Antifibrotic Mechanisms of Resveratrol in Modulating Liver Fibrogenesis

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Abstract: Liver fibrosis results from chronic inflammation to the liver with accumulation of extracellular matrix proteins, eventually leads to cirrhosis. Recently, natural supplements therapy has grown widely. Resveratrol is a polyphenol derived mainly from grapes considered to have a broad spectrum of pharmaceutical activities. The present study investigated the histological incidents of liver fibrogenesis and the cellular mechanisms by which resveratrol prevented and modulated liver fibrosis. Six groups of rats were used. One group served as control. Another was given resveratrol by oral gavage (20 mg/kg body weight/day). The third was injected intraperitoneally with dimethylnitrosamine (10mg/kg 3days/week) to induce liver fibrosis. The fourth was pre-treated with resveratrol then dimethylnitrosamine for 3 weeks. After cessation of dimethylnitrosamine, two subgroups from dimethylnitrosamine intoxicated group were daily post-treated with resveratrol (20mg/kg and 40mg/kg) for a week. Results revealed that resveratrol remarkably recovered body and liver weight and alleviated the histopathological alterations of hepatic fibrosis in time and dose-dependent manner. In conclusion, resveratrol may have employed two mechanisms in modulating liver fibrosis; arrested fibrosis progression through blocking hepatic stellate cell activation and/or reversion as well as stimulated resolution via triggered apoptosis. Resveratrol can be used as antifibrotic and reversing agent in liver disease therapy.
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1. Introduction

Liver fibrosis results from chronic damage to the liver accompanied with accumulation of extracellular matrix (ECM) proteins, which is a characteristic of most types of chronic liver diseases (Friedman, 2003), accounting for more than 1 million deaths each year worldwide (Mokdad et al., 2014). The main causes of liver fibrosis include chronic hepatitisviral infection, alcohol abuse, and nonalcoholic steatohepatitis (NASH). The accumulation of ECM proteins damages the hepatic architecture by forming a fibrous scar, and the subsequent development of nodules of regenerating hepatocytes indicates cirrhosis (Ginès et al., 2004). Several mechanisms exist to trigger immune reactions. Chronic immune reactions lead to liver fibrosis. Uncovering the mechanisms that underlie liver fibrogenesis forms the basis to develop therapies for chronic liver diseases. Hepatic steatosis is a common consequence of metabolic or toxic stress. Liver injury changes hepatocyte gene expression, resulting in increased expression of transforming growth factor β(TGFβ)(Koyama and Brenner, 2017). It has been well documented that the activation of hepatic stellate cells (HSCs) is the key event in hepatic fibrogenesis(Hellerbrand, 2013). HSCs reside in the space of Disse, between liver sinusoidal endothelium and hepatocytes. Physiological roles of HSCs include storage of vitamin A, synthesis of ECM and matrix-degrading metalloproteinases, and regulation of sinusoidal blood flow. Duringchronic liver injury, HSCs are activated, lose lipid-rich vitamin A granules and transdifferentiate into myofibroblasts express α-smooth muscle actin (α-SMA), synthesize increased amount of ECM with impaired ECM degradation. In addition, produce proinflammatory and profibrogenic cytokines leading to liver fibrosis ultimately cirrhosis(Bataller and Brenner, 2005; Blaner et al., 2009; Fujita and Narumiya, 2016; Kisseleva et al., 2012). Therefore, activated HSCs have become an attractive target for antifibrotic therapy in the past few decades. However, recent studies have indicated that HSCs also play a critical role in the process of liver development and regeneration (Yin et al., 2013). Many factors produced by HSCs promote liver regeneration by affecting hepatocytes, progenitor cells or bone marrow-derived mesenchymal stem cells (Bansal, 2016; Suzuki et al., 2003). In addition, HSCs play a potential role in liver regeneration through transdifferentiation into liver progenitor cells(Kordes et al., 2014). Thus, anti-fibrosis therapy targeted to HSCs may affect liver regeneration. Recently, a new study has demonstrated thatportal fibroblast contributes highly to liver fibrosis(Abdu and Al-Bogami, 2018). On the other hand, hepatic macrophages, including Kupffercells, are essential components for maintaining tissue homeostasis and

ensuring fast responses to hepatic injury. These cells either promote the restoration of tissue integrity following liver injury, or contribute to the progression of liver diseases, including hepatitis, fibrosis and cancer (Krenkel and Tacke, 2017). Kupffer cells can activate HSCs through the production of profibrotic cytokines TGFβ and platelet derived growth factor (PDGF)(Pradere et al., 2013). Conversely, Kupffer cells also express multiple matrix metalloproteinases that promote ECM degradation and thus favor the resolution of fibrosis (Fallowfield et al., 2007; Pellicoro et al., 2012).

Reactive oxygen species (ROS) are generated by various liver injuries such as alcoholabuse, hepatitis virus infection and chronic cholestasis and contributeto hepatic fibrogenesis. ROS promote the productionof collagen I in activated HSCs/myofibroblasts (De Bleser et al., 1999).

Resveratrol (RES) (3,4′,5- trihydroxystilbene) hasrecently attracted research attention due to its excitingpharmacological potential. It is a phytoalexin found in many plantsincluding grapes, peanuts, and berries(Aggarwal et al., 2004). RES has been widelyresearched in preclinical studies as a nutraceutical anda therapeutic agent for many diseases. Specifically, forcancer patients because of the high risks associated withtraditional treatments, including surgery and chemotherapy. By targeting multiple pathways, RES is apromising anticancer agent(Sarkar et al., 2009; Yang et al., 2018). Recently, RES has been shown tomimic effects of caloric restriction, exert anti-inflammatory and anti-oxidative effects, and affect the initiation and progression of many diseases including liver inflammation and fibrosis, as well as depressive disorder through several mechanisms(Abdu and Al-Bogami, 2017; Berman et al., 2017; Kessoku et al., 2016; Wang et al., 2018). Understanding the mechanism of inflammation and fibrosis is critically important to developing treatments for chronic liver diseases. This preclinical study emphasizes the events underlying fibrogenesis, and the effect of RES as antifibrotic therapy. Effective antifibrotic therapies may alter the history of chronic liver disease.

