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Modulation of inducible nitric oxide synthase pathway by eugenol and telmisartan in carbon tetrachloride-induced liver injury in rats

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ABSTRACT

Aims: Inducible nitric oxide synthase (iNOS) pathway has been in the limelight since its discovery as a key mediator in the process of liver fibrogenesis. Therefore, the objective of the current study was to elucidate the in vivo molecular mechanism underlying the hepatic preventive relevance of eugenol (EUG) and telmisartan (TEL) through iNOS pathway modulation against carbon tetrachloride (CCl4)-induced hepatic injury.

Methods: Sixty healthy male albino rats were used in this study. Serum aminotransferases activities and NO levels were assessed. Hepatic malondialdehyde (MDA), total nitrite/nitrate content and reduced glutathione (GSH) concentration were estimated. Liver NF-kB, TNF-α, IL-6 and iNOS proteins expressions were investigated by western blot assay. Histopathological examination was done.

Key findings: CCl4 resulted in damage to centrilobular regions of the liver, elevation of serum aminotransferases, rise in oxidative parameters level, and up-regulation of NF-kB, TNF-α, IL-6 as well as iNOS proteins expressions. Treatment of fibrotic rats with either EUG or TEL significantly alleviated CCl4-induced biochemical, inflammatory and histopathological changes. Moreover, the combined administration of EUG with TEL has an ameliorative effect which is greater than either of them alone.

Significance: In conclusion, the combination therapy between EUG and TEL is more effective than either drug alone which is attributed to suppression of NO production and iNOS protein expression. The results support that use of EUG and TEL exerts beneficial effects in the attenuation of CCl4-induced liver fibrosis in rats.

1. Introduction

Liver fibrosis is a critical health problem that necessitates further medical attention as its progress will lead to liver cirrhosis, severe liver failure and death [1,2]. Carbon tetrachloride (CCl4), which is a widespread industrial solvent, is well known as a model for liver fibrosis in animals, like rats and mice, that emulates the manifestations agonizing human subjects [3]. Inflammation and oxidative stress have been demonstrated to be fundamentally involved in the pathogenesis of CCl4-induced liver injury [4,5]. Several cytokines which are secreted as a response to cellular injury like tumor necrosis factor alpha (TNF-α) and interleukin 6 (IL-6) seem to have a central role in the pathogenesis of hepatic fibrosis. They magnify hepatic injury through continuous activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) [6]. Furthermore, although its function might be protective in certain cases, increased level of nitric oxide (NO), which is a potent inflammatory mediator, has been observed in liver fibrosis [7]. Increased NO production might be assigned to NF-kB-induced inducible Nitric oxide synthase (iNOS) expression subsequent CCl4 challenge. NO contributes to nitrosative stress and may mediate lipid peroxidation leading to cellular dysfunction and tissue damage [8,9]. NOS is responsible for NO production from L-arginine. Three hepatic isoforms of NOS were known: iNOS, endothelial (eNOS) and neuronal (nNOS) [10]. Overexpression of iNOS in liver diseases was observed after viral infection leading to production of NO which induces its harmful effects either directly or via the production of reactive oxidants [11].

The interplay of inflammatory cytokines, such as TNF-a, IL-6 and NF- kB which are secreted as a response to cellular injury and considered as hepatic profibrogenic factors, increases the production of iNOS in liver fibrosis. They are stimulated through extracellular matrix components and activation of hepatic stellate cells by oxidants and nitrosative conditions in a cytokine signaling network [12].

Based on this background, blocking the iNOS pathway will inhibit NO synthesis, fibrogenic cytokines expression and reduce hepatic inflammation, offering a potential therapeutic strategy for inflammatory liver diseases.

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Studies have shown that several antioxidants and anti-inflammatory agents are beneficial in dominating the progression and development of liver injury [13,14]. Telmisartan (TEL) is a well-settled angiotensin II receptor blocker, used as an antihypertensive drug. In experimental models, TEL significantly suppressed inflammatory response but using of TEL in chronic liver diseases is not fully investigated [15]. Eugenol (EUG) is the active component of essential oil isolated from clove. EUG possesses various biological and pharmacological characters, including anti-inflammatory, antioxidant, chemopreventive and anti-microbial actions. It has wide clinical applications in alcoholic liver disorders, liver cirrhosis, toxic and drug induced liver diseases [16,17]. It was shown that eugenol, by down-regulation of SREBP1 gene expression, protects against hepatic fibrosis and steatosis [18]. Also, its pretreatment inhibited the hepatic injury caused by thioacetamide through its antioxidant ability and the inhibition of CYP2E1 activity [16].

The present work was undertaken with the main aim of evaluating the in vivo molecular mechanism implied the hepatoprotective effects of EUG and TEL through iNOS pathway modulation against carbon tetrachloride (CCl4)-induced hepatic fibrosis.

2. Materials and methods

2.1. Drugs and chemicals

All chemicals were of analytical grade and were secured from commercial sources. Eugenol (99.0%, Sigma Aldrich Company, St Louis, Missouri, USA) was prepared in olive oil [16] at concentrations 10 and 100 mg/ml and stored in dark at 4 °C. Telmisartan (Sigma Pharmaceutical Company, Egypt) powder was freshly prepared at 2 mg/ml in carboxymethyl cellulose (0.5% aqueous solution, pH 7.0).

