Caffeine intake decreases oxidative stress and inflammatory biomarkers in experimental liver diseases induced by thioacetamide: Biochemical and histological study

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Abstract

Liver disease remains a significant global health problem. Increased caffeine consumption has been associated with a lower prevalence of chronic liver disease. This study aimed to investigate the modifying effects of caffeine on liver injury induced by thioacetamide (TAA) administration in male rats and the possible underlying mechanisms. Forty adult male rats were equally classified into four groups: control group, received only tap water; caffeine-treated group, received caffeine (37.5 mg/kg per day); TAA-treated group, received intraperitoneal (i.p.) TAA (200 mg/kg b.w.) twice a week; and caffeine + TAA-treated group, received combined TAA and caffeine in the same previous doses. After eight weeks of treatment, blood samples were collected for biochemical analysis and liver specimens were prepared for histological and immunohistochemical studies and for assessment of oxidative stress. TAA induced liver toxicity with elevated liver enzymes and histological alterations, fatty changes, apoptosis, and fibrosis evidenced by increased immunohistochemical reaction to matrix metalloproteinase-9 (MMP-9) and collagen type IV in hepatocytes. Also, the levels of pro-inflammatory cytokines (TNF-α, IL-1β, and IL-6) in serum were significantly elevated. Co-treatment with caffeine and TAA restored normal liver structure and function. Caffeine provided an anti-fibrogenic, anti-inflammatory, and antioxidant effect that was associated with recovery of hepatic histological and functional alterations from TAA-induced hepatotoxicity.

Background

The liver is an important organ in the regulation of metabolic balance in the body and detoxification of harmful chemicals and drugs.[1] Liver disease remains a significant global health problem that may result from variety of causes inducing oxidative stress, inflammation, and necrosis of liver cells.[2] Thioacetamide (TAA), originally used as a fungicide, was reported to induce liver fibrosis and cirrhosis in experimental animals.[3–6] Despite great advances in modern medicine, there is no effective drug available that protects the liver from damage or regenerates it.[7]

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Caffeine is a liposoluble substance absorbed quickly and efficiently through the gastrointestinal tract with 100% bioavailability.[8] It is present in many types of foods such as chocolate, teas, energetic drinks, and coffee. It is considered the most used psychoactive substance in the world and about 80% of the population makes daily use.[9,10]

The potential beneficial health effects of caffeine are controversial. Despite a common perception that coffee consumption may have negative health consequences, Lopez-Garcia et al.[11] found that increasing coffee intake led to a reduced rate of cardiovascular death. Benoit et al.[12] illustrated that moderate consumption of three to four cups of instant or roasted coffee per day, assuming an average caffeine concentration of 60–85 mg per cup, is good for human health. However, most of the previous studies on the beneficial properties of caffeine on humans have been observational. Scientists at the National Institutes of Health have reported that increased coffee consumption is associated with reduced incidence of hepatocellular carcinoma.[13,14]

Liver diseases are common heath problems that can lead to fibrosis and cirrhosis and different population surveys detected a possible beneficial association between coffee and liver disease with no adequate studying of liver structure and function. This study therefore aimed to investigate the possible effect of caffeine on TAA-induced liver disease in male rats and the possible underlying mechanisms.

Materials and methods

Drugs and chemicals

TAA and caffeine were purchased from Sigma Chemical Co, (St. Louis, MO, USA).

Animals

This study had been conducted in the Animal House of the Faculty of Medicine, Zagazig University, Egypt, in accordance with the guidance of Ethical Committee for Animal Handling at Zagazig University. Male albino rats (200–250 g) were used after two weeks for proper acclimatization to the standard housing conditions (25 ± 2°C temperature and 12-h light/dark cycle) and were supplied with standard rodent chow and tap water ad libitum.

Experimental design

Forty adult male rats were equally classified into four groups: group 1 (control group): received only tap water in the same amount given to the other groups; group 2 (caffeine-treated group): received caffeine 37.5 mg/kg per day by catheter;[15] group 3 (TAA-treated group): received intraperitoneal (i.p.) TAA at a dose of 200 mg/kg b.w., twice a week;[16] and group 4 (caffeine + TAA-treated group): received combined TAA and caffeine in the same previous doses.

