



Research article

Hepatoprotective effect of *Saccharomyces Cervisiae* Cell Wall Extract against thioacetamide-induced liver fibrosis in ratsZeinab A. El-Gendy^a, Salma A. El-Marasy^a, Rania F. Ahmed^a, Seham A. El-Batran^a, Sahar S. Abd El-Rahman^b, A. Ramadan^{c,*}, S.A.H. Youssef^c^a Department of Pharmacology, National Research Centre, Giza, Egypt^b Department of Pathology, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt^c Department of Pharmacology, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt

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ABSTRACT

Fibrosis represents a common outcome of almost all chronic liver diseases and leads to an impairment of liver function that requires medical intervention. The current study aimed to evaluate the potential anti-fibrotic effect of *Saccharomyces cervisiae* cell wall extract (SCCWE) against thioacetamide (TAA)-induced liver fibrosis in rats (200mg/kg b.w. i.p. twice weekly for 6 weeks) using Ursodeoxycholic acid (UDCA) as a reference anti-fibrotic product. SCCWE at two doses (50 and 100 mg/kg) significantly ameliorated the rise in serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT) activities, total bilirubin and direct bilirubin, increased total protein and albumin. SCCWE significantly reduced glutathione depletion (GSH), Nitric oxide (NOx) and malondialdehyde (MDA), thioredoxin (Trx) contents and elevated nuclear factor erythroid 2-related factor 2 (Nrf-2) content. Its anti-inflammatory effects were confirmed by observing a decrease in nuclear factor- κ B (NF- κ B), interleukin-1 β (IL-1 β) and inducible nitric oxide synthase (iNOS) content. The anti-fibrotic effects of SCCWE were explored by assessing fibrosis related markers as it significantly reduced transform growth factor- β (TGF- β) and autotaxin (ATX) contents. Administration of SCCWE significantly decreased matrix metalloproteinase-3 and 9 (MMP-3 and -9). Furthermore, it also decreased alpha smooth muscle actin (α -SMA) and caspase-3 as assessed immunohistochemically those results were similar to that of the standard drug UDCA. This study shows that SCCWE protects against TAA-induced liver fibrosis in rats, through attenuating oxidative stress, and inflammation, ameliorating MMPs, combating apoptosis and thereby fibrotic biomarkers in addition to improving histopathological changes.

1. Introduction

Liver fibrosis is a major global health problem resulting from several chronic liver injuries such as hepatic viral diseases, biliary diseases and chemical-induced liver disorders (Bataller and Brenner, 2005; Yanguas et al., 2016; El-Mihi et al., 2017). Chronic liver diseases affected millions of patients worldwide; 30% of patients are likely to develop fibrosis and cirrhosis. Also, this condition is evolved towards liver cancer and an elevation in the mortality rate (El-Baz et al., 2021). Globally, more than 100, 000, 000 people suffer from hepatic fibrosis, thereby it represents a major cause of mortality worldwide (Ramadan et al., 2018).

Liver fibrosis is characterized by overproduction of extracellular matrix (ECM) and replacement of liver parenchyma tissue with fibrotic one (Tsuchida and Friedman, 2017; Sadek et al., 2018) Hepatic stellate

cells (HSCs) are the main producers of ECM. Activation and proliferation of HSCs are mediated by reactive oxygen species (ROS) and inflammatory cytokines resulting in deposition of ECM and fibrotic tissue (Bataller and Brenner, 2005). Activated HSCs promotes the expression of fibrotic biomarkers alpha-smooth muscle actin (α -SMA) and autotaxin (ATX) (El-Batch et al., 2011; Peverill et al., 2014).

Oxidative stress, inflammation and apoptosis are involved in the pathogenesis of liver fibrosis (Ma et al., 2015). Matrix metalloproteinases (MMPs) are present in the ECM and are implicated in cell proliferation, differentiation and apoptosis, thereby they play crucial role in liver fibrosis (Duarte et al., 2015).

Thioacetamide (TAA) is a well-established hepatotoxin used to induce liver fibrosis in rats. It mimics human chronic hepatic disease accompanied by toxic damage (Yanguas et al., 2016). TAA undergoes bio

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activation process to form TAA sulfur dioxide, responsible for hepatic necrosis and fibrosis (Hajovsky et al., 2012). Treatment of fibrosis is considered a challenging area for developing drugs, with good tolerance and minimal side effects. Reversal of fibrosis is considered a lengthy and expensive process (Gong et al., 2020; Trivella et al., 2020).

Since the pathophysiology of fibrosis include gut dysbiosis, efforts have been directed towards the use of probiotics and prebiotics as anti-fibrotic therapy (Milosevic et al., 2019). Probiotics are living microorganisms and prebiotics are indigestible polysaccharides that are responsible to restore and improve the gut microflora (Chen et al., 2005; Velayudham et al., 2009; Ferrere et al., 2017).

Saccharomyces Cervisiae Cell Wall Extract (SCCWE) is a prebiotic that resist gastric acidity hydrolysis and stimulates intestinal bacteria growth that is essential for human wellbeing (Park et al., 2016; Holscher, 2017). SCCWE is rich in prebiotics as it is considered a vital source of β -glucans, mannoproteins, chitin and glycopospholipid proteins. β -glucans has shown to exhibit beneficial effects on human health such as, anti-inflammatory, immune-stimulating, anti-diabetic, anti-viral, anti-microbial, anti-tumoral, and cholesterol-lowering effect (Worrasin-chai et al., 2006; Santipanichwong and Suphantharika, 2009).

Although SCCWE have shown hepatoprotective activity (Darwish et al., 2011; Kusmiati and Dhewantara, 2016), its effect on TAA-induced liver fibrosis has not been yet investigated. Therefore, this study aimed to assess the possible protective effect of SCCWE against TAA-induced liver fibrosis. Furthermore, the possible mechanistic pathway underlying the role of oxidative stress, inflammation, MMPs and apoptosis in the protective effect of SCCWE was studied. Ursodeoxycholic acid (UDCA) was used as a standard drug for comparison.

2. Materials and methods

2.1. Animals

Adult male Wistar albino rats, weighing 200–250 g were obtained from the animal house colony, National Research Centre, Giza, Egypt. All animals were housed in metal cages in a well-ventilated environment at (22 ± 3 °C, $55 \pm 5\%$ humidity and 12h dark & light cycles); received standard rat food pellets ($\geq 21\%$ proteins, $\geq 3.48\%$ fats, $\leq 3.71\%$ raw fiber and 1% multivitamins) with water *ad libitum*. Ingredients Yellow corn, soybean meal 44%, corn gluten 60%, di-calcium phosphate (8987), limestone, hulled sunflower cake, crude soybean oil, and a mixture of vitamins and minerals, methionine, and anti-fungicide. Water was provided *ad libitum* throughout the experimental period. The study protocol and procedures have been implemented in compliance with the National Institutes of Health guidelines (NIH publication No. 85–23, revised 2011) and in accordance with the Ethics Committee of the National Research Centre, Egypt (registration number 18/146).