2, Materials and Methods

2.1 Materials (Chemicals)

N-Nitrosodimethylamine (dimethyl n-nitrosamine; DMNA) Cat no: 591068 N-Nitrosodimethylamine-d6,98 atom% d sigma. Carboxymethylcellulose sodium salt (CMC) from Sigma (St. Louis, MO, USA).

Resveratrol (RES); Cayman USA Cat no: R5101 RES ≥99%.

2.2 Experimental Animals

Forty two male Wistar albino rats weighing (90-116 g) were used in the experiment in accordance with the guidelines of the Biochemical and Research Ethical Committee at King Abdulaziz University, Jeddah, Saudi Arabia. Animals were housed in a well-ventilated temperature-controlled room at 22±23°C with 12 hours light and dark cycles. Food consisted of standard laboratory rat chow with free access to water. All experimental procedures were performed between 08:00am and 11:00am and care was taken to avoid all stressful conditions. Institutional Animal Ethical Committee permission was obtained before performing the experiments.

2.3Methods

Rats were divided into six groups. The control (n=7) received saline and 0.5% CMC solution administered orally via gastric tube. The RES (n=7) treated daily by oral gavage (20 mg/kg body weight/day) for 3 weeks(Ahmad and Ahmad, 2014). The DMNA (n=21) administered 10 mg/kg/day (10 µl DMNA diluted to 1 ml with 0.15 M sterile Na Cl) via intraperitoneal injection in the first three days of each week for three weeks(Lee et al., 2003). The RES pre-treatment (n=7) (RES + DMNA) were given RES, then after 2 hours lag DMNA for 3 weeks. The RES post-treatment low dose (n=7). After DMNA cessation of the DMNA group, post- treated with RES 20 mg/kg body weight/day for a week. Thelast group was RES post-treatment high dose (n=7). After DMNA cessation of DMNA group, post treated with RES 40 mg/kg body weight/day for a week. Animals were weighed at the beginning of each week.

2.4 Sample collection and preparation

Animals were sacrificed under ether anesthesia after 21 days (group 1, 2, 3, and 4), while groups 5 and 6 were sacrificed after 28 days. The livers of all animals were rapidly removed rinsed in cold saline and weighed. The liver specimens were fixed in 10% neutral buffered formalin for histological study by light microscopy(Bancroft and Gamble, 2002) or fixed in 2.5% glutaraldhyde for electron microscopy study (Robards and Wilson, 1993). Liver sections 3-5 µm were deparaffinized and processed routinely for Masson Trichrome stain (MTS) for collagen fibers and Hematoxylin & Eosin (H & E) stain for general histopathological study.

2.5Statistical analysis

Values for weight are expressed as mean value ± SD. Statistical analysis was performed by one-way analysis of variance (ANOVA) using SPSS 23.0 software (SPSS Inc., USA). Statistical significance was estimated by one way ANOVA. $P<0.05$ was considered statistically significant. Two-way ANOVA was used in statistical analysis of two doses.

$$\text{Ratio of the total increase of b. wt.} = \frac{\text{final b. wt.} - \text{original b. wt.}}{\text{original b. wt.}} \text{ x } 100$$

$$\text{Relative liver. wt.} = \frac{\text{liver wt.}}{\text{b. wt.}} \text{x} 100$$

3. Results:

3.1 Body and Liver Weights

After 21 days the ratio of the total increase in body weight of DMNA treated group was significantly low (44.9%) compared to the control (88.5%) and RES pre-treatment (91.8%) (table1). However, DMNA treatment caused the highest increase (5.8%) in the relative liver weight compared to the control (5.2%) and RES pre-treatment (5.5%). The results demonstrated that RES pre-treatment has suppressed the loss of body weight as well as the increase in relative liver weight. On the other hand, post-treatment with RES in both groups low and high dose after cessation of DMNA for one weekcaused a significant increase in the ratio of the total body weight (23.04% and 24.28% respectively) compared to the control (9.63%) (table1).

Table 1. Body and liver weight of all groups treated for 21 and 28 days.

Body weight B.W.	Control G1	RES G2	DMNA G3	RES pre-treatment G4	RES post-treatment (20 mg) G5	RES post-treatment (40mg) G6
Day 0	99.80±3.83	105.40±5.98	110.80±4.96	93.20±2.58	-	-
Day 7	120.60±12.72	134.40±5.72	121.40±8.96	122.20±11.41	-	-
Day 14	153.40±9.71	164.80±9.17	148.80±12.67	143.00±13.01	-	-
Day 21	188.20±18.55	193.20±11.12	160.60±8.38*	178.80±13.55**	-	-
Day 28	206.33±32.88	-	-	-	197.60±18.98	199.60±13.81
B.W.Ratio increase Day 21	88.5%	83.3%	44.9%*	91.8**%	-	-
Day 28(1 week)	9.63%	-	-	-	23.04%***	24.28%***
Liver W. Day 21						
Day 28	9.81±.47	9.50±.76	9.32±1.20	9.85±1.63	-	-
	10.31±.82	-	-	-	10.66±1.195	10.486±1.17
Relative Liver W.						
Day 21	5.2%	4.9%	5.8%	5.5%	-	-
Day 28(1week)	5.0%	-	-	-	5.39%	5.26%

Statistical analysis of rat's body and liver weights. Body weights were measured weekly throughout the study. Results are analyzed after 21 day by one way ANOVA and after 28 day by two way ANOVA and are presented as mean±SE $P<0.05$. *Indicates a significant difference between rats treated with DMNA and the control. **Indicates a significant difference between rats pre-treated with RES and rats treated with DMNA alone. *** Indicates a significant difference between rats post-treated with RES and the control.