The chosen caffeine dose in this study (37.5 mg/kg per day) is equivalent to 6 mg/kg per day in humans. It is estimated that an adult human (mean weight, 70 kg) with a moderate coffee intake consumes ~400 mg of caffeine per day, which is 6 mg/kg, per day.[17] Furthermore, 6 mg/kg of caffeine is equivalent to three cups of coffee.[18]

All solutions were prepared daily and offered to the rats ad libitum in aluminum foil-wrapped bottles to avoid light decomposition. Body weight and food/liquids intake were measured daily throughout the experimental period.

After eight weeks of treatment, rats were sacrificed and blood samples were collected from the retro-orbital plexuses for biochemical analysis. Liver was carefully excised and cut into two specimens; one was fixed in 4% paraformaldehyde for histological and immunohistochemical studies and the other was stored in liquid nitrogen until analysis.

Biochemical assay

Blood samples were collected and centrifuged at 4000 rpm for 15 min. The serum was stored at −80°C for biochemical estimation and enzyme-linked immunosorbent assay (ELISA) analysis.

Assessment of liver function

For estimation of liver function, serum levels of transaminases (alanine transaminase [ALT], aspartate transaminase [AST]), gamma glutamyltransferase (GGT), alkaline phosphatase (ALP), and total bilirubin were measured using a biochemistry autoanalyzer (Olympus AU2700, Japan).

Determination of inflammatory cytokines

To determine whether caffeine can suppress inflammation caused by TAA, the serum levels of tumor

necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6) were measured by ELISA kits (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions.

Assessment of oxidative stress

Liver samples from all groups were homogenized with cold phosphate buffer (pH 7.4) and centrifuged at 4000 rpm for 30 min at 4°C. The supernatant was used to assess liver tissue malondialdehyde (MDA) and protein carbonyl (PCO), an indicator of lipid peroxidations and the activity of glutathione peroxidase (GSH-Px), and reduced glutathione (GSH). Also, thiobarbituric acid reactive substances (TBARS) enzyme levels were measured in tissue homogenate. Measurements were performed as described previously[19,20] to determine the oxidative status in the liver specimens.

Histological analysis

Specimens for a light microscope were fixed in a 10% formalin solution and processed to prepare 5-μm thick paraffin sections for hematoxylin and eosin (H&E), Mallory trichrome, and immunohistochemical stains[21] for detection of matrix metalloproteinase (MMP-9), collagen type IV, and antioxidant enzyme super oxide dismutase (SOD).

Immunohistochemical analysis was carried out using the streptavidin–biotin–peroxidase complex method. Paraffin sections were deparaffinized, rehydrated in descending grades of alcohol, and incubated overnight with the primary antibody. Sections were rinsed three times with phosphate buffered saline (PBS), then incubated for 1 h with peroxidase-conjugated secondary antibody, and washed three times with PBS. The reaction was developed with 0.05% diaminobenzidine (Dakopatts, Glostrup, Denmark) as the substrate for peroxidase, and finally, the slides were counterstained with Mayer's hematoxylin. Negative control slides were prepared by replacing the primary antiserum with PBS. The primary antibodies used were: anti MMP-9 Antibody AV33090-100UG; Monoclonal anti-Collagen IV, Cat N≠ SAB4200500; and anti SOD; HPA001401-100UL. All were purchased from Sigma Chemical Co., St. Louis, MO, USA.

Morphometric analysis

The image analyzer computer system Leica Qwin 500 (Leica Microsystems Imaging Solutions Ltd., Cambridge, UK) was used to evaluate the area percentage of blue colored collagen fibers in Mallory trichrome stained sections and the area percentage of brown colored immune reaction of MMP and collage type IV. However, optical density of SOD immune reaction was detected in all studied groups. The area percentage and standard measuring frame of a standard area equal to 118. 476.6 were chosen to record ten readings from five sections/rat of randomly chosen five rats/group.

Statistical analysis

Results were expressed as means ± SEM. Statistical significant difference was determined by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for multiple comparisons. Probability values (P) less than 0.05 were considered to be statistically significant.

Results

General condition

As shown in Table 1, there was a significant reduction in body weight gain and food and liquid intake in TAA-treated groups, either alone or in combination with caffeine, throughout the experimental period, when compared with the control group. However, a significant difference was detected between the TAA-treated and caffeine + TAA-treated groups (P <0.05).

