Mesaraline, an osteopontin inhibitor: The potential prophylactic and remedial roles in induced liver fibrosis in rats

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**A R T I C L E    I N F O**

**Keywords:**
Liver fibrosis  
Mesoraline  
Osteopontin  
Antioxidants  
TGF-β1

**A B S T R A C T**

Liver fibrosis is a major health issue leading to high morbidity and mortality. The potential anti-fibrotic activity and the effect of mesalazine on osteopontin (OPN), an extra cellular matrix (ECM) component were evaluated in TAA-induced liver fibrosis in rats. For this purpose, forty-two adult male Wistar rats were divided into six groups. All animals, except the normal control, were intraperitoneally injected with TAA (200 mg/kg) twice per week for 6 weeks. In the hepatoprotective study, animals were administered mesalazine (50 and 100 mg/kg, orally) for 4 weeks before induction of liver fibrosis then concomitantly with TAA injection. In the hepatoprotective study, animals were administered mesalazine for 6 weeks after TAA discontinuation with the same doses. In both studies, mesalazine administration improved liver biomarkers through decreasing serum levels of AST, ALT and total bilirubin when compared to fibrotic group with significant increase in total protein and albumin levels. Mesalazine significantly decreased hepatic MDA level and counteracted the depletion of hepatic GSH content and SOD activity. Additionally, it limited the elevation of OPN and TGF-β1 concentrations and suppressed TNF-α as well as α-SMA levels in hepatic tissue homogenate. Histopathologically, mesalazine as a treatment showed a good restoration of the hepatic parenchymal cells with an obvious decreased intensity and retraction of fibrous proliferation, while as a prophylaxis it didn't achieve enough protection against the harmful effect of TAA, although it decreased the intensity of portal to portal fibrosis and pseudolobulation. Furthermore, mesalazine could suppress the expression of both α-SMA and caspase-3 in immunohistochemical sections. In conclusion, mesalazine could have a potential new indication as anti-fibrotic agent through limiting the oxidative damage and altering TNF-α pathway as an anti-inflammatory drug with down-regulating TGF-β1, OPN, α-SMA and caspase-3 signaling pathways.

**1. Introduction**

Liver fibrosis is defined as the hepatic dynamic response to repeated and chronic liver injury, accompanying the activation of hepatic stellate cells (HSCs), the over expression of extracellular matrix (ECM) proteins, ultimately lead to cirrhosis, which is characterized by scar tissue, loss of parenchymal architecture and organ failure [1]. It is known that various agents, including chronic infection by hepatotropic viruses; hepatitis B and hepatitis C viruses (HCV), chronic exposure to toxins or drugs (e.g., alcohol abuse), chronic cholestatic diseases and autoimmune chronic hepatitis result in liver damage. When the damage is prolonged, liver fibrosis results [2]. Liver fibrosis afflicts more than 100 million people worldwide and represents one of the most common causes of death in adults [3]. In Egypt, up to 85% of HCV infections persist for life, leading to chronic hepatitis [4]. The typical mechanism underlying the development of hepatic fibrosis is an imbalance between the deposition and removal of ECM. HSCs, the main fibrogenic cell type in the liver, are the predominant producers of ECM and their activation and proliferation are mediated by different cytokines during liver injury process [5]. Following liver injury, hepatic stellate cells undergo trans-differentiation from quiescent vitamin A-rich cells into proliferative, fibrogenic, and contractile myofibroblasts. The major phenotypic changes after activation include proliferation, contractility, fibrogenesis, matrix degradation, chemotaxis, retinoid loss, and WBC chemotraction [6]. Activated stellate cells possess the ability to express myogenic markers as alpha-smooth muscle actin (α-SMA) and secreting large amounts of ECM, with collagen-1 being the most important protein and principal component of scar tissue [7].
HSCs are stimulated by fibrogenic cytokines, one of which is osteopontin, a pro-inflammatory cytokine and matrix protein. The profibrogenic effect of osteopontin is associated with an increased concentration of transforming growth factor β1 (TGF-β1) [8]. Osteopontin (OPN) is a phosphoprotein that is originally described as a structural component of the ECM, which has the ability to bind to proteins and most types of collagen. OPN exists in a secreted form that mediates cell adhesion, migration and survival, and an intracellular non-secreted form [9]. OPN is expressed in a variety of cells, including dendritic cells, endothelial cells, fibroblasts, macrophages, and smooth muscle cells [10]. During hepatic fibrogenesis, activation of quiescent hepatic stellate cells is promoted by OPN then subsequently increases the expression and secretion of collagen I [11]. OPN was suggested to serve as a biomarker of severe fibrosis as well as portal hypertension during Schistosomiasi mansoni and chronic viral hepatitis [12].