2.2. Drugs

TAA (Sigma Company for drug and chemicals, Egypt) was freshly prepared prior to administration by dissolving in 0.9% (w/v) saline solution for intra-peritoneal (i.p.) injection. SCCWE (Antaferm-MG; Dr. Eckel GmbH, Niederzissen, Germany) was freshly prepared prior to administration by dissolving in 0.9% (w/v) saline solution for oral administration. According to the manufacturer, yeast cells are disintegrated, the soluble components are removed and the insoluble cell wall constituents are then purified and dried. The product contains 25% 1–3,1–6-b-d-glucans and 10% d-mannose (Sauerwein et al., 2007).

2.3. Experimental design

Experimental male rats were be classified into 5 groups/6 rats each.

Group 1: (Normal): rats received the vehicle (5 ml/kg, 0.5% CMC, orally) once daily and injected *i.p.* with 0.2 ml sterile saline twice weekly (days 2 and 6) for the whole period of the experiment.

Group 2: (TAA control): Induction of liver fibrosis was done by *i.p.* Injection of freshly prepared TAA (200 mg/kg b.w.) in 0.2 ml sterile saline twice weekly (days 2 and 6) for 6 consecutive weeks (Ftahy et al., 2013).

Groups 3 and 4: rats received daily oral dose of SCCWE of 50 and 100 mg/kg *p.o.*/day respectively, plus TAA twice weekly (days 2 and 6 one hour after β glucans), all for 6 weeks.

Group 5: rats received ursodeox-ycholic acid orally as 20 mg/kg body weight/day/for 6 weeks (Montasser et al., 2017). Plus giving TAA twice weekly (days 2 and 6 one hour after β glucans), all for 6 weeks.

2.4. Preparation of serum samples

After 6 weeks blood samples were collected in clean test tubes, allowed to clot, then were centrifuged for 20 min at 3000 r.p.m. Serum was separated and stored into eppendroff tubes at -20 °C to be used for determination of liver function parameters.

2.4.1. Biochemical measurements in serum

2.4.1.1. Determination of transaminases activity. Serum activities of serum alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were determined colorimetrically using kits of Spectrum® according to the method of Reitman and Frankel (1957). The absorbance was measured at 546 nm.

2.4.1.2. Determination of alkaline phosphatase (ALP). A serum activity of ALP was determined colorimetrically using kits of Spectrum® Kinetic method according to the International Federation of Clinical Chemistry (IFCC) (Zawta et al., 1994). The absorbance was measured at 405 nm.

2.4.1.3. Determination of gamma-glutamyltransferase (GGT). Serum activities of GGT was determined using kits of Spectrum® Kinetic colorimetric according to the method of Szasz (Szasz, 1969). The absorbance was measured at 405 nm.

2.4.1.4. Determination of serum total and direct bilirubin. This test was performed using kits of Spectrum® for the colorimetric determination of total and direct bilirubin in serum as cited in colorimetric Diazo method (Rand and di Pasqua, 1962). The absorbance was measured at 578 nm.

2.4.1.5. Determination of serum total protein. The test was performed using kits of Spectrum® for the quantitative colorimetric determination of total protein in serum as described by Coloremtric method (Biuret reagent) (Gornall et al., 1949). The absorbance was measured at wave length 546 nm.

2.4.1.6. Determination of serum albumin. The test was performed using kits of Spectrum® for the quantitative colorimetric determination of serum albumin at 620 nm according to Modified bromocresol green colorimetric method of Gosselin (1966). Absorbance was measured at 623 nm, or 578 nm.

2.4.1.7. Determination of thioredoxin (Trx). Serum level of Trx was measured using an enzyme-linked immunosorbent assay ELISA kit (Cloud-Clone Corp., Houston, TX, USA) according to the manufacturer's procedure.

2.5. Preparation of hepatic tissue homogenate

After collection of blood samples, rats were sacrificed by decapitation and their livers were immediately removed and divided into parts. For the remaining studies, serum and liver tissue samples were stored at -80 °C.

Part of liver samples was weighed and homogenized in 0.15 M phosphate buffer The aliquot was centrifuged using a cooling centrifuge (2k15; Sigma/Laborzentrifugen) at $1500 \times g$ at 4 °C for 15 min and the

supernatant was collected for the direct assessment of biochemical parameters.

A second part was preserved in formalin 10% for further histopathological and immunohistochemical investigation.

2.5.1. Determination of oxidative stress, anti-oxidant activity and nitrosative stress in liver homogenate

2.5.1.1. Determination of malondialdehyde (MDA). This test was performed using kits of Bio Diagnostic Company for the enzymatic colorimetric determination of MDA at wave length 534 nm according to the method of Ohkawa et al. (1979).

2.5.1.2. Determination of total nitrate/nitrite (NOx). NOx was determined in rat liver homogenate (20%) using a colorimetric method based on the Griess reaction according to the method of Miranda et al. (2001).

2.5.1.3. Determination of reduced glutathione (GSH). This test was performed using kits of Bio Diagnostic Company for the enzymatic colorimetric determination of GSH at wave length 405 nm according to the method of Tietze (1969).

2.5.1.4. Determination of nuclear factor erythroid 2-related factor 2 (Nrf-2). Nrf2 was determined using enzyme-linked immunosorbent assay ELISA kit of Elabscience Biotechnology Co., Ltd, USA.

2.5.2. Determination of inflammatory markers

Liver contents of nuclear factor- κ B (NF- κ B), interleukin-1b (IL-1 β) and inducible nitric oxide synthase (iNOS) content were qualified using ELISA kits according to the manufacturers procedures of (Elabscience® Biotechnology Co.,Ltd, USA), (cohesion Biosciences, Chinas) and (Cloud-Clone Corp., Houston, TX, USA), respectively.

2.5.3. Determination of fibrosis markers

2.5.3.1. Transform growth factor β (TGF- β). Hepatic content of TGF-B was quantified using ELISA kit (Cell science, Canton, MA, USA) according to the manufacturer's procedure.

2.5.3.2. Fibrotic matrix metalloproteases 3 and 9 (MMP-3 and 9). Hepatic content of MMP- 3 and -9 was quantified using ELISA kit from (Fine Test, Wuhan, Hubei, China) and (Cloud-Clone Corp., Houston, TX, USA), respectively and was performed according to the manufacturer's procedure.

2.5.3.3. Autotaxin (ATX). Hepatic content of ATX was quantified using ELISA kit (Cloud-Clone Corp., Houston, TX, USA) performed according to the manufacturer's procedure.

2.6. Histopathological examination

For histopathological studies, autopsy samples were taken from the liver of rats from different groups and fixed in 10% formal saline for twenty four hours. Washing was done in tap water then serial dilution of alcohol (methyl, ethyl and absolute ethyl) was used for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56 °C in hot air oven for twenty four hours. Paraffin bees wax tissue blocks were prepared for sectioning at 4 microns thickness by sledge microtome. The obtained tissue sections were collected on glass slides, deparaffinized, stained by hematoxylin and eosin stain for routine examination then examination was done through the light electronic microscope (Bancroft and Gamble, 2008).

