression was high in the TAA group **(Fig. 8b).** The hepatocytes in the Ertu 5 mg and Ertu 10 mg treatment groups showed a reduced positive immunological response **(Figs. 8 c & d).** Regarding α-SMA, the control group only showed expression in the smooth muscle wall of blood vessels **(Fig. 8a),** whereas the TAA group showed strong expression in the vascular walls and stellate cells of interlobular fibrous septa **(Fig. 8b).** The Ertu 5 mg and Ertu 10 mg treated groups showed significantly lower expression **(Figs. 8c & d).**

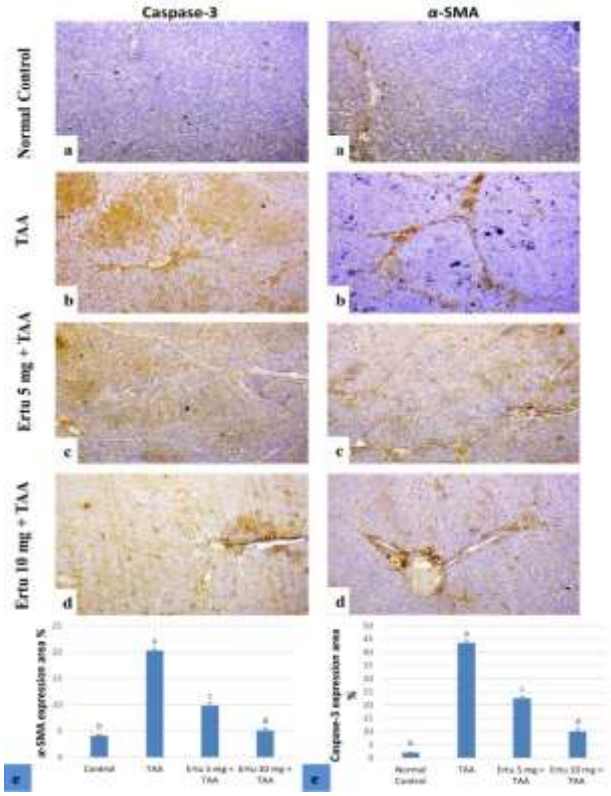

Fig.8: Immuno-staining of caspase-3 and *α*-SMA, liver rat**. (a)** control group showed Immuno-staining of caspase-3 with few immune-reactive cells, *α*-SMA in control group showed expression in smooth muscle wall of blood vessels. **(b)** TAA group showed strong expression of caspase-3 in hepatocytes and vascular walls and stellate cells in interlobular fibrous septa *α*-SMA strong expression. **(c & d)** Ertu 5 mg and Ertu 10 mg groups were demonstrating weak positive immune-reaction of casapase-3 in hepatocytes, expression of *α*-SMA is reduced in these groups **(Caspase-3 and *α*-SMA, X 100)**. **(e)** Caspases-3 and α-SMA area percentages were immune-stained in the liver tissue of several groups; mean ±SE was used to display the results, with different letters denoting significant differences at $p < 0.05$.

DISCUSSION

Thioacetamide (TAA) is an organosulfur chemical that induces both hepatocellular necrosis and lymphatic infiltration, making it a useful model for experimental liver injury **(Farjam *et al.,* 2012)**. In order to reliably cause a dose- and time-dependent hepatic fibrogenesis in rodents that resembles that observed in humans, thioacetamide (TAA) is commonly administered either orally or intraperitoneally **(Abdel-Rahman *et al.,* 2022)**. After undergoing biotransformation via Cytochrome P450, TAA becomes TAA sulfoxide, which is then further broken down to produce the hazardous metabolite TAA

disulfoxide **(Raslan *et al.,* 2021)**. When these chemicals attach to hepatic macromolecules, they can cause oxidative stress and lipid peroxidation, which ultimately leads to hepatic necrosis. Additionally, they have the ability to deplete the body's antioxidant defenses and cause inflammation **(Abdel-Rahman *et al.,* 2021)**.

Due to the oxidation damage generated by TAA, which causes the enzymes to leak into the blood, TAA injection in the present investigation caused an upsurge in ALT and AST and a drop in albumin levels **(Chen *et al.,* 2021; Aslam *et al.,* 2022; Elbaset *et al.,* 2023),** demonstrating hepatocellular injury **(Ramadan *et al.,* 2020)**. These biochemical alterations were validated by histopathological analyses, which showed that the TAA group had obvious liver damage. Furthermore, the results showed that Ertu had a hepatoprotective efficacy against hepatic fibrogenesis caused by TAA because it successfully reduced liver damage by raising serum albumin and lowering liver enzyme levels. Our results are consistent with previous research **(Gallo *et al.,* 2020; Khaliq *et al.,* 2024).**