3.2 Histological Study

Light microscopy study of the control and the RES groups revealed normal liver architecture (Figs. 1A, 1B, 2A & 2B). However, administration of DMNA for 21 days induced liver fibrosis and histopathological changes in the liver tissue. Masson's trichrome stain of DMNA sections revealed dilatation of central veins as well as portal fibrosis. In addition, severe disruption of vascular and lobular architecture in many areas as well as disorganizedportal tracts with bridging fibrosis and extensive proliferation of bile ducts and ductules (Fig. 1C). However, RES pre-treatment group revealed a marked reduction in the deposition of collagen fibers (Fig. 1D). Furthermore, H & E stain in the DMNA fibrotic liver displayed early cirrhosis in some areas, portal tracts were expanded by inflammatory cells. Bridging necrosis and fibrosis between vascular structures as well as vascular branching and wall thickening were seen (Fig. 2C). The hepatocytes were degenerated with indistinct plasma membrane and compressed sinusoids. Lipid droplets were seen inside ducts and veins. Occluded veins in small portal areas were largely replaced by fibrous tissues and may disappear as well as necrosis was evident in many places (Fig. 3A & B). On the other hand, RES pre-treatment

revealed a considerable reduction of the inflammatory cells, necrosis, vascular dilatation and wall thickening, vessels branching, congestion, and duct proliferation (Figs.1D, 2D, 3C & 3D). Examination of RES post-treated groups (low dose & high dose) revealed a slight decline in the pathological level compared to DMNA fibrotic liver. However, the magnitude of the liver damage was more pronounced in RES post-treated high dose (Figs. 1E & 1F, 2E & 2F, 3E,3F, 3G & 3H).

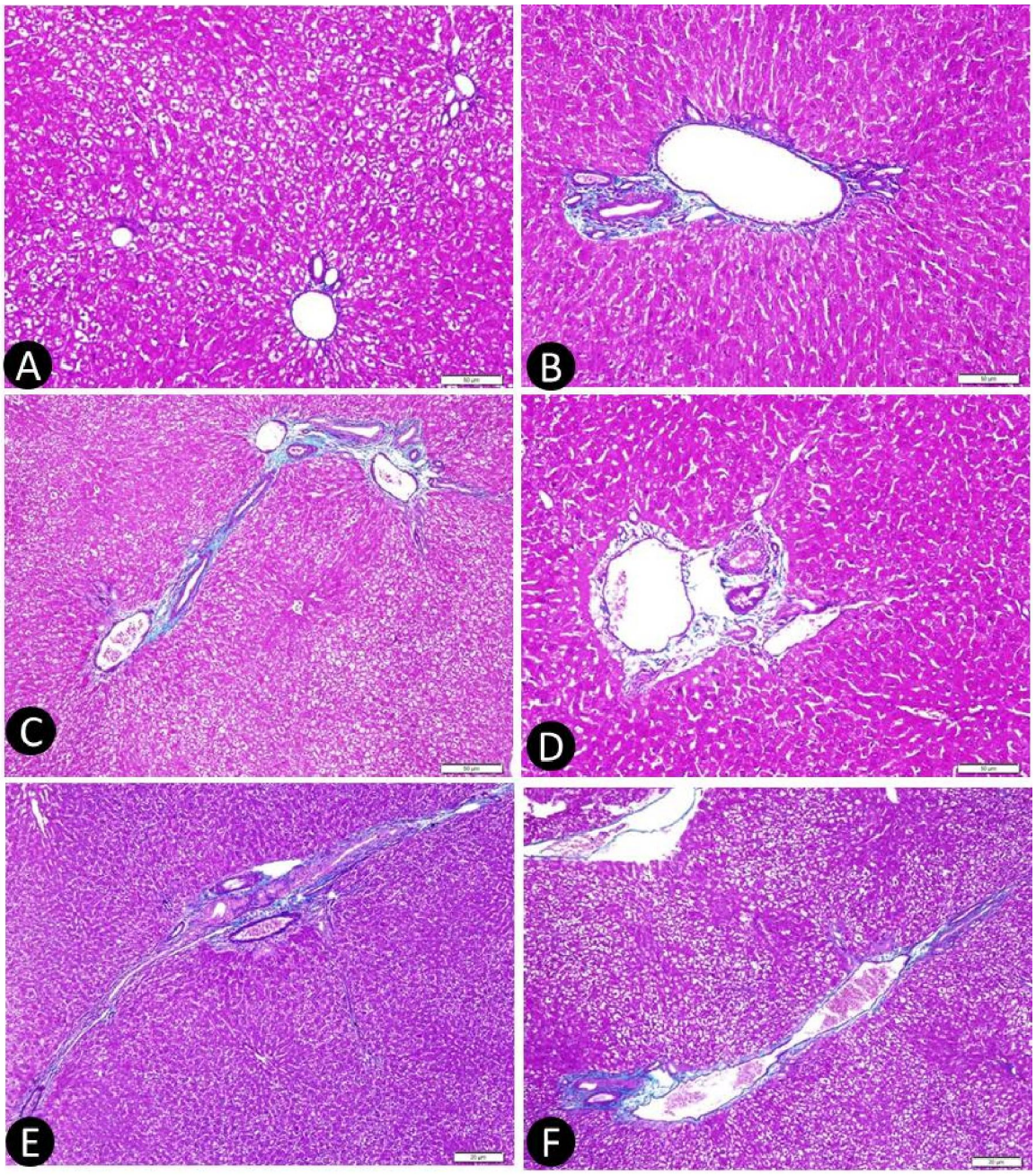

Figure1. Light micrographs of rat liver sections showing collagen fibers.
(A) Control. (B) RES. (C) DMNA, Portal-to-portal bridging fibrosis with early cirrhosis. Note bile ducts proliferation and muscular hypertrophy of vascular structures. (D) RES pre-treatment, exhibiting a remarkable reduction in collagen fibers. (E) RES post-treatment low dose, with minimal fibrosis compared to C, and early cirrhosis. (F) RES post-treatment high dose, with dilated portal area and slight fibrosis (MTS), (A, B, D X200; C, E, F X100)

