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Rosuvastatin Restrains the Headway of Experimentally Induced Liver Fibrosis: Involvement of NF-κB and Nrf2/HO-1 Signaling Pathway

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ABSTRACT

The serious health issue of liver fibrosis causes significant morbidity and mortality. Hepatic fibrosis does not currently have a conventional treatment due to its complicated pathophysiology. Statins are well-known by their Pleiotropic effects such as anti-inflammatory, antiapoptotic and antifibrotic actions. This investigation aimed to mark whether rosuvastatin could prevent the rat liver fibrosis caused by thioacetamide (TAA). Following two weeks of TAA injection, ROS (5 and 10mg/kg, daily) was given orally concurrently for further four weeks. ROS markedly decreased the upsurge in serum aminotransferase activities (AST and ALT) and restored albumin level. The increased hepatic glutathione (GSH) content and super oxide dismutase (SOD) activity, along with the declined malondialdehyde (MDA) level, showed ROS antioxidant capabilities. Further, heme oxygenase-1 (HO-1) and nuclear factor erythroid 2-related factor 2 (Nrf2) were both upregulated by ROS treatment while decreased the levels of nuclear factor-κB (NF-κB), p-NF-κB, tumor necrosis factor-α (TNF-α) and Interleukin 6 (IL-6). Additionally, ROS upregulated considerably the gene expression of Nrf2 and downregulated PI3K gene expression. Moreover, expression of alpha smooth muscle actin (α-SMA) was reduced while Nrf2 protein expression was elevated by ROS. Thus, it can be concluded that rosuvastatin could protect liver tissue against progression of TAA-induced fibrosis. This can be at least partially due to its antioxidant and anti-inflammatory effects via activation of Nrf2/HO-1 pathway and downregulation of NF-κB.

Key words: Liver Fibrosis; Rosuvastatin; Thioacetamide; Nrf2; HO-1.

INTRODUCTION

Liver fibrosis, a prominent cause of mortality and morbidity globally in 2016, was responsible for 2.2% of fatalities and 1.5% of disability-adjusted life years (Cheemerla and Balakrishnan 2021). The most frequent causes of chronic liver disease include viral hepatitis B and C infection, non-alcoholic fatty liver disease, and alcohol-related liver disease (Tsochatzis et al. 2014). The destruction and regeneration of the liver parenchyma are implicated in these aetiologies, which progress to hepatic fibrosis. Due to consequences such portal hypertension, hepatocellular cancer, and organ failure, cirrhosis can be fatal, hence the only treatment option is a liver transplant (Kennedy et al. 2021). New hepatitis C medications may reduce fibrosis progression, but there is no definitive cure for fibrosis (Berumen et al. 2021). Extracellular matrix (ECM) protein accumulation increases as a result of chronic liver damage during a long-term wound-healing response, which causes fibrogenesis and later cirrhosis (Ramadan et al. 2018a). Hepatocyte death, inflammatory cell infiltration, inflammatory cytokine production, and proliferation of nonparenchymal cells that produce ECM, principally hepatic stellate cells (HSCs) are implicated in the fibrogenic process (Puche et al. 2013). HSCs undergo a transformation into myofibroblast-like cells after activation, and these cells produce α-SMA (Abd El-Rahman and Fayed 2019). Several studies showed that the liver fibrosis can be amended either by halting progression and/or promoting resolution. Consequently, understanding the underlying molecular mechanisms is essential for developing antifibrotic remedies (Trautwein et al. 2015).

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Animal models for hepatic fibrosis were induced using the fungicidal drug, thioacetamide (TAA) (Amin et al. 2012). TAA is converted to thioacetamide sulfoxide and then to S, S-dioxide that catalyzed by the cytochrome P450 enzyme (CYP2E2) through oxidative bioactivation. These metabolites cause even more oxidative stress (Hajovsky et al. 2012). Several investigations have shown that continuous exposure to TAA causes biochemical and histological alterations in the liver that are similar to the processes that cause human liver fibrosis (Abd El-Rahman and Fayed 2019).

The etiology of liver fibrosis involves oxidative stress and the generation of free radicals that can be removed via the antioxidant enzymes. A transcription factor known as nuclear E2-related factor 2 (Nrf2) is involved in the activation of elements of antioxidant response, which promote the expression of the antioxidant genes (Wang et al. 2010). When Nrf2 is inactive, its suppressor Kelch-like ECH-associated protein 1 (Keap1) frequently confines it to the cytoplasm (Chen et al. 2013). The cytoplasmic KEAP1 and inactive Nrf2 separate, and the inactive Nrf2 translocates to the nucleus when liver is subjected to oxidative stress. There, it binds to ARE and regulates the production of antioxidant enzymes such as glutathione-s-transferase (GST), NAD(P)H quinone reductase 1 (NQO1), and heme oxygenase (HO-1) (Baird and Yamamoto 2020). Consequently, a possible treatment strategy for preventing liver fibrosis could involve activating the Nrf2/ARE signaling pathway.