2.7. Immunohistochemical studies

Immunohistochemical studies were carried out for detection of caspase-3 and α -SMA expression on paraffin sections of liver of control

and all treated groups using avidin-biotin peroxidase (DAB, Sigma Chemical Co.) according to method described by Hsu et al. (1981). Tissue sections were incubated with a monoclonal antibody for caspase-3 and α -SMA (Dako Corp, Carpinteria, CA, USA) and reagents required for the avidin-biotin peroxidase (Vectastain 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.).

2.8. Statistical analysis

All results were expressed as mean \pm standard error of the mean. Data analysis was achieved by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test using software program Graph Pad Prism (version 5.00). Difference was considered significant when $p < 0.05$.

3. Results

3.1. Effect of SCCWE on serum biochemical parameters

Results in Table 1 revealed that i.p injection of TAA twice weekly for six weeks significantly increased liver enzymes ALT, AST, ALP and GGT levels by 1.41, 1.47, 2.27, and 1.60 folds, respectively when compared to the normal group. Oral administration of SCCWE (50 and 100 mg/kg p.o) resulted in a significant decrease in serum ALT, AST, ALP and GGT levels by 33.58, 41.39 %, 23.36, 37.28%, 47.65, 58.43% and 25.50, 39.92%; respectively as compared to control TAA group. Similarly UDCA significantly reduced serum ALT, AST, ALP, and GGT by 38.11, 27.07, 44.11, and 33.22%, respectively as compared to control TAA group (Table 1).

Results in Table 2 revealed that TAA control group significantly elevated serum total and direct bilirubin levels by 1.73 and 1.82 folds, respectively and significantly reduced serum albumin and total protein levels by 1.48 and 1.4 folds, respectively when compared to the normal group. Treatment with SCCWE 50 and 100 mg/kg showed a significant decrease in serum total bilirubin level by 32.35, 37.25%, respectively; a significant decrease in serum direct bilirubin level by 38.71, 35.48%, respectively; a significant increase in serum albumin level by 18.91, 32.23%, respectively and a significant increase in serum total protein level by 11.85, 20.98% respectively, as compared to control TAA group. Similarly UDCA significantly reduced serum total and direct bilirubin level by 37.25, 41.93%, respectively and significantly increased serum albumin and total protein level by 32.26, 20.49%, respectively as compared to control TAA group (Table 2).

Results demonstrated in Figure 1 revealed that control TAA group increased significantly serum Trx level by 2.21 folds when compared to the normal group. Treatment with SCCWE 50 and 100 mg/kg showed a significant decrease in serum Trx level by 38.89, 59.26%, respectively as compared to control TAA group. Similarly UDCA 20 mg/kg showed a significant decrease in serum Trx level by 45.2 % as compared to the control TAA group.

3.2. Effect of SCCWE biochemical parameters in liver homogenate

Results in Table 3 revealed that TAA intoxicated group significantly increased hepatic contents of MDA and NOx by 2.28, 1.49 folds, respectively and significantly decreased liver content of GSH and Nrf2 by 2.43, 2.64 folds, respectively when compared to the normal group. Treatment with SCCWE 50 and 100 mg/kg showed a significant decrease in MDA hepatic content by 37.95, 45.79% folds, respectively; a significant decrease in NOx hepatic content by 32.3, 41.2% and a significant increase in GSH liver content by 50, 90% and Nrf2 content by 163.8, 219.56%, respectively as compared to control TAA group. Similarly UDCA significantly reduced hepatic content of MDA and NOx by 54.88, 30.1% respectively and significantly increased hepatic content of GSH

Table 1. Effect of SCCWE on liver enzymes in TAA-induced liver fibrosis in rats.

| Parameters | ALT (U/L) | AST (U/L) | ALP (U/L) | GGT (U/L) |
|-------------------|----------------------------|----------------------------|----------------------------|----------------------------|
| Treatments | | | | |
| Normal | 28.17 [#] ± 0.28 | 35.43 [#] ± 0.61 | 59.97 [#] ± 1.57 | 9.7 [#] ± 0.09 |
| Control (TAA) | 39.7* ± 0.78 | 51.93* ± 0.95 | 136.4* ± 3.16 | 15.53* ± 0.37 |
| SCCWE (50 mg/kg) | 26.37 [#] ± 0.82 | 39.8* [#] ± 0.44 | 71.4* [#] ± 2.01 | 11.57* [#] ± 0.08 |
| SCCWE (100 mg/kg) | 23.27* [#] ± 0.67 | 32.57* [#] ± 0.81 | 56.7 [#] ± 2.32 | 9.33 [#] ± 0.15 |
| UDCA (20 mg/kg) | 24.57 [#] ± 0.76 | 37.87* [#] ± 0.48 | 76.23* [#] ± 1.63 | 10.37 [#] ± 0.1 |

One-way ANOVA followed by Tukey's multiple comparison tests were used for carrying out statistical analysis. Mean ± SEM (n = 6) expressed the results. Significant difference from normal group expressed by * at p < 0.05. Significant difference from control (TAA) group was expressed by # at p < 0.05.

Table 2. Effect of SCCWE on liver bilirubin and proteins in TAA-induced liver fibrosis in rats.

| Parameters | Total Bilirubin (mg/dl) | Direct Bilirubin (ng/dl) | Total Protein (mg/dl) | Albumin (mg/dl) |
|-------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| Treatments | | | | |
| Normal | 0.59 [#] ± 0.03 | 0.17 [#] ± 0.01 | 5.67 [#] ± 0.09 | 3.21 [#] ± 0.01 |
| Control (TAA) | 1.02* ± 0.03 | 0.31* ± 0.01 | 4.05* ± 0.01 | 2.17* ± 0.03 |
| SCCWE (50 mg/kg) | 0.69 [#] ± 0.02 | 0.19 [#] ± 0.01 | 4.53 ± 0.27 | 2.58* ± 0.09 |
| SCCWE (100 mg/kg) | 0.64 [#] ± 0.01 | 0.2* [#] ± 0.01 | 4.9* [#] ± 0.18 | 2.87 [#] ± 0.09 |
| UDCA (20 mg/kg) | 0.64 [#] ± 0.01 | 0.18 [#] ± 0.01 | 4.88 [#] ± 0.21 | 2.87 [#] ± 0.12 |

One-way ANOVA followed by Tukey's multiple comparison tests were used for carrying out statistical analysis. Mean ± SEM (n = 6) expressed the results. Significant difference from normal group expressed by * at p < 0.05. Significant difference from control (TAA) group was expressed by # at p < 0.05.