Mesalazine (mesalamine) is considered as an anti-inflammatory drug that is used for the treatment of inflammatory bowel disease. It is well tolerated by most patients and can induce mucosal healing specifically in ulcerative colitis [13]. It may exert an apoptotic effect on colon cancer cells and may also interfere with the cell cycle of necrobiotic cells [14]. Mesalazine is the active moiety of sulfasalazine, which is metabolized to sulfapyridine and mesalazine. Most patients with adverse effects from sulfasalazine will tolerate mesalazine. It was found that OPN expression in inflammatory bowel diseases was significantly reduced after treatment with sulfasalazine [15].

Hence, the present study aimed to investigate the probable protective as well as the therapeutic efficacies of mesalazine against liver fibrosis induced by thioacetamide (TAA) as a new indication for mesalazine with focusing on OPN, as a biomarker for assessing the severity of liver fibrosis.

2. Materials and methods

2.1. Drugs and chemicals

Mesalazine (5-aminosalicylic acid), was purchased from Minapharm Pharmaceuticals Co., Egypt, it was dissolved in 0.5% carboxymethyl cellulose (CMC) for oral administration. Thioacetamide (TAA) was purchased from Sigma-Aldrich, USA; it was dissolved in 0.9% (w/v) saline solution for intra-peritoneal (i.p) injection. All other chemicals used throughout the experiment were of the highest analytical grade available. All drugs and chemicals were freshly prepared prior to use.

2.2. Experimental animals

Forty two adult male Wistar rats weighing 180–200 g were obtained from the Animal House Colony of the National Research Centre (Dokki, Giza, Egypt). Rats were housed in stainless steel cages under controlled conditions; 23–26 °C. The animals were allowed for free access to water and a standard rodent chow diet. Rats were allowed to adapt to the laboratory environment for one week prior starting the experiment. All experiments using animals were performed according to the protocol approved by the Institutional Animal Care and Use Committee at Cairo University (IACUC) as well as the guidelines of the Ethical Committee of the National Research Centre (NRC), Egypt and followed the National Institutes of Health Guide Recommendations Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

2.3. Experimental design

Rats were randomly allocated into six groups (7 rats each). Group1; rats (normal control) received vehicle (0.5% CMC). Liver fibrosis was induced in the remaining 5 groups by intraperitoneal injection of TAA at a dose of 200 mg/kg b.wt, twice a week for six weeks [16]. Group2 served as (TAA group). In the protective study, rats of groups 3 and 4 rats were administered mesalazine at a dose of 50 and 100 mg/kg/day, respectively, for 4 weeks then given concomitantly with TAA injection [17]. In the therapeutic study, rats of groups 5 and 6 received daily oral dose of mesalazine at doses of 50 and 100 mg/kg for 6 weeks after TAA discontinuation. The experimental designs are shown in Fig. 1.

2.4. Blood and tissue samples

Blood samples were collected from the retro-orbital venous plexus of each rat under light diethyl-ether anesthesia after 10 weeks in the protective study and 12 weeks in the therapeutic study. Sera were separated from the collected blood samples and were kept at −20 °C for further biochemical investigations. Immediately after blood sampling, animals were sacrificed by cervical dislocation under gentle diethyl-ether anesthesia and livers were rapidly removed, washed in ice-cooled saline, blotted dry and weighed. Specimen from the left lobe of each liver of rats of all groups was dissected out and placed in 10% buffered neutral formalin for histopathological and immunohistochemical examinations, another weighed part was kept frozen at −20 °C pending further investigations.

Fig. 1. The animals’ groupings, the used chemicals and their doses.
Preparation of hepatic tissue homogenate:

The later weighed part of each hepatic tissue was homogenized with ice-cooled saline using a homogenizer (Medical instruments, MPW-120, Poland), to prepare 20% w/v homogenate. The homogenate was then centrifuged at 4000 rpm for 5 min at 4 °C in a cooling centrifuge (Laborzentrifugen, 2k15, Sigma, Germany) to remove cell debris. The aliquot was kept at −80 °C for further biochemical analysis.