Biochemical results

Effect of caffeine on liver function

Statistical comparison among all studied groups as regards liver function tests revealed a non-significant difference between caffeine and control groups. In the TAA-treated group, serum levels of liver enzymes (ALT, AST, GGT, ALP) and total bilirubin were significantly increased, compared to controls (P <0.001), whereas in the caffeine + TAA co-treatment group, the enzyme levels were much lower than the TAA group (P <0.001) (Table 2).

Effect of caffeine on pro-inflammatory cytokine response

As shown in Table 3, the levels of TNF-α, IL-1β, and IL-6 in serum were significantly elevated in TAA-treated rats compared with that of the control

Table 1. Effect of caffeine, TAA alone, or in combination on body weight (BW), food, and water consumption (n = 10).

	Control	Caffeine	TAA	Caffeine + TAA
Initial BW (g)	230 ± 16.7	228.5 ± 16.4	228 ± 17.1	230.7 ± 15.2
Final BW (g)	398 ± 27.1	376 ± 37.3	200 ± 18.2*	214 ± 9.2*†
Reduction	0	0	28	17.7
Food consumption (g/rat/day)	22.4 ± 2.3	23.1 ± 1.9	15.2 ± 4.6*	20.2 ± 1.5*†
Liquid consumption (mL/rat/day)	25.44 ± 2.5	25.2 ± 2.1	20.2 ± 5.7*	16.7 ± 2.4*†

Data are presented as means ± SEM; n = number of rats in each group. Statistical analysis was done using one-way ANOVA followed by Tukey's post-hoc test for multiple comparisons.
*P <0.05 vs. control and caffeine groups.
†P <0.05 vs. control, caffeine, and TAA groups.

Table 2. Effect of caffeine, TAA alone, and in combination on liver enzymes in albino rats (n = 10).

	Control	Caffeine	TAA	Caffeine + TAA
ALT (μ/L)	48.90 ± 2.86	47.8 ± 2.43	782.27 ± 30.8*	200.7 ± 25.59*†
AST (μ/L)	98.54 ± 6.1	94.27 ± 9.2	987.54 ± 158.45*	217.95 ± 20.57*†
GGT (μ/L)	4.10 ± 0.19	3.89 ± 1.01	19.25 ± 1.01*	6.98 ± 1.51*†
ALP (μ/L)	319.80 ± 18.92	298.12 ± 25.3	2180 ± 135.2*	306.05 ± 12.32*†
Total bilirubin (μm mol/L)	6.31 ± 0.62	6.30 ± 0.56	19.68 ± 5.01*	11.12 ± 0.85*†

Data are presented as means ± SEM; n = number of rats in each group. Statistical analysis was done using one-way ANOVA followed by Tukey's post-hoc test for multiple comparisons.
*P <0.001 vs. control and caffeine groups.
†P <0.001 vs. TAA groups.

group (P <0.05). In contrast, co-treatment of caffeine with TAA decreased these pro-inflammatory cytokines levels, which suggested that caffeine inhibited the inflammatory response induced by TAA administration. However, caffeine per se had no effect on pro-inflammatory cytokines levels in serum.

Effect of caffeine on oxidative stress biomarkers in liver tissue

TAA caused a substantial increase in liver MDA content with a concomitant significant fall in liver GSH content and GSH-Px compared to control group (Table 4). Administration of caffeine alone showed a non-significant decrease in liver MDA content while exhibited a significant increase in GSH content and significant increase in GSH-Px compared to control group (Table 4). Combined administration of caffeine and TAA resulted in a significant reduction in the liver MDA content with significant increase in liver GSH content and GHS-Px content compared to TAA-treated group (Table 4). There was also a statistically significant increase in the hepatic tissues levels of PCO in TAA-treated rats when compared to the control

group (P <0.001). However, a significant decrease was found in the caffeine + TAA-treated group compared with the TAA-treated group.

Moreover, there was significant increase in the hepatic tissues levels of TBARS in TAA-treated rats in comparison to the control rats. However, a significant decrease was observed in the caffeine + TAA-treated group when compared with the TAA-treated rats.

Histological and morphometric results

H&E-stained sections in rat liver from both the control and caffeine groups demonstrated normal hepatic architecture with hepatic cords radiating from clear central veins, and are separated by sinusoids, without inflammation or necrosis (Figure 1a, b). In contrast, the TAA-treated group showed significant changes in liver structure with vascular congestion of both central and portal veins and blood sinusoids. Also, infiltration of inflammatory cells in centrilobular regions was obvious (Figure 1c, d). Fatty changes and apoptosis were manifested in most hepatocyte parenchyma. Conversely, treatment with caffeine + TAA restored

Table 3. Effect of caffeine, TAA alone, and in combination on pro inflammatory cytokines in albino rats (n = 10).