Nonalcoholic fatty liver disease and nonalcoholic steatohepatitis patients have demonstrated that statins, which block 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, have beneficial impact, improve the structure and function of the liver. Furthermore, even at large doses, they are safe to use in individuals with chronic liver disorders and compensated cirrhosis (Ping et al. 2016). Statins' potential as anti-fibrotic drug is gaining popularity, depending on statin compounds' ability to inhibit the activation and proliferation rate of HSC (Shirin et al. 2013). Because it activates the Nrf2 and HO-1 signaling pathways, the statin rosuvastatin (ROS) possesses anti-inflammatory and antioxidant effects (Yeh et al. 2015). There is evidence that rosuvastatin, a frequently prescribed HMG-CoA reductase inhibitor, is more effective than other members of its family in the treatment of dyslipidemia (LDL-C) (Adams et al. 2014). Rosuvastatin has also been demonstrated to reduce ROS through a variety of methods, including suppression of endothelial nitric oxide synthase uncoupling, inhibition of NADPH oxidase, activation of enzymatic defense through antioxidants, and protection against hydrogen peroxide-induced DNA damage (Koc et al. 2015).

In the current research, we evaluated the antifibrotic efficacy of rosuvastatin against hepatic fibrosis model via regulating Nrf2/HO-1 and NF-κB signalling pathways.

MATERIALS AND METHODS

Ethical Approval and Animals

Male, adult Wistar rats, number of which is 24 (6–8 weeks; 180–220g) were secured from animal house belongs to the National Research Centre, Egypt. Animals were housed at 25^{o}C ambient temperature and in a light/dark cycle of 12 hour each. The treatment of the animals followed the national and international ethical standards. Institutional Animal Use and Care Committee of Cairo university authorized the experimental procedures. (Approval number: Vetcu23052022441).

Chemicals

Thioacetamide was obtained from “Sigma-Aldrich, USA”. Rosuvastatin (CRESTOR®) was purchased from “AstraZeneca, Egypt. The best analytical grade chemicals were used for the study's additional compounds.

Work protocol

Following a week of adaptation period, rats were separated into 4 groups at random: **Group 1**: the negative control group, in which rats received three intraperitoneal injections of saline weekly over a period of six weeks. **Group 2**: TAA group; rats received three intraperitoneal injections of 100mg/kg of TAA weekly for six successive weeks to cause liver fibrosis (Ra et al. 2019). **Group 3** and 4: treated groups; rats were administered ROS (5 and 10mg/kg) daily by oral route (Shirin et al. 2013) for 4 weeks starting the third week of TAA administration and continued concurrently with TAA for four weeks.

Making ready blood samples and tissue specimens

Samples of blood of each rat were assembled from retro-orbital venous plexus at the termination of the experiment while rats being lightly sedated with ketamine. Next to blood sampling, while rats were under sedation, they were cervically dislocated for euthanization, and their livers were speedily taken, washed in ice-cold saline and blotted dry. Each rat's liver's left lobe was removed and kept for histological and immunohistochemical analyses in buffered neutral formalin at a 10%. Another portion was weighted and kept at -80°C for molecular and biochemical investigations. While samples of the prepared serum were kept at -20°C for biochemical testing.

Assessment of liver functions biomarkers

Serum aspartate aminotransferase (AST) (Catalog #AS1061, Biodiagnostic®, Egypt) and alanine amino-transferase (ALT) (Catalog #AL1031, Biodiagnostic®, Egypt) activities, as well as serum albumin levels (Catalog #AB1010, Biodiagnostic®, Egypt). All were colorimetrically examined following the kits’ instructions.

Assessment of oxidative stress markers

In hepatic tissue homogenate, super oxide dismutase (SOD) (Catalog # K335-100, BioVision, Milpitas Boulevard, Milpitas, USA), reduced glutathione (GSH) (Catalog # K464-100, BioVision, Milpitas Boulevard, Milpitas, USA), and malondialdehyde (MDA) (Catalog # K739-100, BioVision, Milpitas Boulevard, Milpitas, USA), levels were colorimetrically estimated according to the manufacturing instructions.

ELISA, Enzyme-linked immunosorbent assay of liver inflammatory and pro-fibrotic biomarkers

Nuclear factor-kappa B (NF-κβ) (Catalog # MBS453975, MyBioSource, Inc., San Diego, USA) and its phosphorylated form (p-NFκB) (Catalog NBP2-29661, Novus Biologicals, LLC, USA), tumor necrosis factor-

alpha (TNF-α) (Catalog# 438204, BioLegend, Inc., San Diego, USA) as well as interleukin 6 (IL-6) (Catalog #SEA079Ra, Cloud-Clone Corp. (CCC), Wuhan, China) were estimated estimate in liver homogenate according to the producing company guidelines.

ELISA, Enzyme-linked immunosorbent assay of liver nuclear factor erythroid 2-related factor 2 (Nrf2) and heme oxygenase-1 (HO-1)

According to guidelines provided by Sunlong Biotec Co. LTD, Zhejiang, China, Catalog#SL0985Ra and BioVision, Milpitas Boulevard, Milpitas, USA, Catalog# E4525-100, respectively), Nrf2 and HO-1 were assessed in liver homogenate. Using Direct-zol RNA Miniprep Plus (Cat# R2072, ZYMO RESEARCH CORP. USA), total RNA was isolated from homogenized tissues obtained from various groups.