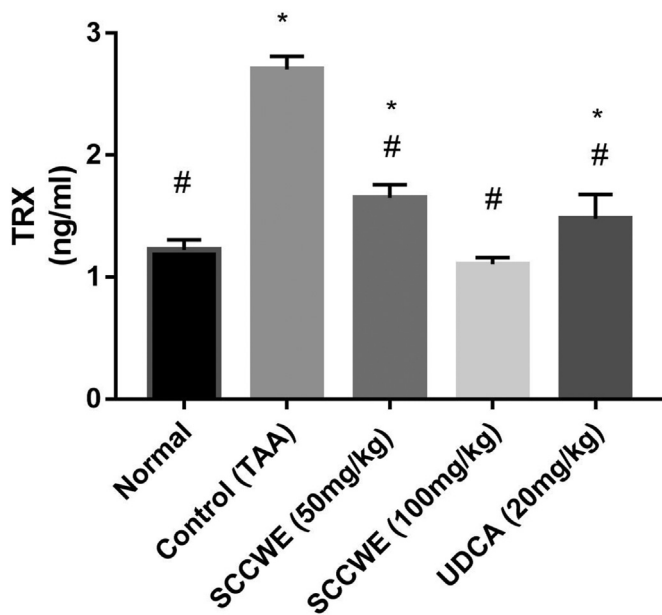


Figure 1. Effect of SCCWE on Thioredoxin level in TAA-induced liver fibrosis in rats. One-way ANOVA followed by Tukey's multiple comparison tests were used for carrying out statistical analysis. Mean ± SEM (n = 6) expressed the results. Significant difference from normal group expressed by * at p < 0.05. Significant difference from control (TAA) group was expressed by # at p < 0.05.

and Nrf2 by 86.67%, 148.21%, respectively as compared to the corresponding TAA group.

Figure 2a,b, c & d revealed that TAA control group significantly increased the hepatic content of, NF-κB, IL-1β, and iNos by 1.52, 3.76 and 4.34 folds when compared to the normal group. Treatment with SCCWE 50 and 100 mg/kg showed a significant decrease in the hepatic content of NF-κB by 20.23, 27.47%, IL-1β by 56.6, 60.73%, and iNOS by 49.16, 73.88%, respectively as compared to control TAA group. Similarly UDCA 20 mg/kg showed a significant decrease in the hepatic content of

NF-κB, IL-1β, and iNOS by 31.41, 72.22, and 71.6% as compared to the control TAA group.

Results demonstrated in Figure 3a, b, c, & d revealed that control TAA group increased significantly the hepatic content of TGF-β, MMP-3, MMP-9, and ATX by 1.56, 2.44 and 2.64 and 2.24 folds when compared to the normal group. Treatment with SCCWE 50 and 100 mg/kg showed a significant decrease in the hepatic content of TGF-β by 28.4, 43.77%, MMP-3 by 37.88, 57.58 %, MMP-9 by 41.84, 50.57%, and ATX by 39.55, 62.71%, respectively as compared to the control TAA group. Similarly, UDCA 20 mg/kg showed a significant decrease in the hepatic content of TGF-β, MMP-3, MMP-9, and ATX by 31.44, 53.03, 59.83, and 46.9% as compared to the control TAA group.

3.3. Histopathological examination

Examination of liver of control rats showed normal histological structure of portal areas, central veins and hepatic parenchymal cells (Figure 4a), while livers of TAA administrated rats showed marked portal triads reaction including; vascular congestion, proliferation of bile duct epithelium, inflammatory cells infiltration and marked fibrous proliferation (Figure 4b) which showed peripheral extension toward the parenchyma as fibrous bands that resulted in parenchymal pseudolobulation (Figure 4c). The hepatic cells within those pseudolobules showed vacuolar degeneration with eccentric nuclei and sometimes cytoplasmic reticulation. Scattered apoptosis as well as inflammatory infiltrates along the fibrous septa were clearly seen (Figure 4d).

Regarding the examination of various treated groups, it revealed that the administration of SCCWE had more curative effect than that observed on using the standard drug particularly at high dose, followed by the lower dose.

Livers of rats administrated UDCA showed moderate degree of fibrous proliferation within the portal areas with peripheral extension of incomplete septa (Figure 4e) accompanied with mild inflammatory cells infiltration and proliferated bile duct epithelial cells. The hepatic cells showed mild degree of degenerative and necrotic changes (Figure 4f).

Livers of low dose SCCWE administrated rats still showed existing fibroplasia in the portal areas but in a decreased intensity with peripheral extension of incomplete septa toward the neighboring areas along with

Table 3. Effect of SCCWE on liver MDA, NOx, GSH, and Nrf2 in TAA-induced liver fibrosis in rats.

| Parameters | MDA (nmole/g tissue) | NOx (μ mole/g tissue) | GSH (nmole/g tissue) | Nrf2 (ng/g tissue) |
|-------------------|--------------------------------|--------------------------------|-------------------------------|--------------------------------|
| Treatments | | | | |
| Normal | 33.6 [#] \pm 0.46 | 65.33 [#] \pm 1.49 | 2.92 [#] \pm 0.04 | 42.90 [#] \pm 1.53 |
| Control (TAA) | 76.53* \pm 0.87 | 97.1* \pm 0.61 | 1.2* \pm 0.03 | 16.21* \pm 0.40 |
| SCCWE (50 mg/kg) | 47.47* [#] \pm 1.05 | 65.73 [#] \pm 1.11 | 1.79* [#] \pm 0.03 | 42.60 [#] \pm 1.15 |
| SCCWE (100 mg/kg) | 41.47* [#] \pm 0.17 | 57.1* [#] \pm 0.16 | 2.27* [#] \pm 0.02 | 51.80* [#] \pm 0.71 |
| UDCA (20 mg/kg) | 34.53* [#] \pm 1.11 | 67.87* [#] \pm 1.32 | 2.25* [#] \pm 0.06 | 40.23* [#] \pm 1.35 |

One-way ANOVA followed by Tukey's multiple comparison tests were used for carrying out statistical analysis. Mean \pm SEM (n = 6) expressed the results. Significant difference from normal group expressed by * at $p < 0.05$. Significant difference from control (TAA) group was expressed by [#] at $p < 0.05$.

proliferated bile duct epithelial cells and few inflammatory cells (Figure 5a). The proliferated bile duct epithelial cells appeared insinuating (Figure 5b) among the parenchymal cells along with the incomplete fibrous strands. The hepatic cells in the vicinity showed moderate degree of vacuolar degeneration, scattered necrosis and apoptosis. While, livers of rats administrated SCCWE in a high dose revealed retraction of fibrotic reaction with its limitation to the portal areas and minimal changes accompanied with moderate degree of hepatocellular vacuolar degeneration particularly in the centrilobular areas (Figure 5c). Generally, the portal triads showed mild inflammatory reaction and bile duct proliferation without any evidence of fibrosis (Figure 5d).

3.4. Immunohistochemistry analysis

Livers of control rats showed mild normal expression of α -SMA around the portal and central veins where the existence of myofibroblasts (Figure 6a). Livers of TAA administrated rats revealed marked increased expression of α -SMA in the portal areas, along the extended fibrous septa (Figure 6b), pericellular and in the perisinusoidal spaces. The administration of SCCWE revealed a dose related decreased expression of α -SMA (Fig. 6c and d). While UDCA administration showed moderate immunorexpression of α -SMA in the portal areas and along the few incomplete septa (Figure 6e). The quantitative analysis of the area percent of the positive brown colour of α -SMA presented as the optical density revealed significant ($p < 0.05$) increased expression in TAA administrated group compared to that of the other treated groups (Figure 6f).