2.5. Biochemical analysis

Serum activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) using kits of EIAab [20] were all analyzed in liver homogenate.

Liver tissue homogenate concentrations of osteopontin (OPN), transforming growth factor β1 (TGF-β1), tumor necrosis factor-alpha (TNF-α), and α-smooth muscle actin (α-SMA) were all determined colorimetrically.

2.6. Histopathological and immunohistochemical examinations

Formalin fixed liver specimens were routinely dehydrated in graded series of alcohol, cleared in xylol and finally embedded in paraffin. Paraffin blocks were serially sectioned at 4–5 μm thickness and stained with Hematoxylin and Eosin (H&E) [21]. The sections were examined using light microscope (Olympus BX50, Japan).

Paraffin sections of liver of control and all treated groups were used for immunohistochemical detection of caspase-3 and α-SMA expression using avidin-biotin peroxidase (DAB, Sigma Chemical Co.) according to method described by Ref. [22]. Tissue sections were incubated with a monoclonal antibody for caspase-3 and α-SMA (Dako Corp, Carpentry, CA) and reagents required for the avidin-biotin peroxidase (Vactastain ABC peroxidase kit, Vector Laboratories) method for the detection of the antigen–antibody complex. Each marker expression was visualized by the chromagen 3,3-diaminobenzidine tetra-hydrochloride (DAB, Sigma Chemical Co.). Immunohistochemical quantification of both caspase-3 and α-SMA expression was carried out by measuring the optical density in 7 high power microscopic fields using image analysis software (ImageJ,1.46a,NIH,USA).

2.7. Statistical analysis

The results were expressed as mean ± SE of the mean. Data analysis was achieved by one-way analysis of variance (ANOVA) followed by Tukey’s multiple comparison test using software program GraphPad Prism (version 5.00). A value of P < 0.05 was considered statistically significant.

3. Results

3.1. Effect on liver function markers

The administration of TAA to rats induced marked liver injury indicated by significant (P < 0.05) increase in the serum levels of AST, ALT and total bilirubin with significant (P < 0.05) reduction in serum levels of albumin and total protein when compared to normal control group (Table 1). Pre-treatment of rats with mesalazine significantly limited the elevation of AST, ALT and total bilirubin levels compared with TAA group. Moreover, significant (P < 0.05) improvement in serum levels of AST, ALT, total bilirubin, total protein and albumin was observed in rats’ groups administered mesalazine post TAA when compared to the corresponding TAA group and both protective groups as well.

3.2. Effect on liver tissue homogenate oxidative stress markers

The results in Table 2 showed that; TAA administration led to significant (P < 0.05) depletion of hepatic GSH and SOD content with significant increase in MDA level compared with those of normal control rats. The pre-treatment of rats with mesalazine (50 and 100 mg/kg) for 4 weeks before induction of liver fibrosis significantly inhibited the increase in hepatic MDA level and counteracted the depletion of hepatic GSH content and SOD activity compared to TAA group. A higher dose related improvement in the oxidative stress markers was noticed on the post-treatment use of mesalazine (50 and 100 mg/kg) for 6 weeks after induction of liver fibrosis as observed by significant (P < 0.05) elevation in hepatic GSH and SOD content accompanied with significant decrease in MDA as compared to TAA group (Table 2).

3.3. Effect on osteopontin (OPN), transforming growth factor beta-1 (TGF-β1), tumor necrosis factor alpha (TNF-α) and alpha-smooth muscle actin (α-SMA) in liver homogenate

TAA administration resulted in significant (P < 0.05) elevation in hepatic OPN, TGF-β1, TNF-α and α-SMA levels compared with that of the normal control group. While, the pre-treated groups with mesalazine (50 and 100 mg/kg) limited the former elevation in OPN, TGF-β1, TNF-α and α-SMA concentrations as compared to TAA group. However, the use of mesalazine (50 and 100 mg/kg) as a therapy for 6 weeks after induction of liver fibrosis resulted in more significant (P < 0.05) suppression in hepatic tissue homogenate levels of OPN, TGF-β1, TNF-α and α-SMA especially with the use of the higher dose as compared to TAA group and the protective groups (Figs. 2 and 3).