qRT-PCR analysis for Nrf2 and PI3K expression in the liver tissues

In order to reverse-transcribe the retrieved RNA, the SuperScript IV One-Step RT-PCR kit (Cat#12594100, Thermo Fisher Scientific, Waltham, MA USA) was used followed by PCR. A 96-well plate, StepOne device (Applied Biosystem, USA) was applied in a thermal protocol as follows: at 45ºC for 10min for reverse transcription, at 98ºC for 2min for RT inactivation, and inceptive denaturation by 40 cycles at 98ºC for 10s, at 55ºC for 10s and at 72ºC for 30s for the amplification step. Cycle threshold (Ct) values were used to express the data for both the housekeeping and target genes following the run of RT-PCR. The oligonucleotide succession of forward and reverse primers used in this study was as follows: Primers sequence for Nrf2 gene was forward AAAATCATTAACCTCCCTGTTGAT, and reverse CGGCGACTTTATTCTTACCTCTC (gene bank accession number NM_010902.5) and for PI3K gene the sequence for the forward TTAAACGCGAAGG CAACGA, and for the reverse CAGTCTCCTCCTGC TGTCGAT, (gene bank accession number is XM_ XM_032898971.1). Target genes (PI3K and Nrf2) expression variation was normalized by using the mean critical threshold (CT) expression.

Histopathological examination

Following a twenty-four-hour fixation in neutral formalin buffered at 10% concentration, specimens collected from livers of rats of diverse groups were consistently processed into paraffin slices (Bancroft and Gamble 2008). The specimens were cleaned in distilled water, dehydrated in ethanol dilutes, clarified in xylene. Finally, paraffin blocks were prepared and chopped into 4-5 μm thick portions. The tissue slices were received on glass slides, after which they underwent deparaffinization and staining with hematoxylin and eosin (H&E). A trained pathologist who was blinded throughout the sample naming procedure to prohibit bias carried out all histopathology investigations.

Immunohistochemical examination

Expression of Nrf2 and Alpha-smooth muscle actin (α-SMA) were checked immunohistochemically using avidin-biotin peroxidase (A-B peroxidase) on the prepared liver paraffin slices of control and treated groups according to method mentioned (Abd El-Rahman and Fayed 2022). A monoclonal antibody for -SMA and Nrf2 (1:200 and 1:100 dilutions respectively) (Abcam, Cambridge, USA) was used to be incubated with tissue slices as well as with the chemicals needed for the A-B peroxidase (peroxidase kit, Vactastain ABC, Vector Laboratories) method for revelation of complex constituted by the union of antigen and antibody. The chromogen 3,3 -diaminobenzidine tetra hydrochloride was used to visualize each marker's expression (DAB, Sigma Chemical Co.). The optical density of expression of each marker was assessed utilizing image analysis tools in seven microscopic fields of high-power magnification (Image J, 1.46a, NIH, USA).

Statistical analysis

The findings were explored by GraphPad Prism version 5 while they expressed as mean±SEM. The Tukey test was employed after the one-way repeated measures analysis of variance. Differences with $P<0.05$ or lower are statistically significant.

RESULTS

Effect of ROS on the serum AST, ALT, and albumin levels in TAA administrated rats

Rats' serum transaminases (AST and ALT) activity was considerably increased ($P<0.05$) after TAA Injection, whereas the serum albumin level was noticeably reduced in comparison with the control one. ROS-treated groups showed marked ($P<0.05$) decline in serum AST and ALT activity as well as marked ($P<0.05$) elevation in serum albumin level as shown in Fig. (1a-c).

Effect of ROS on the liver contents of oxidative stress markers in TAA administrated rats

To inspect how ROS affects the oxidative stress that TAA causes in the liver, hepatic MDA content and antioxidant activities by measuring (GSH content and SOD activity) were assessed. Contrary to the control rats, injection of TAA caused a significant ($P<0.05$) drop in hepatic GSH content and SOD activity as well as an elevated level of hepatic MDA. In a dose-dependent manner, treatment with ROS considerably ($P<0.05$) halted the decline of GSH content and SOD activity as well as significantly ($P<0.05$) restored the elevated MDA levels in the liver (Fig. 1d-f).

Effects of ROS on the liver content of profibrotic and inflammatory markers in rats receiving TAA

TAA considerably ($P<0.05$) raised the levels of IL-6, TNF-α, NF-kB, and p- NF-kB in the rats' livers when compared to the control group (Fig. 2). IL-6, TNF-α, NF-kB, and p- NF-kB levels in rats' liver were significantly ($P<0.05$) reduced after treatment with ROS depending on the dose, compared to the TAA-treated rats. The greatest benefits on the suppression of inflammatory response were seen with ROS at a dosage of 10mg/kg.