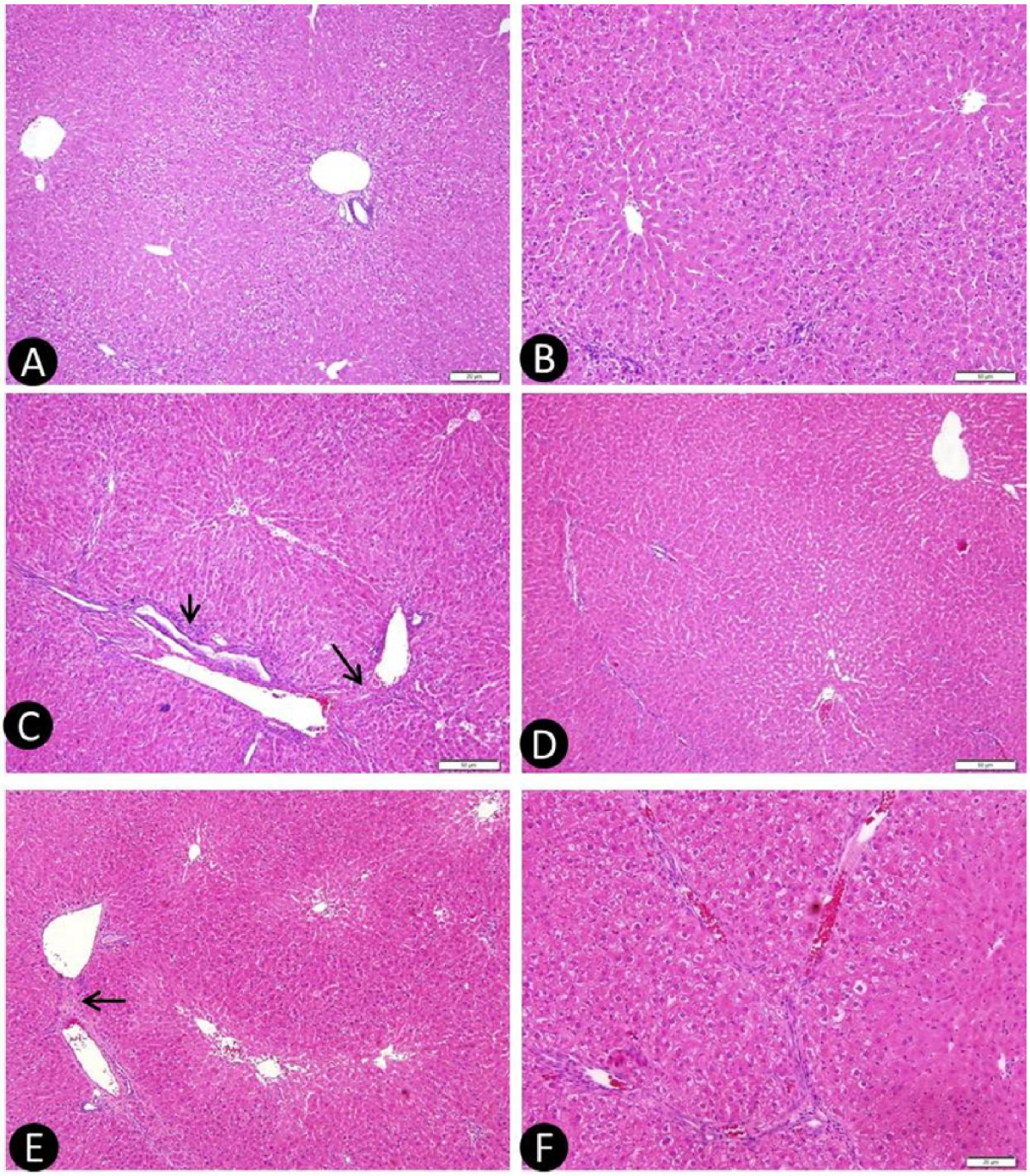

Figure 2. Light micrographs of rat liver sections showing vascular alterations.
(A) Control. (B) RES. (C) DMNA Shunt vessels. Bridging necrosis and early fibrosis extending between two adjacent portal tracts (arrow). Note lymphocytic infiltration and deformation of bile ducts (short arrow). (D) RES pre-treatment, exhibiting markedly reduced inflammation and vascular damage. (E) RES Post-treatment low dose with reduced lymphocytic inflammation and bridging necrosis (arrow) compared to DMNA in C. (F) RES post-treatment high dose, trifurcate and congested vascular structures defining lobules and progress to cirrhosis. (H & E) (A, C, D, E, X10; B, F, X 20).