3.4. Histopathological assessment

Examination of different liver sections of control rats revealed normal histological hepatic structure (Fig. 4a). However, thioacetamide administration resulted in severe tissue alterations as; marked hepatocellular swelling with vacuolar to ballooning degeneration and cytoplasmic reticulation (Fig. 4b). Some hepatocytes showed karyocytomegaly, others appeared with pyknotic nuclei, while some others appeared necrotic. The portal triads in those livers showed congested blood vessels, proliferation of the cholangiolar epithelium with multiple newly formed bile ductules as well as fibrous proliferation with its extension peripherally toward the parenchyma (Fig. 4c). The later extension resulted in marked portal to portal fibrosis and pseudolobulation of the hepatic parenchyma (Fig. 4d). The hepatic cells inside the lobules showed vacuolar degeneration with cytoplasmatic reticulation and pyknotic nuclei, many necrotic cells appeared homogenous eosinophilic masses with the appearance of multiple apoptotic bodies (Fig. 4e). The fibrous septa of the pseudolobulation contained newly formed bile ductules and mononuclear inflammatory cells (Fig. 4f).

In regards to livers of thioacetamide and mesalazine treated rats, the examination of which revealed that; the use of mesalazine as a prophylaxis didn’t achieve enough protection against the harmful effect of TAA, only it decreased the intensity of hepatocellular vacuolation but the portal to portal fibrosis and pseudolobulation appeared clearly, although in a lower intensity particularly in the high dose group. Hepatocellular swelling and karyomegaly with mild vacuolar degeneration were noticed (Fig. 5a). Moderate degree of pseudolobulation at which the fibrous septa appeared thinner, mostly incomplete and contained congested capillaries, few newly formed bile ductules and mononuclear inflammatory cells (Fig. 5b). Most of the hepatic cells in between the septa appeared markedly swollen with a moderate degree of vacuolar degeneration and necrosis (Fig. 5c).

While the use of mesalazine as a therapy showed a dose related good restoration of the hepatic parenchymal cells with an obvious decreased intensity and retraction of fibrous proliferation (Fig. 5d) as well as marked easing of fibrotic reaction with appearance of some apoptotic...
cells and near to normal appearance of the hepatic parenchymal cells with mild vacuolation (Fig. 5e).

Livers of some rats showed mild fibrous proliferation with the appearance of mild incomplete pseudolobulation (Fig. 5f) in some areas appeared as groups of hepatocytes without enclosing with fibrous proliferation.

3.5. Immunohistochemical analysis of α-SMA and caspase-3

Microscopic examination of liver of control rats revealed normal positive expression of α-SMA around the central and portal veins (Fig. 6a) where normal myoﬁbroblasts exist. While livers of thioacetamide administered rats revealed marked increased immunopositivity of α-SMA along the portal to portal ﬁbrous stands, extending among the parenchyma, in the perisinusoidal space and surrounding individual hepatocytes (Fig. 6b). In the portal triads α-SMA was markedly

Table 1

| Effect of pre and post-treatment with mesalazine (50 and 100 mg/kg) for 6 weeks on serum biochemical parameters in TAA induced liver fibrosis in rats’ model. |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Albumin (g/dl)  | Total Protein (g/dl) | Total Bilirubin (mg/dl) | ALT (U/L) | AST (U/L) | Dose (mg/kg) | Groups |
| 4.8 ± 0.08ᵇ     | 7.5 ± 0.16ᵇ     | 0.29 ± 0.01ᵇ     | 19.4 ± 1.08ᵇ | 21.4 ± 0.94ᵇ | –              | Normal control |
| 2.2 ± 0.03ᵇ     | 5.1 ± 0.07ᵇ     | 1.85 ± 0.03ᵇ     | 117.10 ± 3.41ᵇ | 126.2 ± 3.78ᵇ | –              | TAA control |
| Hepato-protective study |
| 2.7 ± 0.03ᵇ     | 5.7 ± 0.05ᵇ     | 1.41 ± 0.01ᵇ     | 85.2 ± 2.06ᵇ | 96.2 ± 1.88ᵇ | 50              | Mesalazine |
| 3.1 ± 0.03ᵇ     | 6.3 ± 0.03ᵇ     | 0.93 ± 0.02ᵇ     | 59.8 ± 0.66ᵇ | 70.8 ± 0.61ᵇ | 100             | Mesalazine |
| Hepato-therapeutic study |
| 3.17 ± 0.02ᵇ   | 6.50 ± 0.04ᵇ   | 1.18 ± 0.03ᵇ   | 59.70 ± 0.86ᵇ | 68.90 ± 1.33ᵇ | 50              | Mesalazine |
| 3.61 ± 0.03ᵇ   | 6.80 ± 0.03ᵇ   | 0.67 ± 0.03ᵇ   | 30 ± 0.71ᵇ  | 39.30 ± 0.54ᵇ | 100             | Mesalazine |