	Control	Caffeine	TAA	Caffeine + TAA
TNFα (pg/mL)	10.15 ± 5.15	9.05 ± 2.8	120 ± 16.7*	40.4 ± 10.1*†
IL-6 (pg/mL)	40.8 ± 12.3	32.9 ± 19.8	420 ± 15.8*	201.5 ± 20.5*†
IL-1B	20.8 ± 11.9	15.8 ± 5.4	460.05 ± 60.96*	272.20 ± 84.32*†

Data are presented as means ± SEM; n = number of rats in each group. Statistical analysis was done using one-way ANOVA followed by Tukey's post-hoc test for multiple comparisons.
*P <0.001 vs. control and caffeine groups.
†P <0.001 vs. TAA groups.

Table 4. Effect of caffeine, TAA alone, and in combination on lipid perioxidation and antioxidant enzyme activities in liver tissue (n = 10).

	Control	Caffeine	TAA	Caffeine + TAA
MDA (nmol/mg protein)	4.65 ± 0.15	4.57 ± 0.09	17.8 ± 0.5*,†	7.9 ± 0.75*†‡
GSH (umol/mg protein)	130 ± 5.2	159 ± 3.4*	67 ± 5.2*,†	118 ± 4.4*†‡
GSH-Px (U/mg protein)	14.7 ± 0.5	19.12 ± 0.3*	7.52 ± 0.6*,†	12.34 ± 0.9*†‡
PCO (nmol/g)	25.45 ± 2.23	23.26 ± 3.16	33.62 ± 3.24*,†	24.74 ± 5. 18‡
TBARS (nmol/100 mg)	1.28 ± 0.6	1.1 ± 0.7	2.31 ± 0.2*,†	1.98 ± 0.4*†‡

Data are presented as means ±SEM; n = number of rats in each group. Statistical analysis was done using one-way ANOVA followed by Tukey's post-hoc test for multiple comparisons.
*P <0.05 vs. control.
†P <0.05 vs. caffeine groups.
‡P <0.05 vs. TAA groups.

normal liver histology, although showing slight inflammatory cell infiltration and vascular congestion (Figure 1f).

Examination of Mallory trichrome-stained sections (Figure 2) revealed few collagen fibers around the central and portal veins in both the control (Figure 2a) and caffeine (Figure 2b) groups. In the TAA group, the collagen fibers were markedly increased around portal tract and within liver parenchyma (Figure 2c). However, co-treatment with TAA and caffeine restored collagen distribution nearly similar to that of control group (Figure 2d).

The previous results were confirmed morphometrically. The area percentage of blue stained collagen fibers was increased significantly in the TAA group compared with both the control and caffeine-treated groups. Also, caffeine administration in the caffeine + TAA group caused a significant decrease in the area percentage of collagen when compared with the TAA group (mean ± SEM, 0.24 ± 0.14 and 2.41 ± 0.52 in the caffeine + TAA and TAA groups, respectively; P <0.05) (Table 5).

Immunohistochemical analysis revealed similar results in both the control and caffeine groups. The control group was therefore used to be compared with the other studied groups. Also, statistical analysis of the area percentage of brown stained immunoreaction of both MMP-9 and collagen type IV revealed non-significant changes between both groups (P >0.05) (Table 5).

Immunolocalization of MMP-9 in liver specimens revealed a positive cytoplasmic reaction in hepatocytes around the central veins in the control group (Figure 3a). The reaction was markedly increased after administration of TAA to involve all liver parenchyma (Figure 3b). In the caffeine + TAA group, the reaction was decreased compared with the TAA group, to be restricted to pericentral hepatocytes (Figure 3c). A significant increase in the area percentage of MMP-9 immunoreactions was confirmed by morphometric analysis in the TAA group when compared with the control and caffeine + TAA groups (mean ± SEM, 4.52 ± 1.5, 1.9 ± 0.03, 2.3 ± 1.02 for TAA, control, and caffeine + TAA, respectively) (Table 5).