Regarding the caspase-3 stained sections, livers control rats showed negative expression (Figure 7a) of caspase-3, while those of TAA-administrated rats showed widespread expression of caspase-3 in the hepatic cells (Figure 7b). A dose related increased caspase-3 expression in the myofibroblasts was noticed in livers of rats treated with low (Figure 7c) and high (Figure 7d) doses of SCCWE, appeared as multiple apoptotic body along the retracted fibrous proliferation. Livers of UDCA administrated rats showed moderate expression of caspase-3 (Figure 7e) particularly among the myofibroblast cells. The area percent of the positive brown colour of caspase-3 presented as optical density revealed significant ($p < 0.05$) increased expression in TAA administrated group compared to that of the other treated groups (Figure 7f).

4. Discussion

Liver fibrosis represent a threatening health condition affecting millions of people worldwide (Lai and Afdhal, 2019; Zhang et al., 2020). It is usually mediated by oxidative stress and overproduction of inflammatory cytokines and cellular apoptosis (Parola and Pinzani, 2019; Roehlen et al., 2020).

Induction of rat liver fibrosis by TAA is considered a well-established model, resulting in; elevation in liver enzymes (Yogalakshmi et al., 2010; Czechowska et al., 2015; Al-Attar et al., 2016), reduction of serum level of bilirubin (Salama et al., 2013; Hessin et al., 2015), Hypoproteinemia (Alshawsh et al., 2011), marked oxidative stress (Lin

et al., 2017; Lebda et al., 2018). Increased oxidative stress and the resultant lipid peroxidation cause tissue necrosis and inflammation, promote the progression of tissue fibrogenesis (Zhang and An, 2007; Mandegary et al., 2013; Ahmadian et al., 2017; El-Agamy et al., 2019). In accordance with the previous investigators; our present study revealed that TAA ip administration at a dose of 200 mg/kg for 6 weeks resulted in a significant elevation in serum ALT, AST, ALP, GGT, and bilirubin levels. In addition to significant reduction in serum total protein and albumin. The current study reveals that liver fibrosis induced by TAA resulted in marked oxidative stress, manifested by a significant increase in the content of lipid peroxidation end product, MDA and NOx hepatic contents and decrease in the endogenous anti-oxidant GSH hepatic content. Depletion of GSH in hepatic tissue results from the evolution on reactive oxygen species (ROS) and lipid peroxidation (Eftekhari et al., 2018).

Trx is a stress-inducible protein whose production is induced in hepatic fibrosis due to the overproduction of ROS in liver fibrosis (Okuyama et al., 2005). In this manner, TAA-induced liver fibrosis elevated Trx hepatic content, revealing that TAA triggers oxidative stress in liver fibrosis.

Nrf-2 is a regulator of antioxidant system which is known to be activated by cellular oxidative stress and the generation of ROS that make it dissociates from its quencher and translocates into the nucleus then binds to the DNA promoter and causes transcription and activation of different antioxidant response elements (Gordillo-Bastidas et al., 2013; Ma, 2013; Zhang et al., 2015). Nrf2 hepatic content was significantly reduced in TAA administered rats as compared to normal rats, emphasizing the role of TAA-induced oxidative stress in hepatic fibrotic rats.

NF- κ B is a crucial transcription factor involved in cell inflammation, apoptosis, growth and development. NF- κ B regulates the production of various pro-inflammatory mediators as IL-1B, iNOS and the Profibrogenic cytokine TGF- β (Carlsen et al., 2009; Luedde and Schwabe, 2011; Singh and Gupta, 2011; Li et al., 2015; Zhang et al., 2015; Amer et al., 2017; Sun et al., 2017; Kumar et al., 2019). TAA administration to rats was significantly elevated NF- κ B hepatic content which in turn elevated IL-1B, iNOS and TGF- β hepatic contents as compared to normal rats, implicating the role of inflammation in mediating TAA-induced fibrosis in rats.

TAA administration in this study led to increase in MMP-3 and MMP-9 hepatic contents. Previously it was demonstrated that; MMP-3 is involved in activating MMP-9 justifying its role in connective tissue remodeling. Oxidative stress, inflammation and MMP-9 hepatic elevation activates release of TGF- β ; which triggers the activation of HSC and thereby α -SMA expression (Zhou et al., 2010; Quint et al., 2011; Ramachandran and Iredale, 2012; Zhang et al., 2015; Abd-Elgawad et al., 2016; Lin et al., 2018).

TAA administered rats showed a significant increase in hepatic ATX content as compared to normal rats, ATX, is a vital enzyme involved in HSC activation and proliferation; thus it is elevated in liver fibrosis (El-Batch et al., 2011; Yamazaki et al., 2017; Ikeda et al., 2018; Lebda et al., 2018).

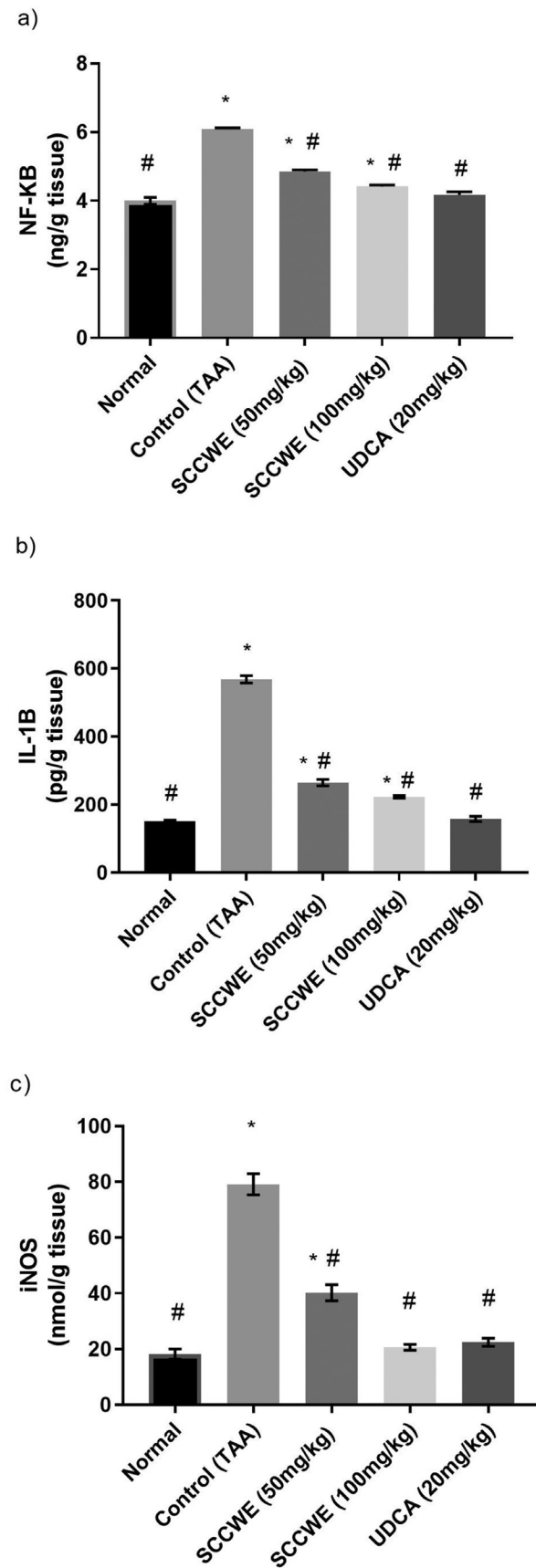


Figure 2. Effect of SCCWE on a) NF-κB, b) IL-1B, and c) iNOS contents in TAA-induced liver fibrosis in rats. One-way ANOVA followed by Tukey's multiple comparison tests were used for carrying out statistical analysis. Mean ± SEM (n = 6) expressed the results. Significant difference from normal group expressed by * at $p < 0.05$. Significant difference from control (TAA) group was expressed by # at $p < 0.05$.