2.2. Selection of animals and care

Sixty healthy Wister male albino rats (180–200 g) were used. Study protocols and animal care were proceeded in accordance with the guidelines affirmed by The Research Ethics Committee, Minia University, Egypt.

2.3. Experimental protocol

Rats were randomly divided to 10 groups, each of them contains 6 rats (n = 6).

Group I (normal control group): Rats received intraperitoneal (i.p.) injection of olive oil (1 ml/kg) for 6 weeks twice a week.

Group II (EUG.10 group): Rats received EUG (10 mg/kg, i.p.), for 6 weeks daily [19,20].

Group III (EUG.100 group): Rats received EUG (100 mg/kg, i.p.), for 6 weeks daily [21].

Group IV (TEL group): Rats received TEL (10 mg/kg, orally), for 6 weeks daily [22,23].

Group V (CCl4 group): Rats received 1:1 (v/v) CCl4 in olive oil (1 ml/kg, i.p.), for 6 weeks twice a week [24].

Group VI: Rats received CCl4 in olive oil (1 ml/kg, i.p., twice a week) and treated with EUG (10 mg/kg, i.p., daily) for 6 weeks.

Group VII: Rats received CCl4 in olive oil (1 ml/kg, i.p., twice a week) and treated with EUG (100 mg/kg, i.p., daily) for 6 weeks.

Group VIII: Rats received CCl4 in olive oil (1 ml/kg, i.p., twice a week) and treated with TEL (10 mg/kg, orally, daily) for 6 weeks.

Group IX: Rats received CCl4 in olive oil (1 ml/kg, i.p., twice a week) and co-treated daily with EUG (10 mg/kg, i.p.) and TEL (10 mg/kg, orally) for 6 weeks.

Group X: Rats received CCl4 in olive oil (1 ml/kg, i.p., twice a week) and co-treated daily with EUG (100 mg/kg, i.p.) and TEL (10 mg/kg, orally) for 6 weeks.

After the last CCl4 dose, animals were anesthetized with light ether (2%) and scarified, then, blood and liver tissues were collected for analysis.

2.4. Specimen collection and preparation

Blood samples (2 ml/rat) were collected in a covered red topped vacutainer tubes (Becton Dickinson) from neck vessels by decapitation. Serum was isolated by allowing the blood to clot by leaving it undisturbed at room temperature for 20 min then centrifuged at 1500 ×*g* for 10 min in a refrigerated centrifuge. The resulting supernatant (serum) was collected into clean polypropylene tubes using Pasteur pipettes and maintained at 2–8 °C while handling. Liver tissues were rapidly dissected out, washed with cold (1 ×) phosphate buffered saline (PBS, pH 7.4, with a final concentration of 10 mM PO_4^{3-}, 137 mM NaCl, and 2.7 mM KCl) in ice, dried and weighed, then liver index was calculated (liver weight/body weight × 100). Finally, parts of them were fixed in 10% formalin for histological study. For western blotting and biochemical determinations, other liver tissue parts were homogenized in lysis buffer (20 mM Tris, 1 mM EDTA, 100 mM NaCl, protease inhibitors mix [25] and 0.5% Triton X-100 buffer) then protein concentrations were determined by Biuret Test [26]. Liver homogenate samples and serum were divided and stored at −80 °C as aliquots until use.

2.5. Biochemical analysis

Activities of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) [27] and NO levels [28] were estimated. Liver homogenate was used for calculating hepatic malondialdehyde (MDA) [29], total nitrite/nitrate content [30] and reduced glutathione (GSH) concentration [31]. The spectrophotometric assessment of all these biochemical parameters was performed according to the manufacturer's instructions using commercially available kits (Biodiagnostic, Egypt).

2.6. Western blotting analysis for hepatic NF-kB, TNF-α, IL-6, and iNOS proteins expression

For direct immunoblotting, liver homogenates (50 μg of total proteins) were boiled for 5 min with loading buffer containing 2-mercaptoethanol and then loaded on 12% sodium dodecyl sulfate- polyacrylamide gel electrophoresis (SDS-PAGE) running for 2 h at 100 V. Following electrophoresis, proteins were transferred to polyvinylideneflouride (PVDF) membranes. After blocking for 1 h in a Tris-buffered saline (TBS-T) blocking solution containing 5% (w/v) non-fat milk and 0.05% Tween-20, they were incubated with primary antibodies for NF-kB, IL-6, TNF-α, iNOS and β-actin (1:1000; Santa Cruz Biotechnology, Santa Cruz, CA) overnight at 4 °C. Horseradish peroxidase-conjugated polyclonal goat anti-rabbit immunoglobulin (Cell Signaling Technology Inc., MA, USA) was used as a secondary antibody at a dilution of 1:5000 in blocking buffer. Bands were visualized by chemiluminescence, using an enhanced chemiluminescence kit (ECL, GE Healthcare, Chicago, IL, USA), according to the manufacturer's instructions and detected using an analyzer for luminescent images (LAS-4000, Fujifilm Co., Tokyo, Japan). Bands corresponding to NF-kB, IL-6, TNF-α and iNOS proteins of the different groups were accessed relative to the normal control group densitometrically after normalization to β-actin using Image J Software.