N = 7; Values are expressed as mean ± SEM.
Data were analyzed by using one way ANOVA followed by Tukey’s multiple comparison test.

ᵃ Significant at P < 0.05 when compared with control group.
b Significant at P < 0.05 when compared with TAA group.

Table 2

| Effect of pre and post-treatment with mesalazine (50 and 100 mg/kg) for 6 weeks on liver tissue homogenate oxidative stress markers in TAA induced liver fibrosis in rats’ model. |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| GSH (μmol/g tissue) | Dose (mg/kg) | MDA (nmol/g tissue) | SOD (U/g tissue) | Groups |
| 5.37 ± 0.25ᵇ     | 18.99 ± 0.52ᵇ     | 9.53 ± 0.73ᵇ     | 73.37 ± 1.31ᵇ | –              | Normal control |
| 0.89 ± 0.03ᵇ     | 2.92 ± 0.18ᵇ     | 48.92 ± 1.00ᵇ | 50              | –              | TAA control |
| Hepato-protective study |
| 1.30 ± 0.02ᵇ   | 4.20 ± 0.11ᵇ   | 36.62 ± 0.49ᵇ   | 100             | Mesalazine |
| 1.94 ± 0.02ᵇ   | 6.20 ± 0.11ᵇ   | 37.32 ± 0.73ᵇ   | 50              | Mesalazine |
| Hepato-therapeutic study |
| 1.84 ± 0.03ᵇ   | 5.91 ± 0.03ᵇ   | 20.37 ± 0.32ᵇ   | 100             | Mesalazine |
| 3.16 ± 0.08ᵇ   | 11.35 ± 0.28ᵇ   | 20.37 ± 0.32ᵇ   | 100             | Mesalazine |

N = 7; Values are expressed as mean ± SEM.
Data were analyzed by using one way ANOVA followed by Tukey’s multiple comparison tests.

ᵃ Significant at P < 0.05 when compared with control group.
b Significant at P < 0.05 when compared with TAA group.

3.5. Immunohistochemical analysis of α-SMA and caspase-3

Microscopic examination of liver of control rats revealed normal positive expression of α-SMA around the central and portal veins (Fig. 6a) where normal myoﬁbroblasts exist. While livers of thioacetamide administered rats revealed marked increased immunopositivity of α-SMA along the portal to portal ﬁbrous stands, extending among the parenchyma, in the perisinusoidal space and surrounding individual hepatocytes (Fig. 6b). In the portal triads α-SMA was markedly
expressed along the proliferated fibrous connective tissue especially pericanalicular around the proliferated bile duct and the newly formed bile ducteoles (Fig. 6c). Livers of rats’ administrated thioacetamide and mesalazine as a prophylaxis showed higher positivity of α-SMA than those administrated mesalazine as a treatment. In the former rats, α-SMA was moderately expressed in the portal areas and along the fine fibrous strands of portal to portal pseudolobulation (Fig. 6d). In regards to livers of thioacetamide and mesalazine (as a treatment) administrated rats’ revealed scattered mild positivity of α-SMA in the portal area and in the parenchyma (Fig. 6e). The immunohistochemical staining of α-SMA was quantified as optical density (OD) of the stained regions using the image analysis software (Fig. 6f). TAA administration caused significant increase in the expression of α-SMA compared to control group, however, a dose related decreased expression was noticed on mesalazine administration that was more significant (P < 0.05) in the therapy groups than in the prophylactic groups.