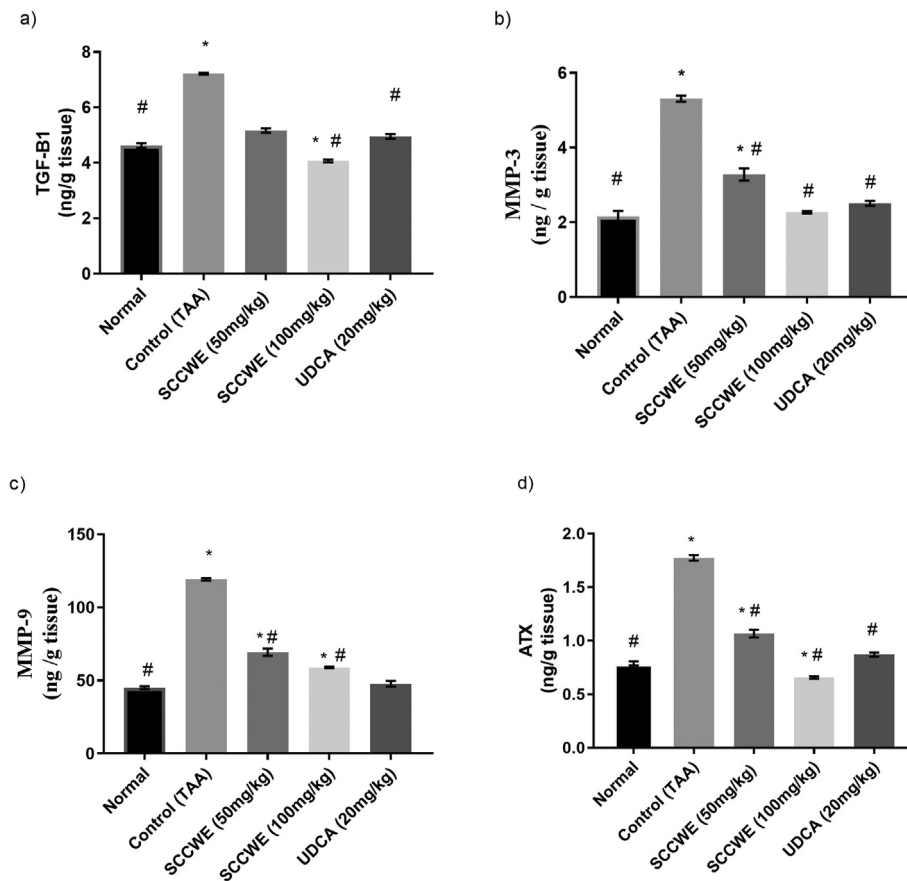


Figure 3. Effect of SCCWE on a) TGF-B1, b) MMP-3, c) MMP-9, and d) ATX contents in TAA-induced liver fibrosis in rats. Statistical analysis was carried out by one-way ANOVA followed by Turkey's multiple comparison tests. Results are expressed as mean \pm SEM (n = 6). Significant difference from normal group expressed by * at $p < 0.05$. Significant difference from control (TAA) group was expressed by # at $p < 0.05$.

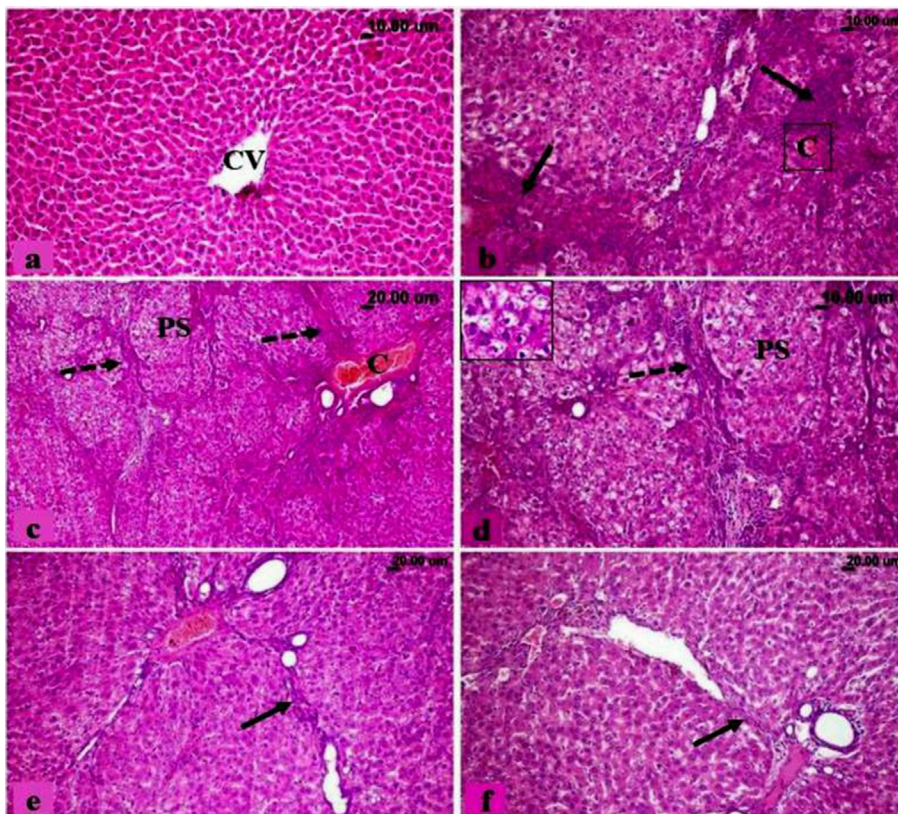


Figure 4. (a) Liver of control rat showing normal central vein (CV) and hepatic parenchymal cells. (b)- liver of TAA-administrated rat showing; (b) portal triad reaction as congestion (C), inflammatory cells infiltration (arrow), fibrous proliferation and proliferated bile duct epithelium, (c) peripheral extension of fibrous septa (dashed arrow) toward the parenchyma with a resultant parenchymal pseudolobulation (PS). (d) vacuolar degeneration of the hepatic cells within the pseudolobules that appeared with eccentric nuclei and sometimes cytoplasmic reticulation (upper corner). (e and f) Liver of rats administrated the stranded drug showing moderate degree of fibrous proliferation within the portal areas with peripheral extension of incomplete septa (arrow) accompanied with mild inflammatory cells infiltration and proliferated bile duct epithelial cells.