2.7. Histopathological examination

After formalin fixation and dehydration in 70% ethanol, the freshly isolated liver sections were embedded in Paraffin wax. Then, the 4 μm-thick sections were stained with hematoxylin-eosin (H&E) and examined, at magnification x40, using the light electric microscope (Leika DMRBE, Germany) for determination of pathological alterations [32].

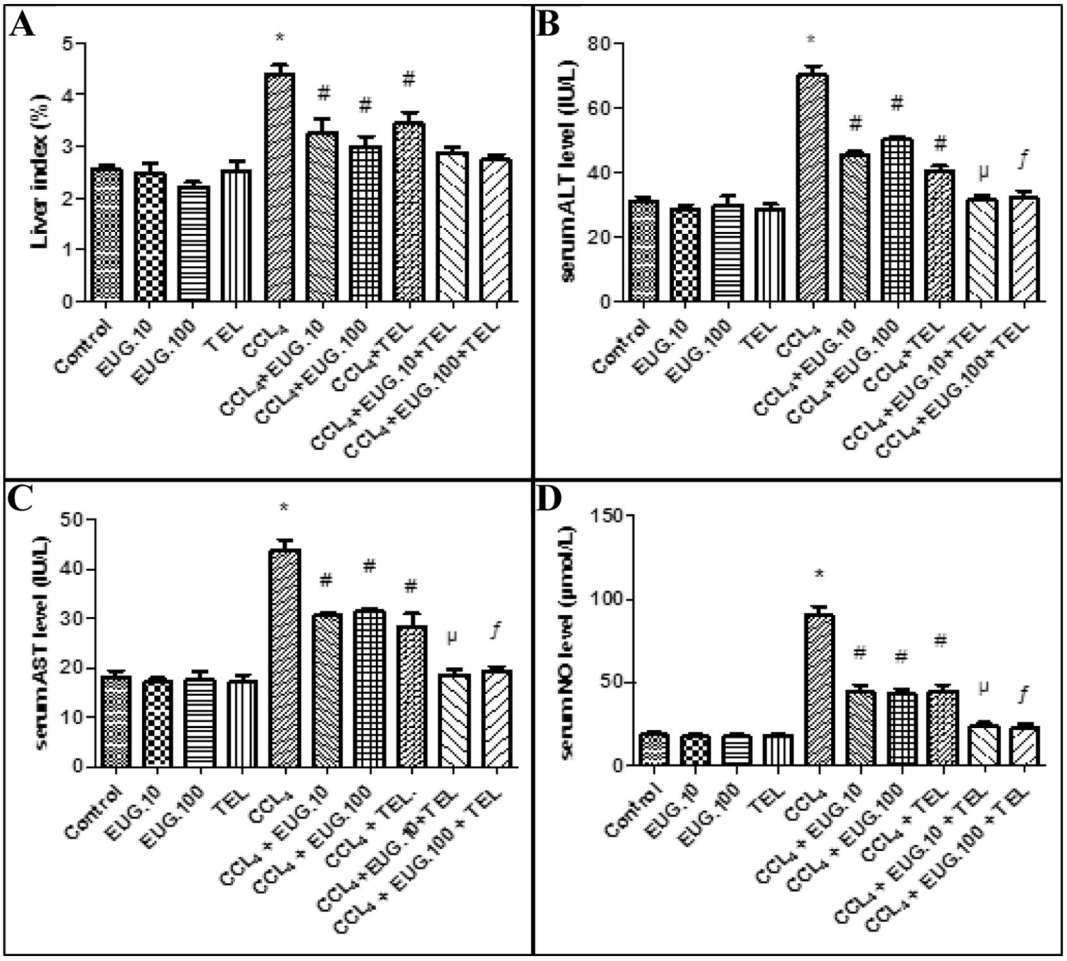

Fig. 1. Effect of EUG.10, EUG.100, TEL and their combination on liver index (A), serum ALT (B) and serum AST (C) levels, as well as serum NO level (D) on normal and CCl_4-injuired rats. Data are expressed as Mean ± S.E.M. (n = 6 rats). *, #, μ and ƒ: significantly different compared to normal control, CCl_4, CCl_4 + EUG.10 and CCl_4 + EUG.100 groups, respectively, at $P < 0.001$.

2.8. Statistical analysis

For statistical analysis, Graph Pad® Prism Software Inc., Program, version 5.0 was used. Data were expressed as mean ± standard error of the mean (S.E.M.), and differences between groups were examined for significance using analysis of variance (ANOVA) and Tukey–Kramer post analysis test. P values were considered statistically significant when they were < 0.05.

3. Results

3.1. Effect of EUG and TEL on hepatic injury indices

As shown in Fig. 1, a significant increase in liver index and activities of both serum ALT and AST could be noticed after CCl4 treatment when compared with normal control group rats. This hepatocellular harmful effect was nearly reversed when treated with EUG at both doses, in a similar pattern to that observed with TEL.