On the other hand, livers of control rats showed negative expression of caspase-3 (Fig. 7a). Marked positive expression of caspase-3 was observed in livers of TAA administrated rats; in the cytoplasm of the swollen hepatocytes (Fig. 7b) as well as in the apoptotic cells, and apoptotic bodies that were spreading along the fibrous septa and even scattered in the parenchyma (Fig. 7c). Although mesalazine administration obviously decreased the expression of caspase-3, but a higher decrement was observed in the therapy groups than in the prophylactic groups which showed scattered immune-positive caspase-3 apoptotic bodies in the hepatic parenchyma (Fig. 7d). While scarce apoptotic bodies expressing caspase-3 were observed in livers of TAA and mesalazine (as a treatment) administrated rats (Fig. 7e). A quantitative analysis of the optical density of the stained regions of caspase-3 (Fig. 7f) was carried out using the image analysis software, which showed significant immune-positivity of caspase-3 in TAA administrated rats compared to controls. A significant (P < 0.05) dose related decrease in caspase-3 expression was noticed in mesalazine administrated rats as compared to TAA administrated rats particularly in the therapy groups than in the prophylactic groups.

4. Discussion

Liver fibrosis is a result of many chronic injuries and often progresses to cirrhosis, liver failure, portal hypertension, and hepatocellular carcinoma. Liver transplantation is the alternative for patients with advanced stages of liver fibrosis. Accordingly, there is a critical need to find novel approaches for anti-fibrotic therapy [23]. Indeed, there are may be several existing antifibrotic drugs with well-established safety profiles and they also have been developed for other indications [24]. The current study investigated the potential
hepatoprotective and hepatotherapeutic effects of mesalazine in rat model of liver fibrosis using thioacetamide which considered as a well-established model of experimental liver fibrosis in rodents [25]. The intraperitoneal injection of TAA induced liver fibrosis within 6 weeks which agreed with Liedtke et al., [26]. TAA had been used as a hepatotoxin in several studies to speculate the mechanism of hepatic fibrogenesis. Following TAA administration, reactive metabolites as thioacetamide sulfoxide (TASO) and thioacetamide-S,S-dioxide (TASO2) results from an extensive metabolism by microsomal CYP2E1 that contributes to the toxic effects of TAA [27]. TAA administration resulted in elevated levels of serum transaminases (ALT and AST) and bilirubin which are considered as relevant indicators of liver injury and hepatotoxicity, the elevation of which may be interpreted as a result of liver cell destruction or changes in the membrane permeability suggesting their leakage from injured hepatocytes into the blood, which agrees with the previously reported results Czechowska et al., [28]. The increased bilirubin is known to be associated with hepatobiliary damage and hepatic cholestasis [29] and is an indication of increased erythrocyte degeneration rate. Hyperbilirubinemia indicates liver failure to conjugate and excrete the heme-product bilirubin [30]. Moreover, TAA administration resulted in significant reduction in serum total protein and albumin levels which comes in parallel with the findings of Hessin et al., [31]. Hypoproteinemia might be attributed to the inflammatory reaction or perturbed protein biosynthesis in the fibrotic liver. Besides, free radicals and toxic metabolites emerged from TAA account for the cellular necrosis and inability of liver to perform its metabolic and excretory function [30].

Results of the present study revealed that, daily administration of mesalazine (50 and 100 mg/kg) prior to induction of liver fibrosis induced significant decrease in serum AST, ALT and total bilirubin combined with significant increase in total protein and albumin levels compared to TAA group, that restorative effect was more pronounced in the therapeutic use than that in the pre-treatment use. The later results are in accordance with those of Oakley et al. [32], who reported that, treatment with mesalazine significantly decreased serum activities of AST, ALT and bilirubin levels in CCL4 induced liver fibrosis model.

In the present study, induction of liver fibrosis through TAA caused marked oxidative stress in rat liver which was evidenced by a significant increase in the content of lipid peroxidation end product (MDA) combined with a significant decline in reduced GSH content and SOD activity. This finding is in agreement with Chen et al. [33], who reported that the increased level of lipid peroxidation in liver after TAA hepatotoxicity is attributed to free radical-mediated lipid peroxidation, leading to tissue injury and failure of the antioxidant defense.