As regards immunolocalization of collagen type IV in liver specimens, no reaction was detected in the hepatocytes of the control group (Figure 3d). However, a strong reaction was seen in the hepatocytes' basement membrane of the TAA-treated group (Figure 3e). Co-administration of caffeine with TAA decreased the reaction compared with TAA alone, to be restricted to pericentral hepatocytes (Figure 3f). Statistical analysis of morphometric study of this
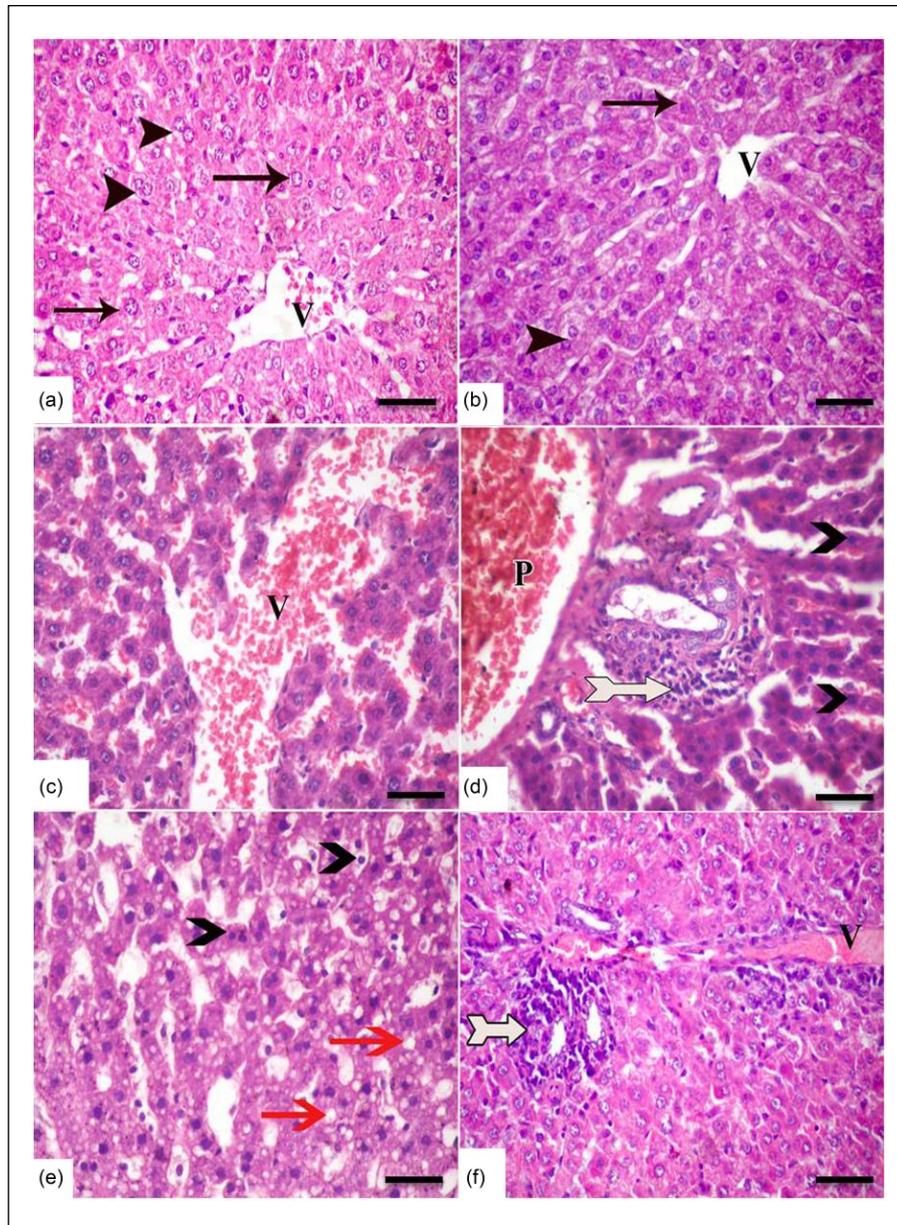
Figure 1. A photomicrograph of H&E-stained sections in rat liver of all studied groups. The (a) control group and (b) caffeine group showing cords of normal hepatocytes (arrows) with acidophilic stippled cytoplasm and vesicular nuclei radiating from central veins (v), and separated by sinusoids (s). Some hepatocytes (arrow head) are binucleated. (c–e) In the TAA-treated group, congestion of both central (v) and portal (p) veins and blood sinusoids was seen. Also, inflammatory cells (bifid arrow) heavily infiltrate the portal area. Well circumscribed cytoplasmic vacuoles indicating fatty changes (red arrow) and apoptotic darkly stained nuclei (arrow head) are manifested in most hepatocyte parenchyma. Conversely, co-treatment with caffeine and TAA restored normal liver histology, although the portal area still shows cellular infiltration (bifid arrow) and vascular congestion (v) (f) (scale bar = 50 μm).

immune reaction revealed a significant increase in the TAA group when compared with the control and caffeine + TAA groups (mean ± SEM, 1.29 ± 0.69, 0.02 ± 0.01, and 0.4 ± 0.01, respectively).