Administration of the combined regimen (EUG plus TEL) showed significant improvement in the mentioned parameters when compared to the monotherapy treated groups.

Similarly, a significant increase in the serum NO levels was observed in CCl4-treated rats when compared to normal control group rats and a significant reduction in its level was achieved by sequent treatment with EUG. This effect was also comparable to that of TEL. Moreover, the combination therapy of EUG and TEL resulted in significant decrease in serum NO levels compared to monotherapy-treated groups.

3.2. Effect of EUG and TEL on hepatic oxidative stress parameters

Upon CCl4 administration, it was observed that there was a significant elevation in liver content of MDA (Fig. 2A) and total NO (Fig. 2B) and a significant depletion of GSH (Fig. 2C) compared to normal control rats. Interestingly, co-administration of EUG with CCl4 was significantly reduced MDA and total NO levels as well as significantly increased GSH concentration in liver homogenate in a dose-independent manner. The effect of EUG was comparable to TEL that was also effective in replenishing GSH and in decreasing MDA and total NO levels regarding to CCl4-treated group. The levels of MDA and NO was significantly diminished by co-administration of EUG and TEL which also improved the hepatic GSH content compared to fibrotic groups treated with EUG or TEL alone.

3.3. Expression of hepatic NF-κB, IL-6, TNF-α and iNOS proteins

Western blotting showed a significant hepatic up-regulation of NF-kB, TNF-α, IL-6 and iNOS proteins expression in CCl4-treated rats compared to the normal control rats, after normalizing the intensities of bands to β-actin. There were no significant differences between both doses of EUG upon comparing each other. Interestingly, treating fibrotic animals with either EUG or TEL resulted in significant down-regulation of all four proteins expression compared to their expression level in fibrotic group rats. Furthermore, the expression level of the above mentioned proteins in combination therapy-treated fibrotic rats (EUG plus TEL) was markedly lower than that in monotherapy-treated