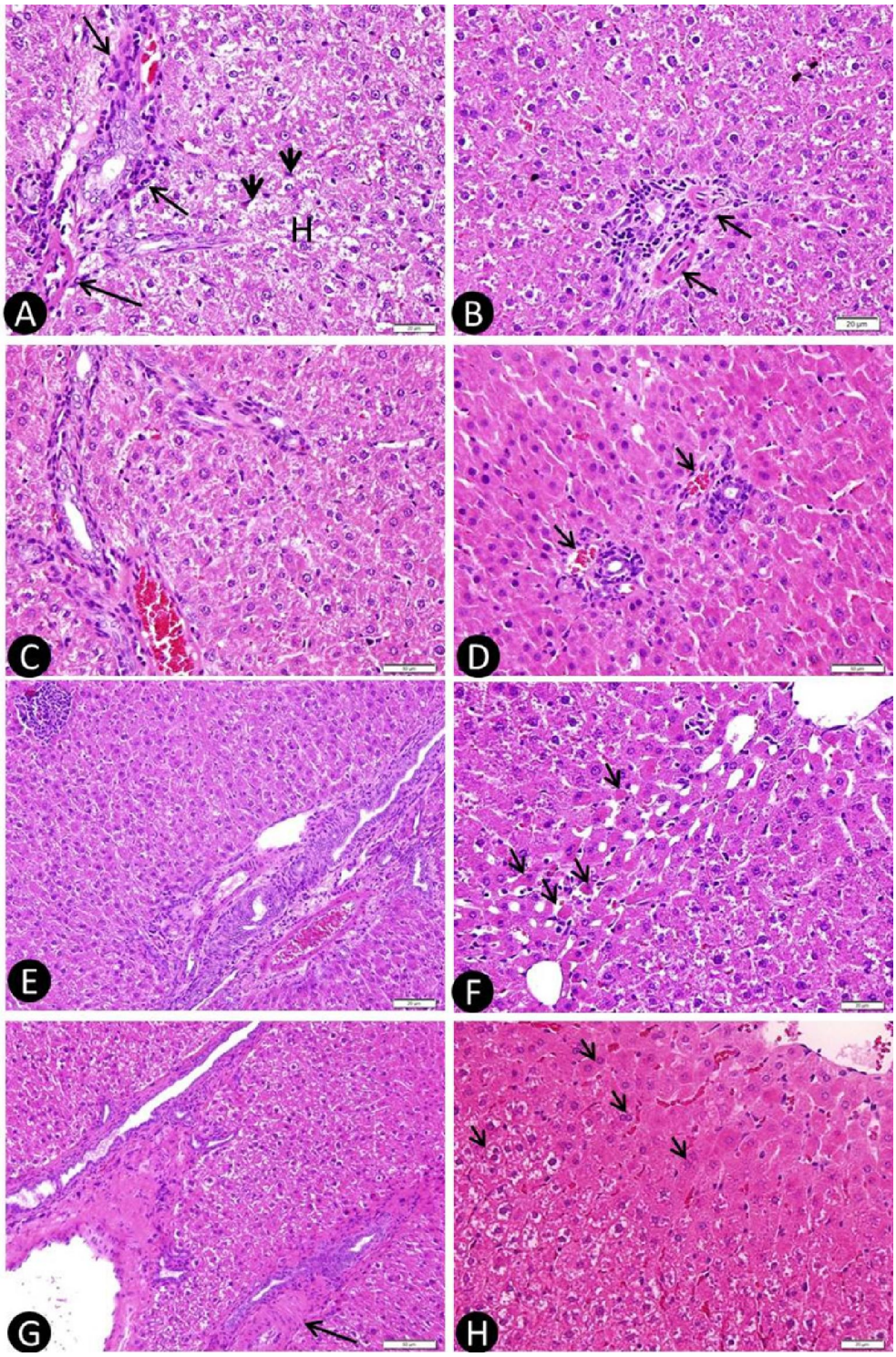

Figure3. Light micrographs of rat liver sections showing levels of inflammation.

(A) DMNA, hepatocyte injury triggers the recruitment of inflammatory cells and necrosis (arrows). Note damaged bile ducts, fat droplets inside hepatocytes, duct and veins. Vacuolated (H) and necrotic (thick arrow) hepatocytes, thick wall hepatic vein (long arrow). (B) DMNA, two small portal tracts closely opposed with partially obstructed veins and necrotic cells. The small veins are largely replaced by fibrous tissue. (C & D) RES pre-treatment, markedly reduced inflammation and fibrosis compared to figs. A & B. Note, the thin wall blood vessels (arrow) compared to thick wall in B. (E & F) RES post-treatment low dose, slightly reduced hepatocytes inflammation with ducts proliferation and marked fibrous portal expansion. Apoptosis is frequently noticed in the RES post- treatment low dose (arrows). (G & H) RES post-treatment high dose, aberrant duct proliferation as well as vein wall thickening and obstruction (arrow). Note, congested sinusoids. Hepatocytes increased regeneration (arrows) in H depicts cirrhosis progression. (H & E) (A, B, C, D, F, H, X40; E, G, X20).