Effects of ROS on the liver content of Nrf2 and HO-1 in TAA administrated rats

Rats with liver fibrosis caused by TAA showed marked ($P<0.05$) decline in the levels of Nrf2 (Fig. 3a)

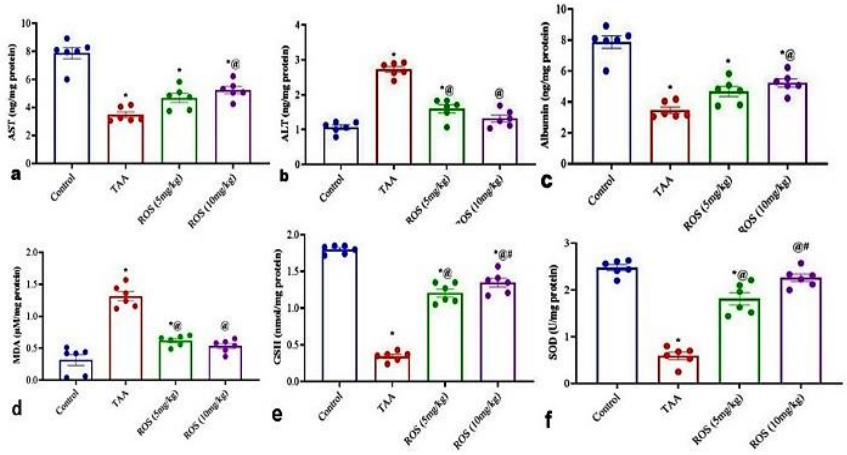

Fig. 1: Rosuvastatin amended serum transaminases (a) AST, (b) ALT, and (c) albumin as well as oxidative stress markers, (d) MDA level, (e) GSH content, and (f) SOD activity in liver tissue homogenate of TAA rats' model of fibrosis. Data are presented as mean±SE. *vs control group, @vs TAA group, #vs ROS (5mg/kg) at P<0.05.

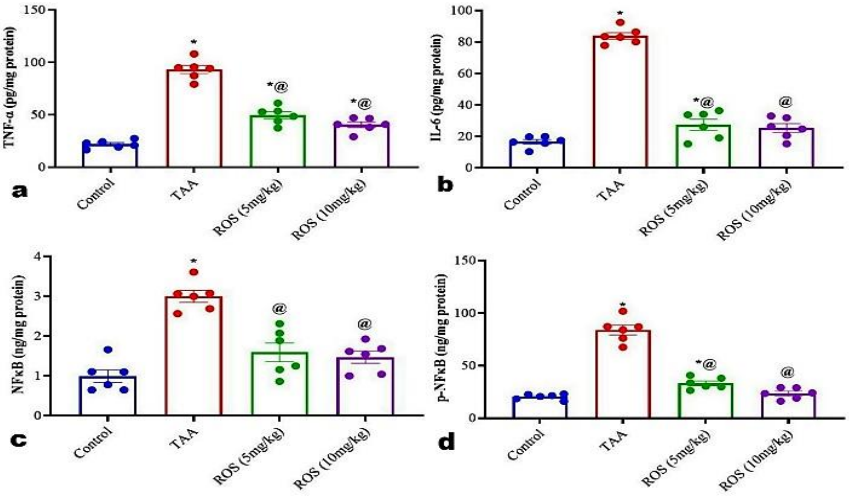

Fig. 2: Effect of ROS on pro-fibrotic and inflammatory markers levels; (a) TNF-α, (b) IL-6, (c) NF-kB, and (d) p-NF-kB in TAA rats' model of fibrosis. Data are presented as mean±SE. *vs control group, @vs TAA group, #vs ROS (5mg/kg) at P<0.05.

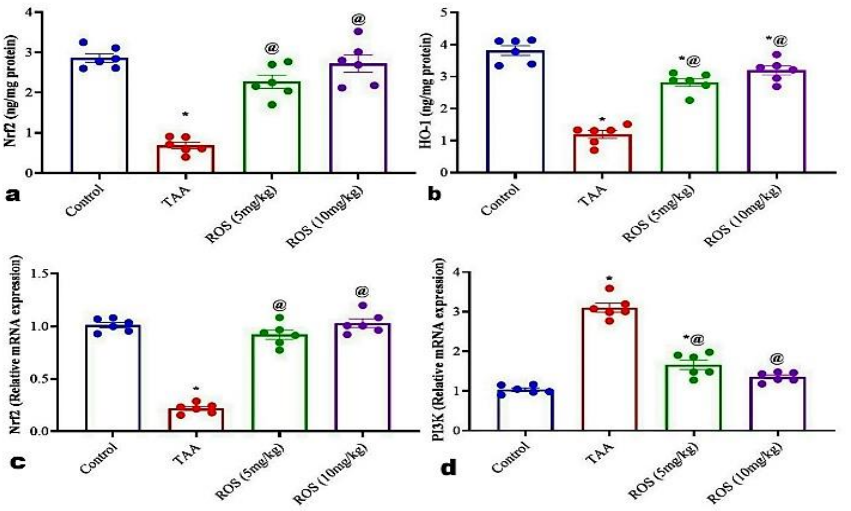

Fig. 3: Effect of ROS on (a) Nrf2 and (b) HO-1 markers' contents in hepatic tissue homogenate as well as the gene expression of (c) Nrf2 and (d) PI3K of TAA rats' model of fibrosis. Data are presented as mean±SE. *vs control group, @vs TAA group, #vs ROS (5mg/kg) at P<0.05.

and its target gene, HO-1 (Fig. 3b) than those in the control group. As opposed to the TAA group, rats treated with both dosages of ROS displayed a significant elevation in the antioxidant defense pathway (Nrf2 and HO-1) levels.

Effects of ROS on gene expression of Nrf2 and PI3K in TAA administrated rats

The TAA rats displayed a marked (P<0.05) drop in the expression of the Nrf2 gene (Fig. 3c) in comparison to the control group as well as a marked increase in the PI3K gene expression (Fig. 3d). In comparison to the TAA group, ROS treatment increased the mRNA expression of Nrf2 significantly (P<0.05) while significantly suppressing the PI3K expression.