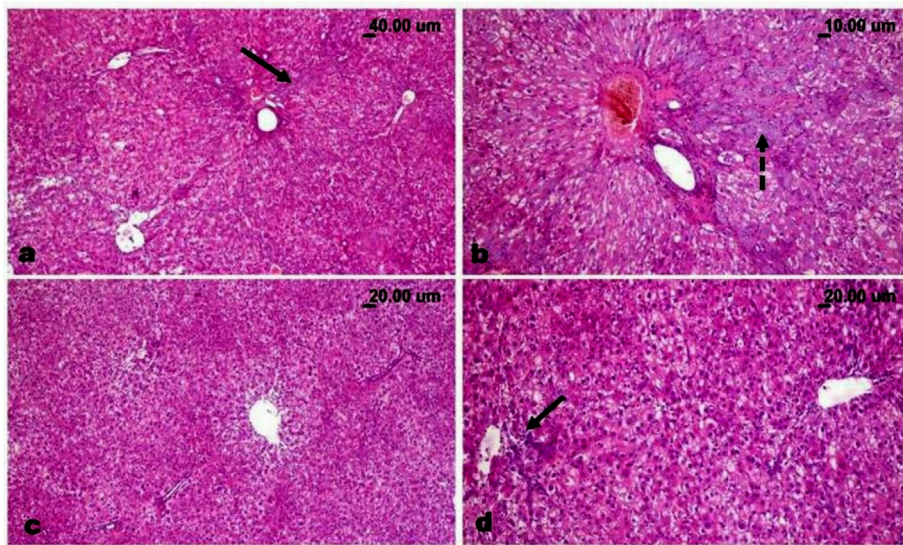


Figure 5. (a and b) Liver of low dose SSCWE administered rat showing mild fibroplasia in the portal areas and peripheral extension of incomplete septa (arrow) with proliferated bile duct epithelial cells insinuating among the parenchymal cells (dashed arrow). (c and d) liver of rat administered high dose of SSCWE showing retraction of fibrotic reaction and moderate centrilobular hepatocellular vacuolar degeneration (c) as well as mild inflammatory reaction and bile duct proliferation without any evidence of fibrosis (d).

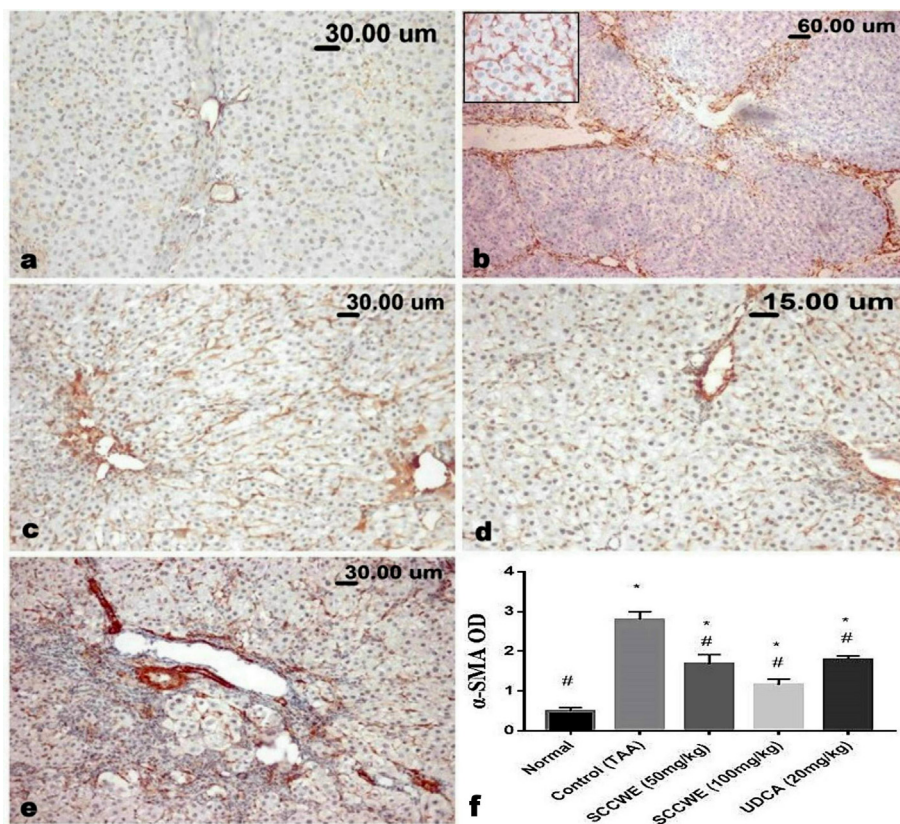


Figure 6. Immunohistochemical analysis of α -SMA. (a) normal mild expression of α -SMA around portal vein, (b) increased expression of α -SMA in the portal areas, along the fibrous septa, pericellular and perisinusoidal (upper corner). (c and d) an obvious dose related decreased expression of α -SMA in livers of rats treated with low and high doses of SSCWE. (e) moderate immunorexpression of α -SMA in the portal areas and along the few incomplete septa in liver of standard drug administered rat. (f) Quantitative image analysis of the area percent of the positive brown colour of α -SMA presented as the optical density. Values are expressed as the mean \pm SE and were analyzed using one-way ANOVA with Tukey's multiple comparisons test. * $p < 0.05$ versus normal control group, # $p < 0.05$ versus the TAA group.

Finally; histopathological examination of the liver sections revealed altered distorted hepatocellular architecture, with marked inflammatory cell infiltration and fibrous tissue proliferation. It has been documented that oxidative stress triggers apoptosis and HSCs activation (Kisseleva and Brenner, 2007; Ahmadian et al., 2018). Besides; Caspase-3, a major enzyme in the apoptotic cascade and an indication of apoptotic cell death (Ho and Hawkins, 2005) that is elevated in TAA-induced fibrosis in rats (Furtado et al., 2012; Jing et al., 2015). TAA-induced fibrosis in rats elevated hepatic caspase-3 protein expression as compared to normal rats, indicating that apoptosis mediated TAA-induced fibrosis in rats.

Alteration in gut microbes play a vital role in the progression of liver fibrosis. Anti-microbial response factors such as cytokines, chemokines,

anti-endotoxin core antibodies, and peptides, are released into the circulation to combat microbial products (Stapels et al., 2015; Lee et al., 2019). Anti-microbial proteins which include cathelicidins, defensins, C-type lectins, ribonucleases and S100 proteins are host defense factors with anti-microbial properties that were reported to be deficit in chronic liver diseases and restored by the use of prebiotics (Hendriks and Schnabl, 2019). The prebiotic SCCWE has long been considered as a chief source of β -glucans which proved to be an effective as; anti-inflammatory, immune-stimulation, and anti-apoptotic agent (Dietrich-Muszalska et al., 2011; Pengkumsri et al., 2017).