The pathogenesis of TAA hepatotoxicity is thought to begin with oxidative stress, which is mostly brought on by natural defense systems' inability to effectively eliminate free radicals (ROS), which contributes to liver damage **(Ibrahim *et al.,* 2023)**. It was shown that administering TAA to rats significantly increased “MDA”, the lipid peroxidation indicator, and reduced the levels of the antioxidants “SOD and GSH” **(Abdelmageed and Abdelrahman 2023)**. Consistent with the previous research, our study demonstrated that TAA compromised the antioxidative status, as evidenced by elevated lipid peroxidation levels and a decline in GSH and SOD in the hepatic tissues. Importantly, cells' defense mechanism, Nrf2 and its downstream target HO-1, protects against inflammation and oxidative damage **(O’Connell and Hayes 2015)**. Upon oxidative stress, Nrf2 separates from Keap1, the negative regulator, and translocates into the nucleus to attach to the ARE, which promotes the synthesis of cytoprotective genes and antioxidant enzymes like SOD and HO-1 **(Wu *et al.,* 2015; Naif ALSuhaymi 2025)**. The antioxidant defense mechanism malfunctions as a result of excessive ROS generation interfering with the Nrf2's equilibrium **(Ikram *et al.,* 2019)**. The therapy of various oxidative stress-related illnesses, including TAA-induced liver fibrosis, depends on the proper functioning of Nrf2/HO-1 **(Hussein *et al.,* 2021)**. Additionally, the Nrf2 protein reduces inflammation by inhibiting the activation of NF-κB-mediated pro-inflammatory signaling pathways **(Yanaka 2018; Abu-Risha *et al.,* 2023).** Since Nrf2 is crucial for hepatocyte protection, it is thought to be a viable target for the prevention and/or treatment of a number of liver disorders **(Shin *et al.,* 2013)**. According to our research, TAA reduced HO-1 expression and Nrf2 levels and expression in hepatic tissue. Similar results showed that TAA significantly suppressed the Nrf2 pathway in liver tissues **(Demirel *et al.,* 2012; Hassan *et al.,* 2019).**

The results of this investigation demonstrated that Ertu markedly increased liver SOD, GSH, Nrf2, and HO-1 while decreasing hepatic MDA. The present data align with earlier research indicating that Ertu reduced oxidation stress in rats with an Alzheimer's disease (AD) model. **(Pang *et al.,* 2023)**. In line with our results, Zhang et al.'s study **(Li *et al.,* 2019)** showed that SGLT2 inhibitors lower oxidation stress by triggering Nrf2/ARE signaling and stimulating Nrf2 translocation to the nucleus in the heart of diabetic mice. Therefore, by lowering ROS generation and Nrf2/HO-1 signaling activation, Ertu may reduce oxidative stress.

To determine how ertugliflozin reduces inflammation, we measured the levels of "TLR4, IL-6, and TNF-α" in liver tissues. TLR4 signals trigger the cascade of “PI3K, NF-kB, and mitogen-activated protein kinase (MAPK)”. These pathways regulate how pro-inflammatory genes and cytokines are expressed, which impacts cell survival and death **(Bai *et al.,* 2014).** TLR4 stimulation elicits a cascade of events that includes NF-kB p65 translocation to the nucleus, which leads to the production of inflammatory cytokines (TNF-a, INF-g, and IL-6) **(Tian *et al.,* 2017).** One of the primary mediators of inflammation is TNF-α, which is produced by Kupffer cells and activated T cells **(El-Kashef and Serrya 2019)**. It has been shown that IL-6, anotherant pro-inflammatory cytokine, enhances acute inflammation and the immune system **(Mei *et al.,* 2012).** HSCs from both normal and cirrhotic livers emit IL-6, which increases the expression of TGF-β in cirrhotic livers, resulting in collagen production and hepatic inflammation **(Fu *et al.,* 2008).** Long-term TGF-β1 cascade signaling promotes HSC proliferation, which results in the production of ECM and fibrous scarring **(Brenner 2009)**. Great interest has been shown in targeting TGF-β1 to cure liver fibrosis **(Lee *et al.,* 2019)**. TGF-β1 causes myofibroblasts to differentiate, leading to liver fibrosis, through “phosphotidylinositol-3-kinase/Protein Kinase B/PKB” (PI3K/Akt) signaling **(Kulkarni *et al.,* 2011).**

PI3K triggers Akt by phosphorylating two sites, Thr308 and Ser473 **(Assinder *et al.,* 2009).** The serine/threonine kinase Akt is involved in "glucose metabolism, inflammation, cell division, and survival," among other physiological functions, when it is activated **(Yamada and Araki 2001)**. The present study found that “TLR4, TNF-α, IL-6, TGF-β1, and PI3K” levels were markedly elevated following TAA injections. Findings from earlier research were

comparable **(El-Kashef and Serrya 2019; Mi *et al.,* 2019; Abdelmageed and Abdelrahman 2023).** Ertugliflozin, on the other hand, has been shown to reduce the previously mentioned indicators. The results showed that ertugliflozin may have an anti-inflammatory effect on hepatic fibrogenesis by modulating the TLR4/TGF-β1/PI3K signaling pathways. To the best of our knowledge, there is no information on how ertugliflozin affects these pro-inflammatory cytokines in a model of liver fibrosis caused by TAA. In line with our results, a study by Abd Uljaleel demonstrated that ertugliflozin reduces lung dysfunction during endotoxemia in male mice by hindering oxidation stress and downstream inflammatory "IL-6, IL-1β, TNF-α, and TLR4" signaling pathways **(Abd Uljaleel and Hassan 2023).**