Effect of ROS on the liver histopathological findings in TAA administrated rats

The livers of control rats had a normal histological structure, including the hepatic cells, central veins, and portal areas (Fig. 4a). Whilst rats administrated TAA, their livers showed marked fibroplasia, which started as portal-to-portal bridging fibrosis with increased proliferating fibrous tissue in portal areas (Fig. 4b). The later portal areas showed in addition, cholangiolar epithelial proliferation, congestion, inflammatory cells infiltration including

histocytes and lymphocytes (Fig. 4c) as well as oval cells hyperplasia. Outward extension of variably sized fibrous strands was noticed toward the parenchyma, causing pronounced parenchymal pseudo-lobulation (Fig. 4d). Within those pseudo-lobules, hepatocytes showed eccentric nuclei and vacuolar degeneration, necrosis (Fig. 4e) and scattered apoptosis. Mononuclear inflammatory infiltrates along with congested vessels and proliferated bile ductules were all observed along the fibrous septa.

Microscopic examination of various livers' sections of treated groups revealed different degrees of regression of fibrosis extension. The group receiving rosuvastatin showed the best results, especially with the high dose (10mg/kg) treated rats. Regarding livers of low dose Rosuvastatin-treated (5mg/kg) group (Figs. 4f-h) revealed mild portal triads' fibrosis, with proliferating cholangiolar epithelial cells and infiltration of a few inflammatory cells. Some portal triads showed incomplete thin fibrous strands peripherally extended without bridging. Hepatocellular degeneration, dispersed necrosis, and apoptosis were all present in a moderate degree. The high dose of Rosuvastatin treated group showed good restoration of the hepatic cells, scarce portal areas fibrous proliferation, and minimal changes (Figs. 4i-k).