Our study aimed to provide a new perspective on the hepatoprotective effect of SCCWE (50, 100 mg/kg) on TAA-induced hepatic

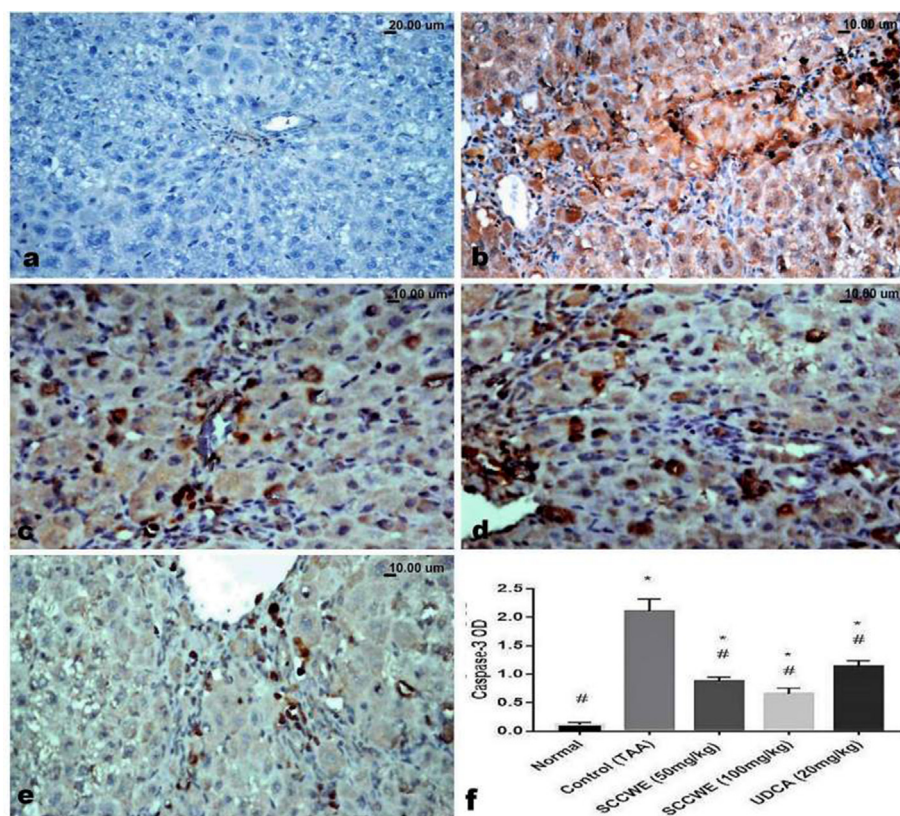


Figure 7. Immunohistochemical analysis of caspase-3. (a) liver of control rat showing negative expression (b) liver of TAA-administrated rat showing severe caspase-3 expression in hepatic cells, (c and d) a dose related increased caspase-3 expression in the myofibroblasts in liver of rats treated with low (c) and high (d) doses of SCCWE. (e) moderate expression of caspase-3 among the myofibroblasts in liver of the standard drug administered rat. (f) Quantitative image analysis of the area percent of the positive brown colour of caspase-3 in 5 microscopic fields presented as optical density. Values are expressed as the mean \pm SE and were analyzed using one-way ANOVA with Tukey's multiple comparisons test. * $p < 0.05$ versus normal control group, # $p < 0.05$ versus the TAA group.

fibrosis in rats. According to the authors' knowledge this is the first study to investigate the role of liver enzymes, oxidative stress, inflammation, MMPs, apoptosis, and histopathological changes in the hepatoprotective effect of SCCWE on TAA-induced liver fibrosis in rats.

In our study, oral administration of SCCWE in this study significantly reduced ALT, AST, ALP, GGT, and bilirubin levels and elevated serum total protein and albumin levels similar to UDCA the standard drug which reveal its ability to overcome the chemically induced hepatotoxicity and decrease necrotic conditions of hepatocytes. Those findings are in agreement with those of [Darwish et al. \(2011\)](#) and [Poloni et al. \(2020\)](#) who stated that SCCWE exerted hepatoprotective effect against Aflatoxin-induced toxicity in various animal species. Treated rats with 50 and 100 mg SCCWE and UDCA revealed the hepatoprotective effect of SCCWE against TAA-induced liver fibrosis as it was able to attenuate the altered hepatic histopathological changes compared to TAA group.

SCCWE treatment significantly decreased MDA hepatic content similar to UDCA the standard drug which come in accordance with [Nada et al. \(2010\)](#) who reported that *Saccharomyces cerevisiae* inhibited aflatoxin induced oxidative stress in rats that it may act as an adjuvant in preventing oxidative damage. Also its ability to decrease NOx and increase GSH hepatic contents proves its anti-oxidant effect against TAA-induced hepatic fibrosis. SCCWE was similar to UDCA treatment in reducing Trx hepatic content, suggesting that SCCWE helped in preventing the production and scavenging of ROS.

SCCWE-treated rats showed a significant increase in Nrf2 hepatic content similar to UDCA, the standard drug, therefore the anti-oxidant effect of SCCWE may be due to up regulation of Nrf2. SCCWE treatment was similar to UDCA and significantly reduced NF-KB hepatic content which in turn reduced IL-1 β , iNOS and TGF- β hepatic contents as compared to normal rats, indicating that SCCWE exerted anti-fibrotic effect through its anti-inflammatory effect which may be partly mediated by its anti-oxidant effect.

SCCWE treatment significantly reduced MMP-3 and MMP-9 hepatic contents to be similar to UDCA, indicating enhancement of ECM

degradation and anti-fibrotic effect of SCCWE. Furthermore, it has been demonstrated that Nrf2 directly regulates MMP-9 inhibition or indirectly via Nrf2-inhibiting NF-KB activation, thereby suppressing MMP-9 ([Ahmed et al., 2017](#)). Therefore, SCCWE suppressed MMPs activation directly via activating Nrf2 or indirectly through NF- κ B inhibitory pathway.

It has been reported that the reduction in α -SMA is accompanied by decrease in the number of the activated HSCs and amelioration of liver fibrosis ([Sokar et al., 2017](#)). The current data revealed that SCCWE treatment showed reduced hepatic α -SMA protein expression compared to TAA group similar to UDCA the standard drug, revealing decrease in activated HSCs number and anti-fibrotic effect of SCCWE. SCCWE treatment in the current study significantly reduced ATX hepatic content similar to UDCA, reflecting anti-fibrotic action of SCCWE is mediated by reduction in ATX hepatic content.

SCCWE and UDCA-treated rats significantly reduced hepatic caspase-3 protein expression, implying that SCCWE exerted anti-fibrotic effect via its anti-apoptotic effect mediated by its anti-oxidant effect.

5. Conclusion

SCCWE exerted hepatoprotective effect against TAA-induced fibrosis in rats possibly by attenuating hepatotoxicity biomarkers, exerting anti-oxidant, anti-inflammatory and anti-apoptotic effects in addition to ameliorating MMPs and histopathological changes. More studies are needed to depict the role of anti-microbial response factors and the exact molecular pathways involved in the hepatoprotective effect of SCCWE against TAA-induced fibrosis in rats.

Declarations

Author contribution statement

Zeinab A. El-Gendy; Salma A. El-Marasy; Rania F. Ahmed; Seham A. El-Batran; Sahar Abd El-Rahman; A. Ramadan; S.A.H. Youssef: Conceived

and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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The data that has been used is confidential.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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