The primary mechanisms of apoptosis are p53 and the caspase cascade, which cause chromatin condensation and cellular shrinkage **(El-Kashef and Serrya 2019)**. Immunostaining in this investigation revealed that TAA markedly elevated caspase-3 expression in the hepatic tissue. Increased oxidative stress during TAA hepatotoxicity causes Bax to migrate to the outer mitochondrial membrane, enhancing the permeability of mitochondria and causing cytochrome c to be released into the cytoplasm, resulting in caspase 9 and more caspases downstream, like caspase-3, to be stimulated, which in turn causes caspase-dependent apoptosis **(Eleftheriadis *et al.,* 2016; Ghosh *et al.,* 2016; El-Kashef and Sharawy, 2018)**. Ertugliflozin therapy considerably reduced these changes. Our results are consistent with those of Abbas et al. **(Moellmann *et al.,* 2022)**, who found that ertugliflozin decreased left ventricular fibrosis by reducing apoptosis in a model of cardiac hypertrophy in mice.

ECM production begins when "quiescent HSCs" are activated and transformed into "myofibroblasts" that express α-SMA **(Zhou *et al.,* 2014)**. The TAA-treated group in our study exhibited a significant elevation of fibrotic markers, including the production of α-SMA and collagen by MTC. Similar findings were demonstrated in Đurašević **(Đurašević *et al.,* 2021)** and El-Gendy **(El-Gendy *et al.,* 2021)** studies**.** Ertugliflozin's antifibrotic action was indicated by MTC down-regulation of collagen expression and α-SMA. In agreement with our results, the Qiang study **(Qiang *et al.,* 2015)** showed that SGLT-2 inhibitors have anti-fibrotic effects by lowering α-SMA, TGF-β, collagen1a1 and collagen1a2 expressions.

CONCLUSION

Collectively, these findings demonstrated that ertugliflozin prevented TAA-induced liver fibrosis by a number of mechanisms, including antioxidant defense (shown by a marked increase in hepatic Nrf2 and its target genes, HO-1, SOD, and GSH), anti-inflammatory effect (shown by a significant decrease in the hepatic TLR4 pathway and the downstream cytokines TNF-α and IL-6), anti-apoptotic activity (shown by a marked decrease in hepatic caspase3 expression), and anti-fibrotic activity (shown by a marked decrease in hepatic TGF-β1, PI3K, and α-SMA expression). To confirm the hepatoprotective efficacy of ertugliflozin therapeutically and explore further molecular mechanisms, more research is needed.

Future Perspectives and Conclusive Remarks

Findings of this study have significant clinical relevance, particularly for liver fibrosis patients who may also present with type 2 diabetes mellitus. The efficacy of ertugliflozin in the prevention of fibrosis of the liver by multiple mechanisms suggests that it may prove to be a dual-purpose therapeutic agent, regulating blood glucose at the same time as offering protection to the liver. This double mechanism could be particularly beneficial in patients with diabetes and liver disease, potentially making it possible to decrease the number of drugs required. The dose-dependent effects observed in this study are important for possible clinical dosing regimens. Future studies should focus on the following areas: (1) long-term safety and efficacy in humans with both diabetes and liver fibrosis; (2) determination of drug-drug interactions with Ertugliflozin and commonly prescribed medication for liver disease; (3) its impact on the different causes of liver fibrosis beyond TAA-induced; (4) monitoring of possible prophylactic action of Ertugliflozin in high-risk patients; and (5) comparison between the drug by itself and combined therapy with existing antifibrotic drugs given to patients with liver fibrosis with an eye toward establishing any possible synergism.

Limitations of this study

Several limitations must be kept in mind while interpreting this study's results. Firstly, while the TAA-induced liver fibrosis model is well documented, it cannot accurately reflect the sophisticated pathophysiology of human liver fibrosis, which takes years to develop and has several variable etiologies. Secondly, the study was performed in male rats only, and gender differences in drug response cannot be ruled out. Third, the brief study duration (six weeks) may not have been sufficient to capture the long-term effects and safety profile of ertugliflozin in chronic liver disease. Fourth, even though the study demonstrated remarkable effects on many molecular pathways, the overall mechanism of action may also involve pathways that were not examined by this study. Finally, the study did not examine the potential effects of ertugliflozin on other organs or systems that would be important to understand about its overall safety profile in the management of liver disease. These are limitations that deserve more detailed, longer studies,

particularly in patients, before final clinical guidelines can be issued.

Author Contributions

Each author made an equal contribution to this work: they planned the investigation, carried out the experimental portion, examined the data, made suggestions for the procedures, and wrote, revised, and submitted the paper.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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