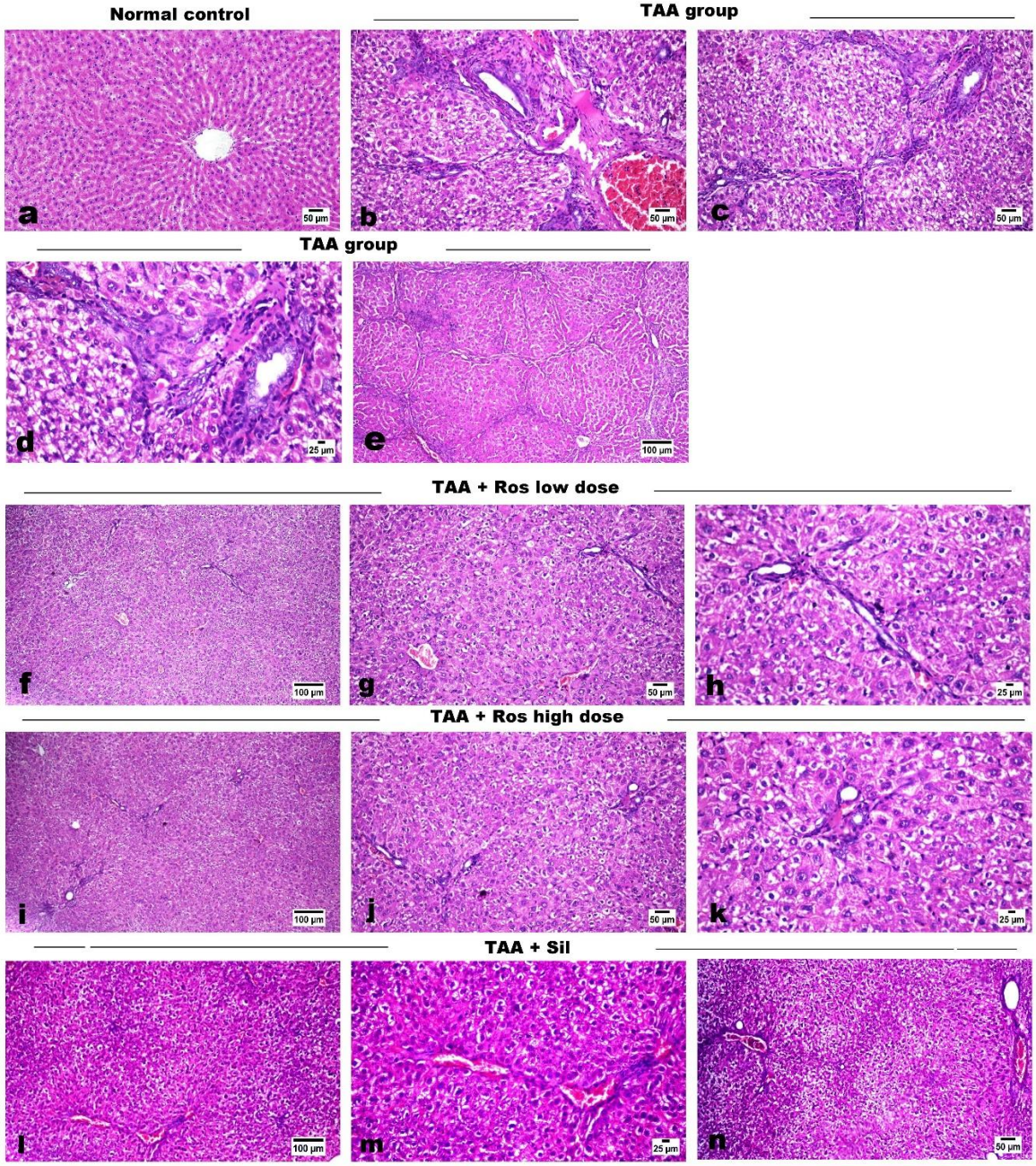

Fig. 4: H&E-stained liver sections; (a) Liver of control rat shows normal central vein (CV) and hepatic parenchymal cells (HCs). (b-e) liver of TAA-administrated rat showing; (b) marked fibroplasia of the portal areas with portal-to-portal bridging fibrosis, (c) bile duct epithelial proliferation, vascular congestion (Co), mononuclear inflammatory cells infiltration (IF), and fibrosis (F), (d) marked parenchymal pseudo-lobulation (PS), (e) hepatocellular vacuolar degeneration (dashed arrow) and scattered apoptosis (arrow). (f –h) livers of ROS (low dose) treated group showing mild fibrosis in portal areas (short arrow) with peripheral extension of incomplete thin fibrous strands (arrow) from some areas with moderate degree of hepatocellular vacuolation (dotted arrow). (I - K) ROS (high dose) treated showing scarce fibrous proliferation in the portal areas (arrow) without any peripheral extension, notice the mild hepatocellular vacuolation (dotted arrow).

Effects of ROS on the immunohistochemical α-SMA and Nrf2 expression in rats receiving TAA

Fig. 5 presented noticeably higher α-SMA expression in rats' group administrated TAA compared to control group. While ROS (5 and 10mg/kg) treatment two-week post the start of liver fibrosis induction displayed a dose-related reduced α-SMA expression compared to TAA group. Regarding the Nrf2 expression, TAA administration resulted in mild increased immuno-expression of Nrf2 compared to control and treated rats' groups. The treatment with ROS significantly increased expression of Nrf2 particularly in livers of high dose treated group (Fig. 6). The quantitatively analyzed positive brown color of both Nrf2 and α-SMA, displayed as staining score, demonstrated that the TAA rats significantly ($P<0.05$) overexpressed α-SMA and somewhat boosted the expression of Nrf2 in comparison to the other treated groups.

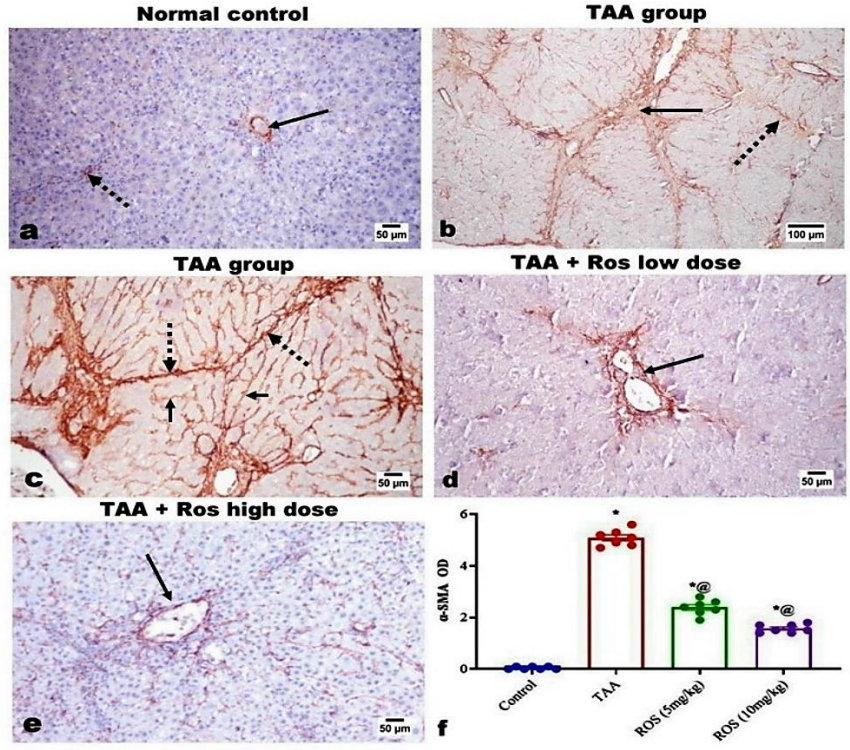

Fig. 5: Immunohistochemical analysis of α-SMA in liver sections. (a) control group showing normal expression in portal areas (arrow) and around central veins (dashed arrow), (b and c) TAA administrated group showing marked expression in portal areas (arrow), peri-cholangiolar, along the fibrous septa (dotted arrow) and peri-cellar (short arrow). (D and f) ROS (5 and 10mg/kg) treated groups showing weak expression of α-SMA in the portal areas and very rare extension in the parenchyma in the low dose group. For statistical analysis, one-way ANOVA was performed, followed by Tukey's multiple comparison tests. Data are presented as mean±SE. *vs control group, @vs TAA group, #vs ROS (5mg/kg) at $P<0.05$.