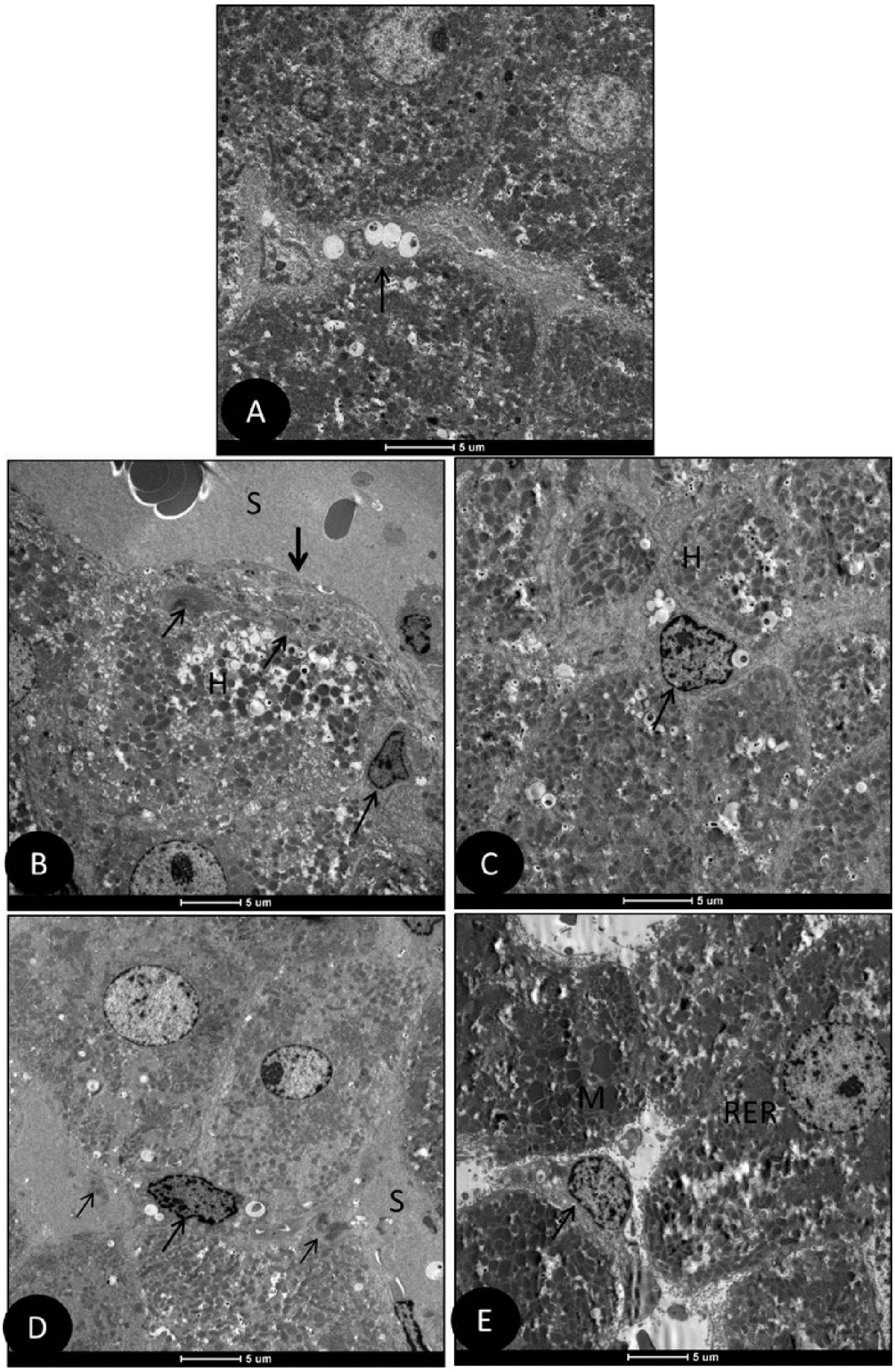

Figure4. Transmission electron micrographs low magnification of liver cells.

(A) Control, hepatic stellate cell (HSC). (B) DMNA, HSC activated and transdifferentiated into a myofibroblast (arrow) surrounded by fibers (thin arrows). Hepatocytes (H) with fat drops and lost microvilli, the endothelial cell thickened and lost their fenestrae (thick arrow). Sinusoid (S). (C) RES pre-treatment, inactivated HSC retains an intermediate phenotype with numerous vitamin A droplets (arrow). Note regenerated hepatocytes (H). (D) RES post-treatment low dose shows reverted HSC with few vitamin A droplets. Sinusoids with dense ECM, fibers (thin arrow). (E) RES post-treatment high dose, HSC with few undefined vitamin A droplets (arrow). Slightly degenerated hepatocytes, with dense cytoplasm, giant dense abnormal mitochondria (M) with lost cristae.

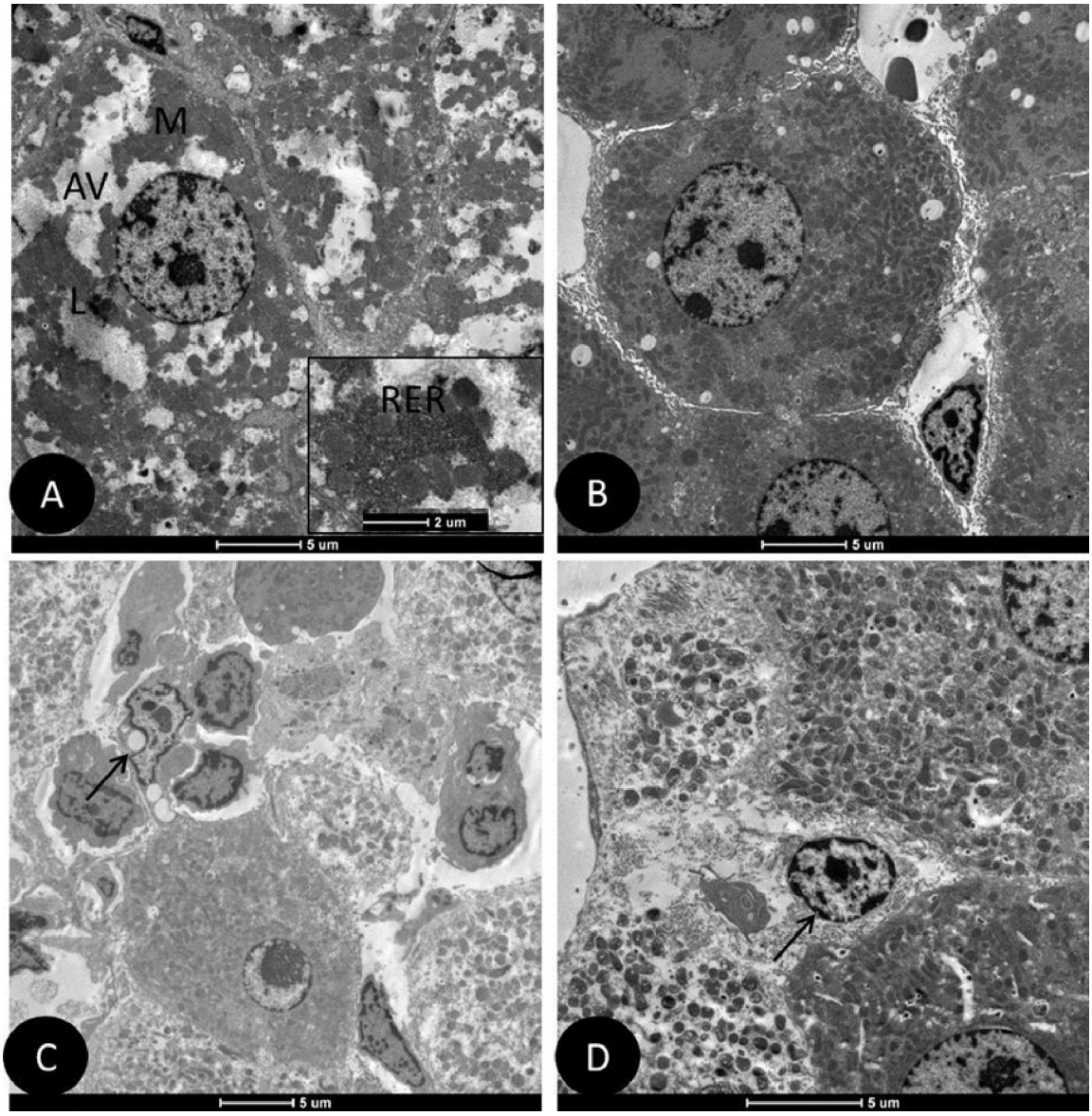

Figure 5. Transmission electron micrographs medium magnification of liver cells

(A) DMNA. Degenerated hepatocyte with large number of autophagic vacuoles (AV), RER, degenerated mitochondria (M), autolysosome (L). Inset RER. (B) RES pre-treatment, maintains almost normal ultrastructure. (C) RES post-treatment low dose, induces apoptosis of both hepatocytes (A) and the activated HSC (arrow) (D) RES post-treatment high dose, Myofibroblast (arrow) is surrounded by fibers.