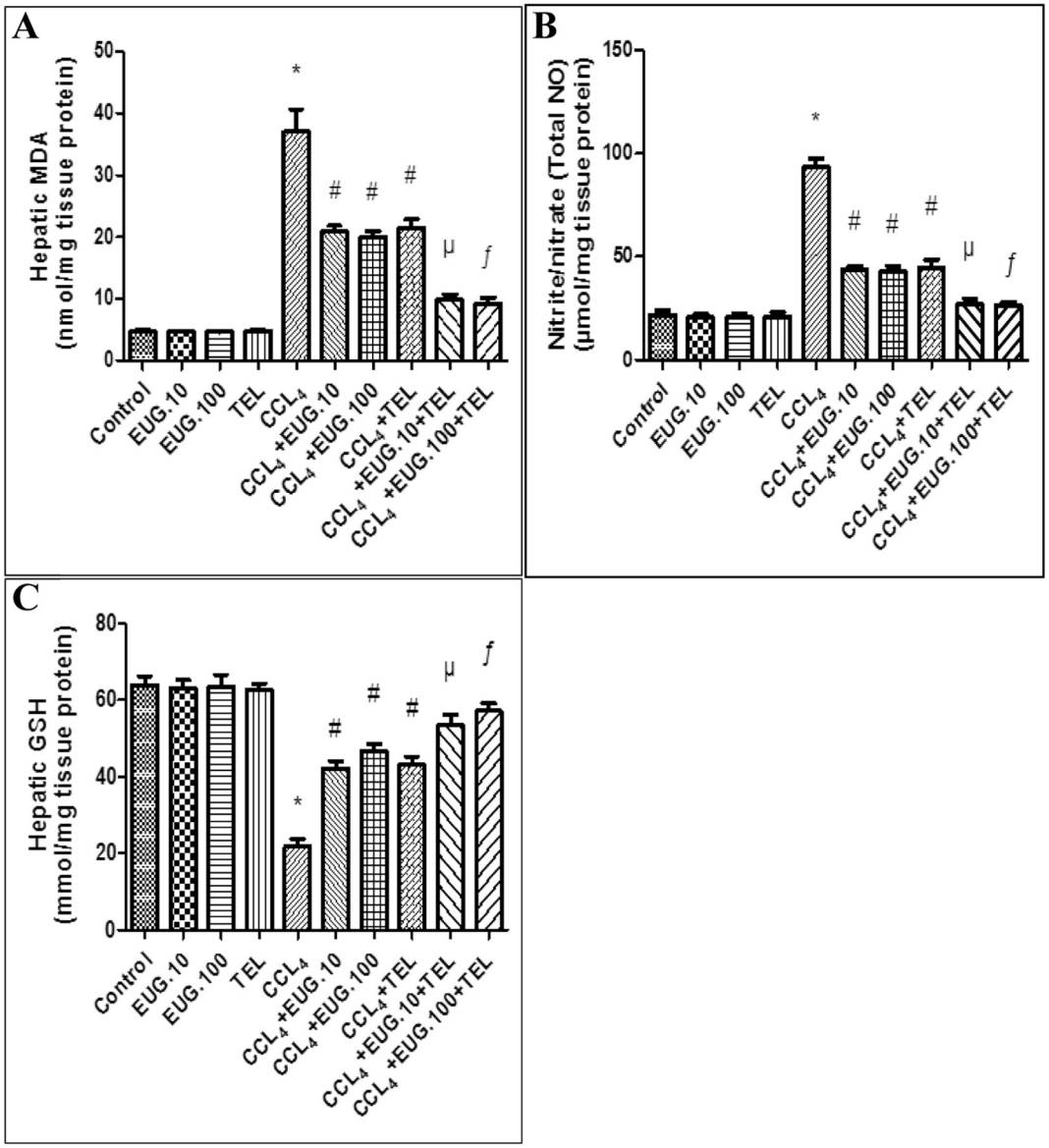

Fig. 2. Effect of EUG and TEL on hepatic oxidative stress parameters

(A) Effect of EUG.10, EUG.100, TEL and their combination on hepatic malondialdehyde (MDA) on normal and CCl_4-injuired rats. Data are expressed as Mean ± S.E.M. (n = 6 rats). *, #, μ and ƒ: significantly different compared to normal control, CCl_4, CCl_4 + EUG.10 and CCl_4 + EUG.100 groups, respectively, at $P < 0.001$.

(B) Effect of EUG.10, EUG.100, TEL and their combination on nitrite/nitrate (Total NO) on normal and CCl_4-injuired rats. Data are expressed as Mean ± S.E.M. (n = 6 rats).

* and #: significant compared to normal control and CCl_4 groups, respectively, at $P < 0.001$.

μ and ƒ: significant compared to CCl_4 + EUG.10 and CCl_4 + EUG.100 groups, respectively, at $P < 0.01$.

(C) Effect of EUG.10, EUG.100, TEL and their combination on hepatic reduced glutathione (GSH) on normal and CCl_4-injuired rats. Data are expressed as Mean ± S.E.M. (n = 6 rats).

* and #: significant compared to normal control and CCl_4 groups, respectively, at $P < 0.001$.

μ and ƒ: significant compared to CCl_4 + EUG.10 and CCl_4 + EUG.100 groups, respectively, at $P < 0.05$.

fibrotic rats, as shown in Fig. 3.

3.4. Effect of EUG and TEL on hepatic histopathological changes

Following different treatments, Paraffin sections were used to compare and evaluate histopathological changes. For normal control rats (Fig. 4A), light microscopic examination showed normal hepatic architecture, no inflammatory infiltration, fatty change or fibrous expansion. Liver sections of rats administered only EUG.10 (Fig. 4B), EUG.100 (Fig. 4C) or TEL (Fig. 4D) showed no histopathological variation and approximately normal hepatic architecture. Hepatic sections of CCl4 group rats (Fig. 4E) showed sever fatty change in a widespread manner all over the hepatocytes accompanied with inflammatory cells infiltration and fibrous expansion of most portal areas. Rats administrated CCl4 and treated with EUG.10 (Fig. 4F) showed vacuolar degeneration which was detected in most hepatocytes surrounding the portal vein while rats administrated CCl4 and treated with EUG.100 (Fig. 4G) showed dilatation in the portal vein, meanwhile rats administrated CCl4 and treated with TEL showed a moderate inflammatory cells infiltration and centrilobular ballooning degeneration surrounding the congested central vein (Fig. 4H). Treatment of the injured rats with the combination regimens (EUG.10 + TEL) (Fig. 4I) and