Concerning immunolocalization of superoxide dismutase in liver sections, there was a cytoplasmic reaction in the hepatocytes of the control group

(Figure 3g). In the TAA-treated group, no reaction was detected in liver parenchyma (Figure 3h), while after co-administration of caffeine with TAA, hepatocytes revealed a strong cytoplasmic reaction (Figure 3i). Statistical analysis of optical density of cytoplasmic reaction of SOD showed a significant decrease in the TAA group when
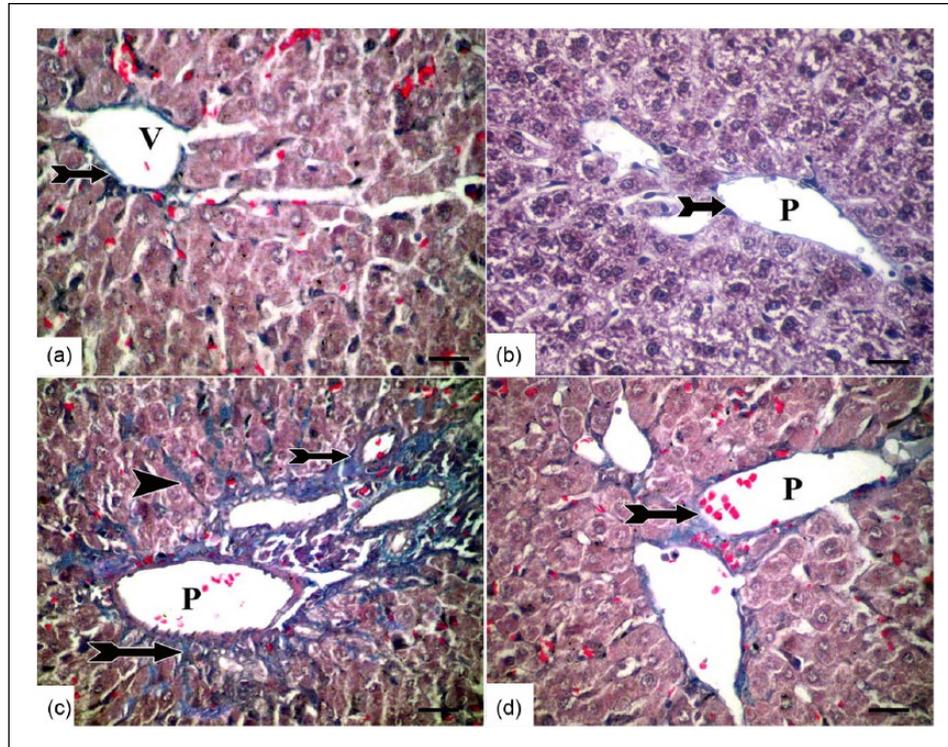
Figure 2. A photomicrograph of Mallory trichrome-stained sections in rat liver of all studied groups. Few collagen fibers (bifid arrow) are seen around the central (v) and portal (p) veins in both the (a) control and (b) caffeine groups. (c) In the TAA group, the collagen fibers are markedly increased around the portal tract (bifid arrow) and within the liver parenchyma (arrow head). (d) In the caffeine + TAA group, collagen distribution is nearly similar to the control group (Mallory trichrome, scale bar = 50 μm).

Table 5. Effect of caffeine, TAA alone, and in combination on histomorphometric results of all studied groups.