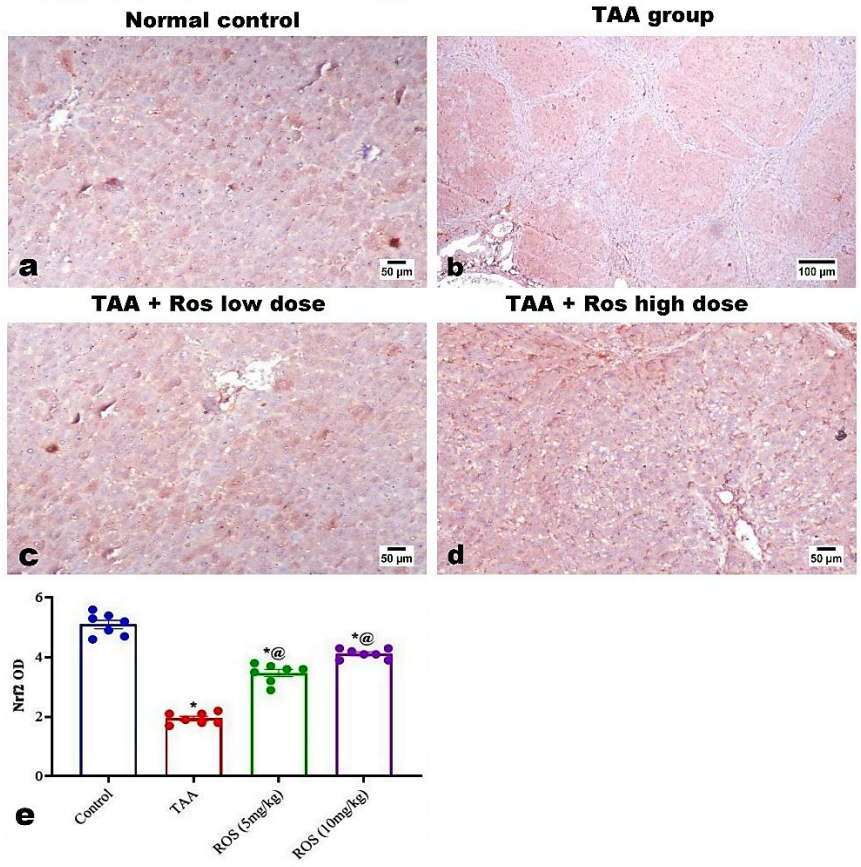

Fig. 6: Immunohistochemical analysis of Nrf2 in liver sections of (a) normal control group showing mild expression in hepatic cells, (b) TAA administrated group showing increased expression of Nrf2, (c and d) ROS (5 and 10mg/kg) treated groups respectively showing significant increased expression. For statistical analysis, one-way ANOVA was performed, followed by Tukey's multiple comparison tests. The results were presented as mean SEM (n=6). At $P<0.05$, symbol a represent the significant difference from the control group while symbol b to represent the significant difference from the TAA group.

DISCUSSION

Chronic liver damage is the main cause of hepatic fibrosis, which occurs prior to the end-stage of cirrhosis (Li et al. 2017). Rosuvastatin is a lipid-depreciation agent that be owned to the statin group (Wang et al. 2019). It has strong anti-oxidative effects and anti-inflammatory properties (Kanno et al. 2018). The possible preventive impacts of rosuvastatin versus rats' liver fibrosis caused by TAA were assessed in the present study. Our findings established that treatment with ROS safeguard liver from fibrosis progression through boosting the Nrf2 pathway and lowering the NF-κB, which detract oxidative stress and inflammation.

TAA employed to encourage fibrosis of liver in rats in this investigation. TAA injection caused severe histological damage to liver tissue, according to H&E staining; however, ROS therapy considerably reduced the severity of liver injury. Furthermore, TAA brought about a marked upsurge in AST and ALT serum activity with a marked decline in albumin serum levels, indicating liver cell damage and a reduction in hepatic albumin synthesis function (Ramadan et al. 2018b). These changes coincide with research conducted by Abd El-Rahman and Fayed (2019). ROS therapy, on the other hand, reduced serum ALT and AST activities, implying hepato-defensive actions versus injury of the hepatic cells. Furthermore, ROS therapy enhanced the synthesis function of liver, as seen by returning to normal albumin levels in serum. Similar findings have been reported by Yokohama et al. (2016).

The pathogenic mechanism that propagates liver injury is inflammation. The inflammatory response is recognized to contribute to collagen deposition and synthesis as a necessary process for future fibrogenesis. As a result, lowering pro-inflammatory cytokine levels may aid in the prevention of liver fibrosis (Hamza et al. 2020). Many cytokines, inclusive of TNF-α and IL-6 can adjust fibrosis by boosting its propagation after attaching to certain receptors on fibroblast, luring inflammatory cells, promoting the production of collagen, and secreting autocrine substances (Liang et al. 2013).

TNF-α and IL-6 levels showed significant rise in TAA group yet markedly diminished in the Rosuvastatin (ROS) treated rats, proposing that ROS may help to prevent liver fibrosis by suppressing the inflammatory cytokines. Over and above, we inspected the activation pathway of NF-κB that is important for production of several cytokines of pro-inflammatory nature, to investigate more about the underlying mechanism of ROS against inflammation.