Ultrastructural study of the control revealed normal liver structure (Figs. 4A & 6A). However, DMNA fibrotic liver displayed pathological changes in liver cells. HSCs were transdifferentiated intomyfibroblasts lost their vitamin A contentwith collagen fibers aroundit (Figs. 4B & 6B). Furthermore, ECM was markedly deposited in the space of Disse as well as in the sinusoids and the portal area. In addition, hepatocytes were separated from the sinusoidal blood flow by collagenous septa (Fig. 4B). The hepatocyteslost many of their microvilli as well as the endothelial cells lost their fenestrations. Some of the hepatocytes were degenerated, with autophagic vacuoles and undefined mitochondria with lost cristae. In addition, extensive short profiles of rough endoplasmic reticulum (RER) and phagolysosomes were evident in the fibrotic group (Fig. 5A).

On the other hand, during RES-pretreatment, the liver parenchyma was preserved in a good shape and fibrosis was greatly reduced (Figs. 4C, 5B and 6C). HSCs were preserved to high extent in its quiescent state with numerous fat droplets, and the ECM deposition was markedly reduced. Hepatocyte's microvilli and endothelial fenestrations were

decapillarized and greatly reserved as well as RER profiles were clearly reduced compared to the fibrotic group.

In RES post-treatment, fibrous content as well as tissue damage was much lower compared to the fibrotic DMNA group. The extent of the damage was lower in the RES post-treatment low dose than the high dose. Activated HSCs was relatively reverted to an intermediate state with few fat droplets (Figs. 4E, 5D & 6E). Hepatocytes apoptosis was more pronounced in RES post-treatment low dose than in the high dose (Fig. 5D).

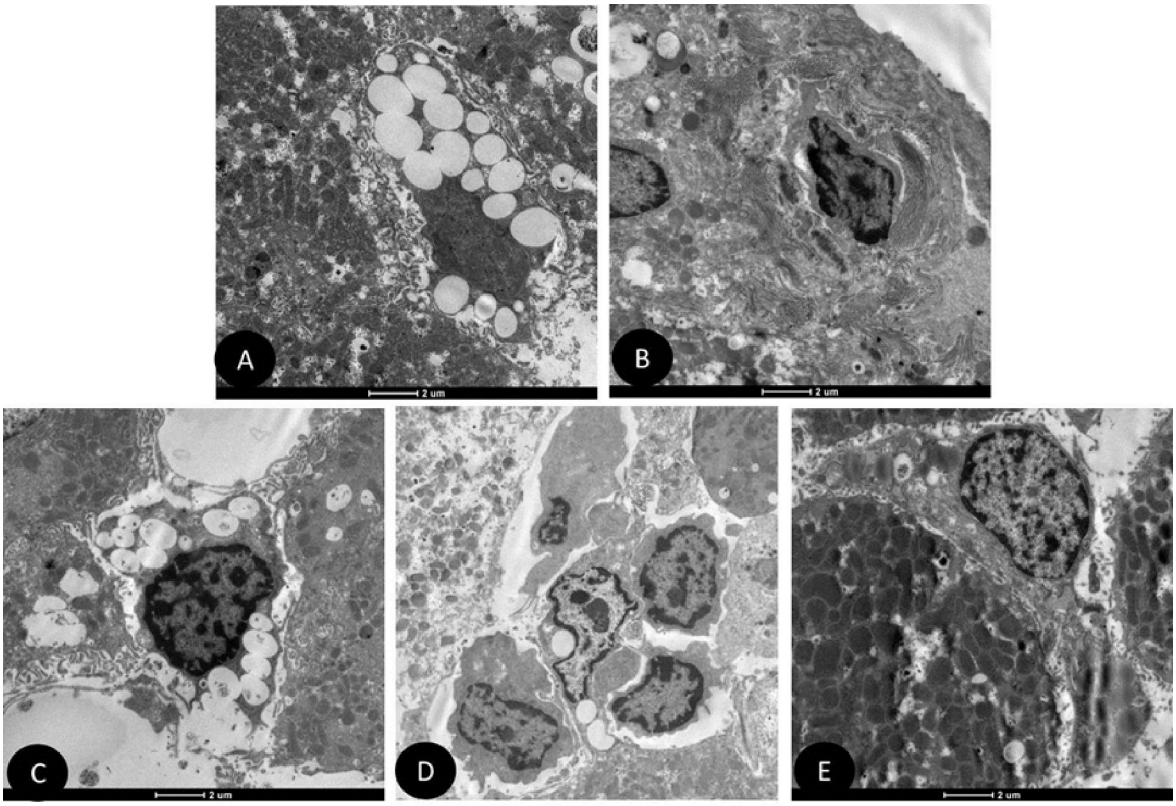

Figure 6. Transmission electron micrographs high magnification of hepatic stellate cell.
(A) Control, HSC (B) DMNA, activated HSC (myofibroblast) surrounded by large amount of collagen fibers and separated from the sinusoid by thickened defenestrated endothelium (arrow). (C) RES pre-treatment, HSC is greatly preserved with vitamin A droplets (D) RES post-treatment low dose, HSC apoptosis as a sign of fibrosis regression. (E) RES post-treatment high dose, HSC intermediate phenotype between myofibroblast and inactivated state. HSC represents a reversion of myofibroblast into inactivated HSC also indicates fibrosis regression.