	Control	Caffeine	TAA	Caffeine + TAA
Area % collagen MT	0.21 ± 0.18	0.2 ± 0.15	2.41 ± 0.52*	0.24 ± 0.14†
Area % MMP-9 immunoreactions	1.9 ± 0.03	1.89 ±0.02	4.52 ± 1.5*	2.3 ± 1.02†
Area % collagen IV immunoreactions	0.02 ± 0.01	0.01 ± 0.09	1.29 ± 0.69*	0.4 ± 0.01†
Optical density SOD immunoreactions	0.43 ± 0.2	0.45 ± 0.3	0.1 ± 0.04*	0.54 ± 0.3†

*P <0.05 vs. control and caffeine groups.
†P <0.05 vs. control, caffeine, and TAA groups.

compared with the control group (mean ± SEM, 0.1 ± 0.04, 0.43 ± 0.2, respectively; P <0.05). Administration of caffeine with TAA caused significant increase in the optical density of SOD immunoreaction (mean ± SD, 0.54 ± 0.3, P <0.05).

Discussion

This study was aimed at the evaluation of the protective utility of caffeine, the world's most popular psychoactive substance, against TAA-induced liver injury in rats. TAA proves highly useful as an experimental liver injury model. It has been used for years as the lesions caused by this hepatotoxic

drug are similar to most cases of liver diseases in human, which makes it a good model to study the mechanism in vivo.[22]

Per our results, TAA-induced liver toxicity in rats was evidenced by elevated liver enzymes and total bilirubin along with histological disturbance of liver tissue. The previous results were considered by Bahashwan et al.[23] as an indication of structural and functional defects in liver cells.

Administration of caffeine with TAA in the present work restored normal liver architecture as well as decreased serum levels of liver enzymes and total bilirubin, compared with the TAA group. In agreement, Ruhl and Everhart[24] reported that caffeine
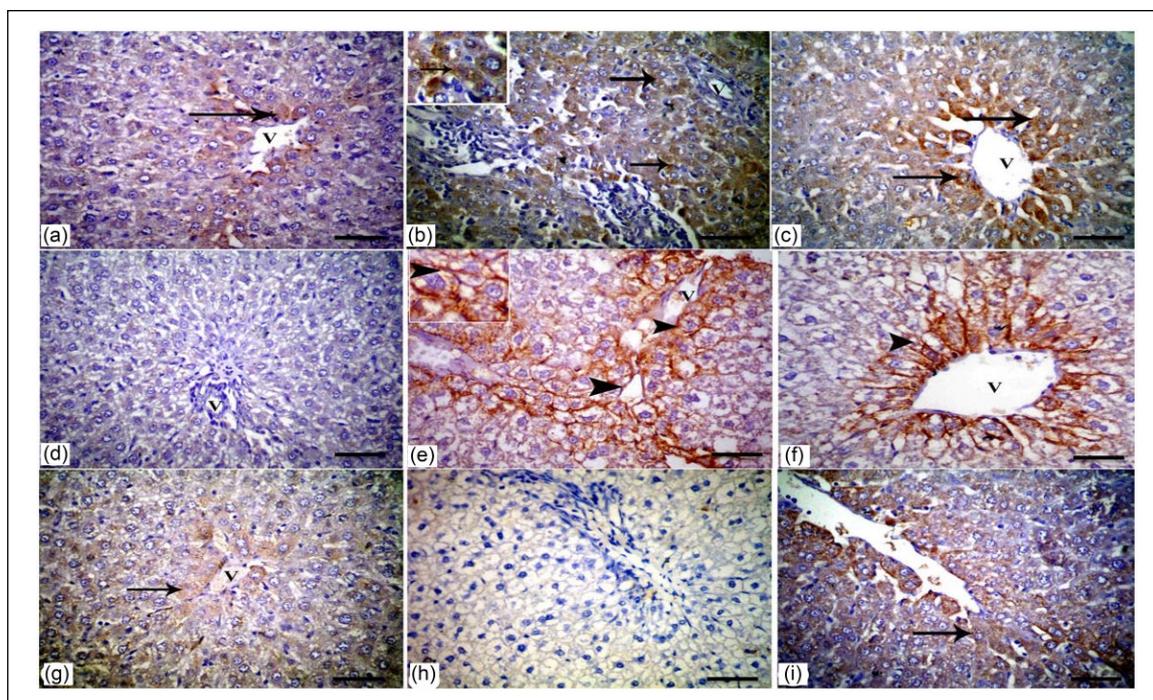
Figure 3. (a–c) Immunolocalization of MMP-9 (arrow) in liver specimens of all studied groups. (a) Control group showing cytoplasmic reaction in hepatocytes around central veins (v). The reaction is markedly increased after administration of TAA to involve all liver parenchyma (b). In the caffeine + TAA group (c), the reaction decreases to be restricted to pericentral hepatocytes (Immunoperoxidase; scale bar = 50 μm). (d–f) Immunolocalization of collagen type IV in liver specimens of all studied groups. No reaction is detected in hepatocytes of the control group (d). However, a strong reaction is seen in basement membrane of hepatocytes (arrow head) of the TAA-treated group (e, inset). Co-treatment with caffeine and TAA decreases the reaction as compared to TAA alone, to be restricted to pericentral hepatocytes (arrow head) (f) (Immunoperoxidase; scale bar = 50 μm). (g–i) Immunolocalization of superoxide dismutase (arrow) in liver specimens all studied groups. Cytoplasmic reaction is seen in the hepatocytes of the control group (g). In the TAA-treated group, no reaction is detected in liver parenchyma (h) while after co-administration of caffeine with TAA (i), hepatocytes reveal a strong cytoplasmic reaction (Immunoperoxidase; scale bar = 50 μm).