NF-κB is a transcription factor that stimulates the reproduction of a number of genes encoding some cytokines, guarantying TNF-α. There are five transcription factors in this family (c-Rel, RelB, p50, p52, and p65) (Hayden and Ghosh 2011). In the NF-κB signaling pathway, p65 and p50 forms are the most significant NF-κB dimmers (Ren et al. 2012). The inhibitory molecule IB is phosphorylated and so degraded, releasing the cytosolic dimer NF-B p65/p50, which then activates NF-κB. The cytosolic dimer binds to DNA after entering the nucleus, triggering the transcription of target genes (Hellerbrand et al. 1998). TAA significantly increased NF-κB invigoration, as shown by the significant rise in phosphorylation of NF-κB p65, TNF-α, and IL-6 in this study. However, remediation with ROS caused a marked decline in NF-κB p65, TNF-α, and IL-6 production that is consistent with prior research on the effect of ROS on lipopolysaccharide (LPS)-induced heart damage (Ren et al. 2021). These findings imply that ROS reduces inflammation in liver via decreasing the NF-κB signaling pathway, at least in part.

Nrf2 performs a stringent role in response of cells to oxidative stress (Zhang et al. 2019). Additionally, Human cell cultures from HO-1 deficient patients were more sensitive to oxidative stress, and models of HO-1 knockout animal had more lipid peroxidation and elevated amounts of oxidized proteins, implying that HO-1 has antioxidant properties (Son et al. 2013). Nrf2 and its downstream proteins play a stringent function in preventing chemical damage and oxidative stress to hepatocytes (Niture and Jaiswal 2013). Nrf2 streamlines over 100 genes, including HO-1 and several cytoprotective proteins and antioxidant enzymes including SOD and GST, leading to cellular protection after binding to elements of the antioxidant response on DNA (Enomoto et al. 2001). The signaling axis of Nrf2/HO-1 can considerably reduce ROS production in mitochondria and regulate mitochondrial functional integrity, in addition to its effects on antioxidant enzyme expression and activity (Zhang et al. 2019). Nrf2 activation has been shown to be an effective pathway for preventing drug- or xenobiotic-induced liver damage (Iranshahy et al. 2018). Results of the present work demonstrated that TAA markedly reduced expression of Nrf2 protein. The destruction of the Nrf2 signaling pathway may result in imbalanced redox equilibrium in the cell, which may lead to elevation of ROS levels. The results of the current investigation showed that TAA significantly reduced Nrf2 expression and that of downstream effectors, including HO-1. ROS therapy effectively curbed TAA-induced hepatic fibrosis by increasing Nrf2/HO-1 signaling, according to our findings. The capability ROS for the reduction of atrial tachycardia-induced cellular remodeling produced similar results (Yeh et al. 2015). Parallel with our findings, research jointly *in vivo* and *in vitro* has elucidated that statins increase the HO-1 expression, an antioxidant and anti-inflammatory protein (Hsu et al. 2006). Furthermore, the antioxidant efficacy of activating the Nrf2/HO-1 pathway was related to diminished NF-κB levels via intervening with its translocation in the nucleus (Baiyun et al. 2018). As a result, we hypothesized that the antioxidant hepatoprotective efficacy could be explained by ROS' power to invigorate the Nrf2/HO-1 signaling pathway while downregulating NF-κB nuclear translocation.

The PI3K/Akt pathway is thought to be important in controlling Nrf2 and elements of antioxidant response dependent on oxidative stress defense (Arisawa et al. 2009). Treatment with ROS significantly increased PI3K protein expression and phosphorylation in this study. This was in consistent with the findings of Yeh et al. (2015), who showed, which ROS activated PI3K/Akt/Nrf2/HO-1 pathway to safeguard atrial myocytes from damage caused

by tachycardia. The findings Imply that Nrf2 activation in TAA-treated rats is linked to PI3K activation.

MDA levels in the blood are a good predictor of lipid peroxidation, and its elevation indicates oxidative stress (Gaschler and Stockwell 2017). The soluble antioxidant GSH is found in the nucleus, cytoplasm, and mitochondria. GSH is well-known for its many physiological roles as an antioxidant against oxidative stress and free radicals in xenobiotic compound detoxification (Mirończuk-Chodakowska et al. 2018).

The current investigation found that repeated TAA injections increased the oxidative stress marker levels, MDA and depleted GSH content along with SOD activity in liver. Similar findings were reported by Abdel-Rahman et al. (2021). ROS treatment, on the other hand, prevented MDA levels from rising and improved liver content of GSH and the activity of SOD. This was consistent with the findings of Habibi et al. (2007) and Yu et al. (2018), who claimed that ROS had an antioxidant effect that protects cells from lipid peroxidation. Therefore, the elevation of Nrf2 expression and subsequent upregulation of its related genes of antioxidation as well as the suppression of the expression of NF-kB and its signalling cascade in the cells, may be responsible for the antifibrogenic impacts of ROS in our study.

Conclusion

Our findings show that ROS inhibits NF-κB and promotes the Nrf2/HO-1 pathways, which helps to limit the progression of hepatic fibrosis. According to this research, ROS may offer a promising therapeutic option for treating liver fibrosis.

Conflict of interest

The authors have declared that no conflict of interests exists.

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Author's Contribution

All authors contributed equally to this work, they designed the study, performed the work research, analyzed the data, contributed to the methods, and wrote the paper.

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