Fig. 5. Livers of TAA and mesalazine treated rats; (a, b, c) as a prophylaxis and (d, e, f) as a treatment showing: (a) hepatocellular swelling and karyomegaly. (b) Moderate degree of pseudolobulation, the incomplete fibrous septa (arrow) contained few newly formed bile ductules and mononuclear inflammatory cells. (c) Moderate degree of vacular degeneration and necrosis of the hepatic cells in between the fine septa. (d) Marked decreased intensity and retraction of fibrous proliferation. (e) Easing of fibrotic reaction and near to the normal appearance of the hepatic parenchymal cells with mild vacuolation. (f) Mild fibrous proliferation with the appearance of mild incomplete pseudolobulation. (H&E, X400, 200, 100).
mechanism. Oral administration of mesalazine significantly reduced the oxidative stress which could be achieved through acting as scavenger of free radicals and providing antioxidant protection of biomolecules that is consistent with previous reports which indicated the scavenging properties of mesalazine for the free radical [34]. In the same context, Campregher et al. [35], reported that mesalazine (5-ASA) and its metabolite N-acetyl-5-ASA showed a significant O-2 scavenging effect.

The present results revealed significant increase in hepatic OPN, TGF-β, TNF-α and α-SMA levels in TAA group. These findings are consistent with those obtained by Arffa et al. [36], who mentioned that TAA treatment caused hepatic fibrosis as demonstrated by up-regulation of OPN and increased expression of α-SMA and TGF-β, and Saleh et al. [37], who reported that, TAA administration led to an increase in serum level of TNF-α. Previous studies have shown that measuring OPN in the plasma of liver disease patients is an effective biomarker for assessing the severity of liver fibrosis. It has been demonstrated that hepatocytes act as a major source of OPN, and has a paracrine role in activating HSCs and increasing collagen-I production. Several studies demonstrated that, neutralizing OPN abolishes the development of fibrosis [36].

OPN is crucially involved in the transformation of fibroblast to myofibroblast. Additionally, for the occurrence of TGF-β mediated myofibroblast differentiation and subsequent fibrosis, OPN expression is necessitated [38]. Comprehensively, OPN is able to activate fibroblasts to assume a myofibroblast or activated HSC phenotype. Several key cytokines as well as plentiful signaling molecules including TNF-α and TGF-β are produced by myofibroblasts generated directly by OPN, or through other mechanisms [39].

Our results showed that pre and post-treatment of rats with mesalazine significantly reduced the expression of OPN and TGF-β1.
compared with TAA group. This result is consistent with Chen et al. [15], who reported that in sodium sulfate-induced colitis in mice, OPN expression increased significantly in plasma and colonic tissues, and this increase was significantly reduced after treatment with mesalazine. Also, Wang et al. [1], reported that mesalazine has the ability to prevent nuclear translocation of NFκB and consequently the expression of TGF-β. In highlight of the current data, it was found that mesalazine abolished the driving action of OPN on inflammatory cells, and prevented its stimulatory action on TGF-β and collagen synthesis. Moreover, mesalamine, 5-aminosalicylic acid (5-ASA), is a potent antioxidant and is known to enhance peroxisome proliferator-activated receptor γ (PPAR) activity in the intestine [40], that PPAR agonist the reduction of OPN gene expression [41].

Tumor necrosis factor-α (TNF-α) is a pleiotropic cytokine implicated in the pathogenesis of chronic liver inflammation that leads to liver fibrosis. During the inflammatory phase, TNF-α results in the activation of resident HSCs into fibrogenic myofibroblasts [42]. Results obtained by Kaomongkolgit et al. [43], showed that TNF-α significantly increased OPN expression. With regard to the effect of mesalazine on TNF-α level, our results showed that daily administration of mesalazine significantly inhibited the elevation of TNF-α as compared with TAA group. This is consistent with the results previously reported by Saber et al. [44], who mentioned that mesalazine has the ability to decrease the expression of TNF-α.

Remarkably, the common hallmark of fibrosis is the activation of myofibroblasts, which produce excessive amount of extracellular matrix leading to the destruction of original tissue architecture and gradual decline of organ function. In response to activation, myofibroblasts express a high amount of alpha smooth muscle actin (α-SMA). Accordingly, measuring α-SMA expression is widely used to determine...