4. Discussion

Hepatic fibrosis is a general consequence of chronic liver disease. Recently, much interest in natural medicine has been focused on the hepatoprotective and antifibrotic effects of compounds such as RES. The present study demonstrated that pre-treatment with RES was significantly effective in retaining the measured parameters of body and liver weight at normal levels

In the current study, the DMNA liver parenchyma displayed several areas of collagen fibers deposition, vascular architecture disruption, as well as hepatocyte sufferance. The later was represented by cellular ballooning, vacuolar degeneration, steatosis, as well as spotty and single cell necrosis. Hepatocytes ballooning and degeneration have occurred mainly in the periportal areas. Accordingly, sinusoids are compressed and disappeared. Therefore, many hepatocytes in that area suffered necrosis. In this study, RES treatment has remarkably suppressed the levels of necrotic liver damage.

Another change in the present study is the obliteration of small portal vein branches, followed by the development of portal vein shunt vessels branching into the adjacent liver parenchyma. This observation is similar to previous report by Hübscher (2011). Vascular obstruction within the liver causes an increase in portal venous pressure termed portal hypertension (Alan et al., 2002). Portal hypertension is a common clinical syndrome related to chronic liver diseases and is described as a pathological increase in portal pressure as a result of an increase in vascular

resistance and an elevated portal blood flow. In the present investigation, muscular hypertrophy of portal vein branches has occurred may be in response to portal hypertension. Hübscher (2011) reported that obstruction of vein branches provokes areas of ischemic hepatocytes. Loss of hepatocytes leads to collapse or abnormally close opposition of portal tracts and hepatic veins. The collapsed parenchyma was replaced by fibrous septa, which form portal –portal linkage. As a result obliterated veins may incorporate into areas of fibrosis. The present study results are in accordance with Hübscher (2011) report. In the current study, fibrosis progression with continued liver cell regeneration eventually led to cirrhosis as also mentioned by (Alan et al., 2002).

At the cellular level hepatic fibrosis is characterized by a multicellular response with the activation of HSC as a critical constituent. Therefore, inhibition of the activated HSC either by modulating their activation or by promoting their apoptosis is the main target in patients with hepatic fibrosis (Schuppan and Kim, 2013). In pathological conditions, HSC transforms to an activated myofibroblast phenotype, starts to proliferate, and express several proinflammatory and profibrogenic genes. The inflammatory activity of liver immune cells, mainly macrophages (Kupffer cells) promotes HSC activation. Macrophage-derived TGFβ activates HSC and considered as the most potent fibrogenic agonist (Fujita and Narumiya, 2016; Hellerbrand et al., 1999). The current electron microscopy study demonstrates that DMNA treatment activates HSC and converts it into a myofibroblast phenotype. On the other hand, RES pre-treatment surprisinglymaintains the HSC in the quiescent phenotype or in active state, as well as prevents the accumulation of ECM.

The present investigation is in agreement with the previous studies by (Iredale et al., 2013; Lee and Friedman, 2011) that regression of liver fibrosis is accompanied by either clearance of activated HSCs through apoptosis followed by resorption of the fibrous scar, or reversion of myofibroblasts into a quiescent phenotype and stop collagen production and partially restore expression of lipogenic genes. Resolution of liver fibrosis is associated with recruitment of macrophages that secrete matrix-degrading enzymes (Fallowfield et al., 2007; Pellicoro et al., 2012). Recruitment of macrophages was clearly noticed in the present study in RES pre-treatment and RES post-treatment low dose. However, repeated liver injury may cause irreversible crosslinking of ECM and formation of uncleavable collagen fibers. As a result advanced fibrosis progresses to cirrhosis and hepatocellular carcinoma (Rockey and Friedman, 2012; Xu et al., 2014). A recent study showed that resveratrol could inhibit the growth of human gastric carcinoma cells through apoptosis induction via activation of mitochondrial pathway (Yang et al., 2018).

The current ultrastructure study suggests that RES employs two mechanisms; blocking HSC activation, and/or triggering apoptosis as a sign of resolution of fibrogenesis. Apoptosis of activated HSCs was frequently seen in the present study in RES post-treatment low dose which means that fibrosis resolution has occurred. Friedman PNAS (Friedman, 2012) proposed that fibrosis regression leads to either apoptosis of stellate cells or reversion to an inactivated state with restored features of quiescence.

However, inactivated stellate cells maintain an intermediate phenotype which may remain in this state forever or slowly return to full quiescence over a longer period. This is in agreement with our findings.

Activated HSC when reverts to a quiescent shape still acquires fibers in its cytoplasm and does not look exactly as the inactivated HSC.

Accumulation of fibrillar ECM leads to capillarization of the sinusoids; a loss of the sinusoidal endothelial fenestrae as well as the hepatocytes microvilli. Eventually vascular structures are linked and the architecture of the liver disrupted significantly (Friedman, 1993). In the present study, DMNA fibrotic reaction results in ECM deposition and consequently capillarization of the endothelial fenestrae as well as the hepatocyte microvilli. Capillarization interferes with the blood flow between sinusoids and hepatocytes, resulting in fibrogenesis and hepatic failure(Wang et al., 2017). However, RES pre-treatment in the present study sustains the integrity of hepatocyte's microvilli and endothelial fenestrations.

In contrary to the study of Abdel-Halim (Abdel-Halim et al., 2015), the present study proved that RES is more potent as a protective (pre-treatment) agent than a curative (post-treatment) agent, may be due to the short period of the post-treatment in the current study.

In the present study, the ameliorative effect is more pronounced in RES pre-treatment than post-treatment. In addition, the post-treatment with RES 20 mg/kg is more effective than 40mg/kg. The present study does not recommend the daily use of 40 mg/kg due to the focal congestion and hepatocytes vacuolation through the liver tissue.

It can be concluded that RES pre-treatment has remarkably prevented liver fibrosis incidence and resveratrol supplementation significantly regulated the pathological alterations of hepatic fibrosis in time and dose-dependent manner. Moreover, resveratrol can be considered as a potent antifibrotic agent. In addition, it is possible that combination therapies that affect

pathogenic pathways will be needed to accelerate the progress of liver disease therapy.

Summary

This study proved that liver fibrosis can be regulated by resveratrol treatment in animal model. It has been shown in the present study that resveratrol employed two mechanisms; blocking hepatic stellate cell activation for prevention, and triggering apoptosis as a sign of resolution of fibrogenesis.

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Competing interests:

No competing interests declared.

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