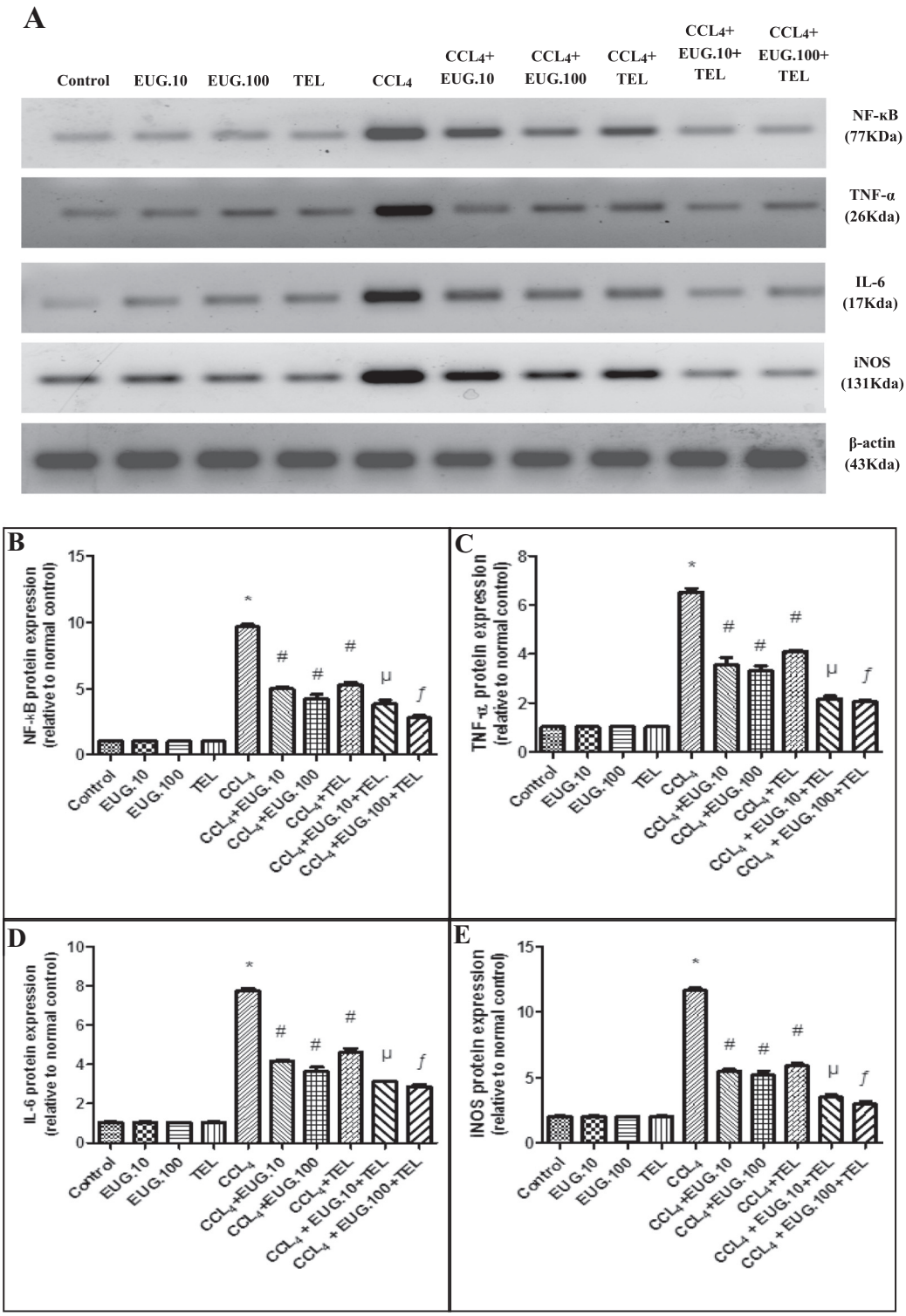

Fig. 3. Effect of EUG and TEL on expression of NF-κB, TNF-α, IL-6 and iNOS proteins

(A) Representative immunoprecipitation blots of NF-κB, TNF-α, IL-6, iNOS and β-actin proteins for all groups.

(B, C, D, E) Expression of NF-κB, TNF-α, IL-6 and iNOS proteins, respectively, was expressed densitometrically, using bands in (A) after normalization to the corresponding internal control β-actin, as fold change relative to that of normal control rats. Data are expressed as Mean ± S.E.M. (n = 6 rats). *, #, μ, ƒ; statistically significant compared to normal control, CCl_4, CCl_4 + EUG.10, and CCl_4 + EUG.100 groups, respectively, at $P < 0.001$.

(EUG.100 + TEL) (Fig. 4J) protected the architecture of hepatic cells and produced no quantifiable histological injury to liver morphology following CCl4 treatment, as shown in Table 1.

4. Discussion

Liver fibrosis is a pathological response that results from inflammatory liver diseases or prolonged exposure to certain drugs. It is a model of the wound healing response to a prolonged or repeated injury,