Fig. 7. Immunohistochemical stainig of caspase-3, the positive expression indicated by intense brown staining (arrows). (a) Liver of control rat showing negative expression of caspase-3. (b and c) Liver of TAA administrated rat showing marked positive expressing of caspase-3 in the cytoplasm of the swollen hepatocytes (b) as well as in the apoptotic cells and bodies (c). (d) Decreased immuno-positivity of caspase-3 in liver of rat administrated TAA and mesalazine as a prophylaxis. (e) Only few scattered apoptotic bodies expressing caspase-3 in liver of rat administrated TAA and mesalazine as a treatment (X100 and 400). (f) Quantitative analysis of the stained regions of caspase-3 as optical density using image analysis software (Values are expressed as the mean ± SE and were analyzed using one-way ANOVA with Tukey's multiple comparisons test. *P < 0.05 versus the control group, #p < 0.05 versus the TAA group).
the presence and activity of myofibroblasts. Kashiwagi et al. [45], stated that OPN significantly correlated with the α-smooth muscle actin-positive myofibroblast appearance in cisplatin-induced renal failure in rat model. In this regard, Corallo et al. [46], demonstrated that OPN levels increase simultaneously with the increasing of α-SMA levels in the pathogenesis of systemic sclerosis in the early stage of fibroblast differentiation process. The observed significant increase of α-SMA expression following administration of TAA was parallel with the findings of Abd-Elgawad et al. [47], and Zhou et al. [48], which was also confirmed by its marked immunopositivity in those liver sections. The oral administration of mesalazine (50 and 100 mg/kg) pre and post induction of liver fibrosis in our study showed a significant decrease in hepatic α-SMA expression as compared to TAA group, a finding which is in agreement with those of Wang et al. [1], and Oakley et al., [32].

Caspases are crucial mediators of apoptosis; among them, caspase-3 is a principal enzyme in the apoptotic cascade and is an indicator of apoptotic cell death [49]. Previous studies found that OPN suppresses caspase-9 and caspase-3-dependent cell apoptosis [50]. In our work, the hepatic caspase-3 showed significant immunopositivity following administration of TAA as compared to normal control group which is in agreement with Jing et al., [51]. However, livers of rats’ administrated mesalazine revealed significant decreased expression of caspase-3 pre-sent by few scattered apoptotic bodies expressing caspase-3.

Histopathologically, hepatic fibrosis induced by TAA is intimately similar to alcoholic liver fibrogenesis with similar histological and metabolic alterations commonly detected in the livers of stricken humans [52]. The oral administration of mesalazine (50 and 100 mg/kg) pre and post induction of liver fibrosis significantly decreased intensity and retraction of fibrous proliferation and preserved the hepatic cells integrity which was supported by restoring the hepatic function markers. The later mitigated effect was more pronounced on the post-treatment use of mesalazine as a marked retraction of fibrous proliferation with appearance of some apoptotic cells which was confirmed by the decreased immunopositivity of both α-SMA and caspase-3 expression. That observed preserved integrity of hepatocyte by mesalazine could be explained by its scavenging properties for the free radicals and toxic metabolites emerged by the free radicals and toxic metabolites by TAA as mentioned by Joshi et al., [34].

5. Conclusion

Mesalazine could have a potential new indication as anti-fibrotic agent through limiting the oxidative damage and altering TNF-α pathway as an anti-inflammatory drug with down-regulating TGF-β1, OPN, α-SMA and caspase-3 signaling pathways.

Lay summary

Mesalazine could be a superior candidate for potential new indication as anti-fibrotic agent and may represent a promising opportunity to develop a novel approach that might overcome the obstacles and risks associated with diseased liver. The later could be achieved through limiting the oxidative damage and altering TNF-α pathway as an anti-inflammatory drug with down-regulating TGF-β1, OPN, α-SMA and caspase-3 signaling pathways.

Conflicts of interest

The authors have declared that no conflict of interests exists between any of the authors.

Author contributions

Ramadan A. and Nehal Afifi conceived, designed the study, analyzed the data, and revised the manuscript. Nemat Z. Yassin and Rehab F. Abdel-Rahman contributed to the reagents/materials/analysis tools, wrote the manuscript and analyzed the data. Sahar Abd El-Rahman analyzed the data, wrote and revised the manuscript. Hany M.Fayed; contributed to the reagents/materials/analysis tools, collected the material, analyzed the data and wrote the manuscript.

Funding sources

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.cbi.2018.05.002.

References