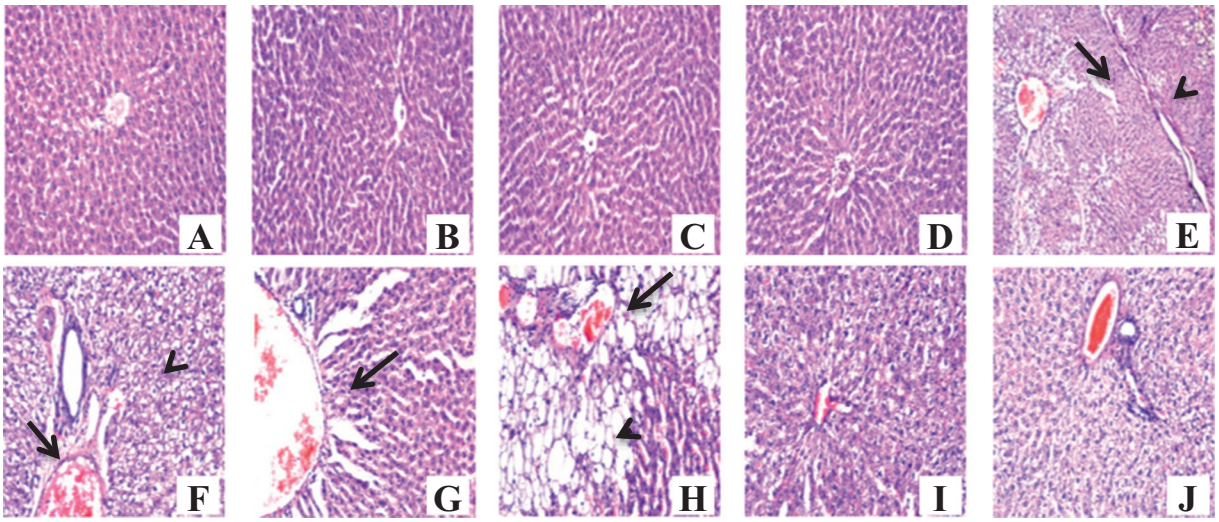

Fig. 4. Histological examination for animal livers of all groups
Hematoxilin-eosin stained sections of livers of the control group rats (A) show normal histological structure of the central vein and no histopathological alteration. Hepatic sections of rats treated with EUG.10 (B), EUG.100 (C) and TEL (D) show no histopathological alteration. Liver sections of CCl4 group rats (E) show obvious fatty change in a diffuse pattern all over the hepatocytes (arrow) accompanied with inflammatory cells infiltration and fibrosis in the portal area (arrow head). CCl4 + EUG.10 group rats (F) show slight congestion in the portal vein (arrow) with vacuolar degeneration in the hepatocytes (arrow head) while CCl4 + EUG.100 group rats (G) show slight dilatation in the portal vein at the portal area (arrow) and CCl4 + TEL group rats (H) show slight dilatation in the central vein (arrow) accompanied with inflammatory cells infiltration found in the portal region (arrow head). The combination group (EUG.10 + TEL) rats (I) and the combination group (EUG.100 + TEL) rats (J) show approximately no histopathological alteration and nearly normal histological structure of hepatocytes and the central vein (Magnification is ×40).

Table 1
Scoring of the histopathological changes of hepatic tissues of rats from all groups.

Groups	Hepatic histopathological alterations				
	Fatty changes in hepatocytes	Portal inflammatory reaction with congestion in portal vein	Vacuolar degeneration in hepatocytes	Congestion in portal vein	Fibrosis in portal vein
Control	−	−	−	−	−
EUG.10	−	−	−	−	−
EUG.100	−	−	−	−	−
TEL	−	−	−	−	−
CCl_4	+ + +	+ + +	−	+ +	+ +
CCl_4 + EUG.10	−	−	+ +	+ +	−
CCl_4 + EUG.100	−	−	−	+ +	−
CCl_4 + TEL	−	−	+ + +	+ +	−
CCl_4 + EUG.10 + TEL	−	−	−	−	+
CCl_4 + EUG.100 + TEL	−	−	−	−	−

Score represents values got from sections of 6 rats of each group and 5 fields per one section. Score level (−) was considered as no significant alterations. Scores (+, + + and + + +) were considered as mild, moderate and severe levels, exhibiting < 25, 50, and 75% histopathological changes of total fields examined, respectively. EUG.10: Eugenol 10 mg/Kg, EUG.100: Eugenol 100 mg/Kg, TEL: Telmisartan.

eventually leading to cirrhosis [33,34]. Thus, searching for an effective hepatoprotective agents for management of liver fibrosis is an urgent necessity. Not only one mechanism participates in progression of hepatic fibrosis, but there is a multi-mechanisms process that involves activation of Kupffer cells in response to tissue injury [35] with the subsequent release of inflammatory cytokines and mediators (including interleukins and TNF-α) [36] that exert paracrine actions on hepatic stellate cells (HSCs) and induction of immune and inflammatory cells amplifying the inflammatory response [37]. Yet, this pathological sequence may be particularly interrupted by other different mechanisms (i.e. inhibiting lipid peroxidation, scavenging free radicals, reducing inflammatory response, restoring hepatic glutathione, or modulating of hepatic iNOS pathway) [9,38–40].

CCl4 is one of the commonly used chemicals for inducing hepatic injury. In this study, CCl_4 caused significant biochemical and histopathological disturbances. Rats administered it, as reported previously [41,42], exhibited disturbed liver enzymes, boosted oxidant stress, elevated rates of lipid peroxidation and promoted inflammatory responses. These hepatotoxic effects of CCl4 are dependent upon its hepatic metabolisation by cytochrome P450 2E1 (microsomal mono-oxygenase system), into trichloromethyl radical CCl_3* (a highly reactive species) which reacts with oxygen producing trichloromethylperoxy radical CCl_3OO* [43]. They launch lipid peroxidation chain reaction producing by-products and free radicals (i.e. MDA, NO) as well as affecting phospholipids synthesis causing disruption of the endoplasmic reticulum membranes composition, altering their permeability and leading to cellular enzymes leakage to the circulation. Free radicals generation leads to depletion of intracellular molecules (i.e. GSH) [44].

In agreement with those findings, CCl4 in this study resulted in significant rise in liver enzymes serum levels, AST and ALT. Also, the levels of MDA and total nitrate/nitrite content in the hepatic tissue of fibrotic rats were significantly higher than the normal control group rats. An opposite paradigm was found with GSH which was depleted in the CCl4- intoxicated rats.

This accumulating oxidant stress ultimately excites an obvious inflammatory reaction with inflammatory cells infiltration and sequent hepatic macrophages (Kupffer cells) activation which respond instantly by liberation transcription factors (i.e. NF-kB) and many pro-inflammatory cytokines (i.e. IL-6, TNF-α) [45,46]. NF-kB, in response to tissue injury, has been activated in CCl_4-induced fibrosis. It contributes to NO production by enhancing the expression of iNOS in hepatocytes, thus enhancing liver inflammation [47]. Accordingly, we observed a marked increase in the expression of activated NF- kB, IL-6, TNF-α and iNOS proteins in livers of CCl4-intoxicated rats.

iNOS pathway has drawn considerable attention for its critical role in progression of liver fibrosis, so its amendment could be a useful therapeutic strategy, which attempts to protect hepatocytes from oxidative stress and inflammation, thus modulating liver fibrosis [9,48]. As the chronic liver disorder proceeds, iNOS represents an important pathway which is mainly triggered by activation of the transcription factor; NF-kB. Previous reports observed the iNOS activation in hepatic fibrosis induced by CCl_4 [49]. The final product of iNOS, NO can couple with O_2 to form peroxynitrite ($ONOO_2$) in a diffusion-dependent manner that is extremely reactive, as compared with NO or O_2 alone, while NO has also been shown to have a chemical potential to cause protein degradation, DNA damage, lipid peroxidation and tissue damage [50–52].

Regarding, the in vivo EUG hepatoprotective effect against CCl4-induced liver injury, the present study showed that treatment with EUG significantly decreased liver index and enzyme activities compared to CCl4 treated group. This effect may be attributed to the potential feature of stabilizing the membrane permeability leading to suppressing the leakage of intracellular transaminases into the blood stream.

According to our data, EUG enhanced the anti-oxidant capacity of the liver by abolishing lipid peroxidation, decreasing total NO levels and preventing glutathione depletion [53]. These findings could be demonstrated on the principle that EUG acts as free radical scavenger depending on its phenolic structure [54,55]. Another possible mechanism is the decreased activity of CYP2E1 enzyme leading to reduced CCl4 biotransformation and hence decreased probability of hepatocytes toxicity to CCl4 [56].

In addition, EUG in this study declined inflammation induced by CCl4 via the down-regulation of NF-kB, IL-6 and TNF-α proteins expression after their up-regulation by CCl4. It also restored iNOS expression in hepatic tissue and retrieved NO formation, which indicate a repercussion of the detrimental effects of the inflammatory mediators and hence ameliorating liver fibrosis [57]. It is obvious that the mechanism of EUG effect might result from the minimized production of ROS, reduction in generation of cytokines by Kupffer cells and diminished inflammatory cell infiltration.

In the same pattern of EUG, TEL was noticed to reduce the expression of NF-kB, IL-6, TNF-α and iNOS in the liver of rats exposed to CCl4 hepatotoxicity. This ameliorative effect is a consequence of activation of peroxisome proliferator-activated receptor (PPAR-γ) receptor that inhibits NF-kB expression, thus, down-regulating most of the pro-inflammatory responses [58,59]. It was reported that Angiotensin II receptor blockers (ARBs) modulated oxidative stress in different cell types. Their anti-oxidant activity may be secondary to angiotensin receptor blockade and inhibition of ANG II-induced generation of ROS and oxidative stress [60]. Interestingly, we found that TEL treatment corrected the levels of serum aminotransferases, effectively minimized MDA and total NO levels and significantly diminished CCl4-induced reduction in GSH level, thus preventing or at least slowing down more hepatic damage induced by CCl4.

Pro-inflammatory cytokines are showing their ability as effective inducers of iNOS in many types of cells, and thus production of NO. In this study it has proposed a cytokine signaling network of TNF-α, IL-6 and NF-kB as molecular markers responsible for the regulation of iNOS pathway in liver fibrosis pathogenesis. Results showed that after CCl4-induced liver injury, a release of pro inflammatory cytokines, TNF-α and IL-6, was occurred followed by the stimulation of NF-kB which in turn induced iNOS with NO production. EUG and TEL ability in modulation of iNOS pathway could be proved through the inhibition of the initial steps (via inhibition of IL-6 and TNF-α proteins expression) or latter steps (via inhibition of NF- kB protein expression) that subsequently lowered iNOS protein expression and NO production.

The findings obtained in the present study were confirmed with the histopathological investigation that was observed in the hepatic sections of treated rats. Both, EUG and TEL elucidated a reduction in fatty changes, hepatic degeneration, together with attenuation of hepatic fibrosis and injury which is probably related to the anti-oxidant and anti-inflammatory effects of either agent.

Collectively, this study revealed that administration of EUG or TEL caused no obvious alteration in the measured biochemical parameters or any histopathological changes confirming their safety while their treatment resulted in improvements in biochemical, inflammatory, and oxidative parameters as well as reduced intensity and incidence of alterations in liver morphology induced by CCl4 administration.

5. Conclusion

The combination therapy comprising EUG and TEL further reverses fibrosis markers in a rat model of liver fibrosis and potentiates the effect of each other. In conclusion, repression of the inflammatory response, mediated by TNF-α and NF-kB, and attenuation of iNOS pathway could be involved, in part, in the hepatoprotective effect of EUG and TEL. Consequently, our findings possess great promise for the usage of EUG and TEL in the management of liver injury and its related complications. However more investigations are required for more understanding of the molecular mechanisms by which EUG and TEL might exert their beneficial hepatoprotective effect.

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Conflict of interest

The authors declare that there are no conflicts of interest.

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