consumption (>2 cups per day) was associated with a lower risk of elevated ALT levels and a lower risk of chronic liver disease, compared with non-coffee drinkers. In addition, previous studies illustrated that coffee and some of its major components (caffeine) alter expression and activity of enzymes involved in xenobiotic metabolism in mice and rats, as well as human hepatoma cell lines.[25,26]

Rezaie et al.[27] indicated that caffeine tended to prevent liver damage by maintaining the integrity of the plasma membrane, thereby suppressing the leakage of enzymes through membranes, exhibiting hepato-protective activity. This might be the reason for the restoration of the activities of the marker enzymes during administration of caffeine.

The biochemical findings in our work were supported by histopathological findings in liver specimens. Microscopic results in the TAA group showed different lesions including apoptosis, fatty degeneration, vascular congestion, and apparent

fibrosis. TAA is proved by other research to induce fibrosis/cirrhosis associated with hepatocyte death and activation of Kupffer cells (KC) and hepatic stellate cells (HSCs).[28–30] Fibrotic liver injury is an active process characterized by abnormal extracellular matrix (ECM) deposition and remodeling.[31] Advanced liver fibrosis disrupts the liver's normal architecture; hepatocytes are replaced with abundant ECM causing hepatocellular dysfunction and portal hypertension.[32] Liver fibrosis is the result of extensive tissue remodeling with a net increase in ECM.[28]

Our study also indicated that TAA-induced liver toxicity is possibly mediated through generation of free radical and oxidative stress, indicated by elevated MDA, PCO, and TBARS. Also, decreased reduced glutathione and GSH-Px were detected in liver tissue and immuno-histochemical expression of SOD was apparently decreased in TAA-treated liver tissue.

TAA is biotransformed by CYP2E1 enzymes located in the microsomes of liver cells that convert it to a highly reactive toxic intermediate, known as thioacetamide sulphur dioxide through oxidation,[33] inducing hepatotoxicity in experimental animals and different grades of liver damage including nodular cirrhosis and necrosis of parenchyma cells.[34] Toxins target metabolically effective hepatic cells causing dysfunction of hepatocytes and discharge of inflammation-related cytokines.[35] This was confirmed in our study by elevation of inflammatory cytokines IL-6, IL-1B, and TNF-α in blood samples of TAA-treated group.

Administration of TAA caused a significant increase in collagen fibers around the portal tract and within liver parenchyma associated with significant increase in expression of MMP-9 and collagen IV in the hepatocytes basement membrane. Similarly, Park et al.[30] found that TAA caused an increase in the expression of MMPs and their endogenous inhibitors which were reported to be the key enzymes responsible for degradation and deposition of all the protein components of ECM and basement membrane.[36,37] HSCs and hepatocytes produce, remodel, and turn over abnormal ECMs of fibrosis via MMPs.[38] MMP-9 is critically involved in the degradation of collagen IV and fibronectin of basement membrane.[39]

Collagen IV is a major constituent of basement membranes in tissues.[40] It is generated during liver fibrogenesis by the activated HSCs in the liver.[41] Moreover, a close association was found between increased MMP level and increased collagen fibers in hepatocytes.[42,43]

In contrast, co-treatment with caffeine and TAA restored liver collagen distribution nearly similar to that of the control group, associated with a significant decrease in area percentage of matrix MMP-9 and collagen type IV immunohistochemical reactions in liver specimens, as compared with the TAA group. The assumption has been that reduced fibrosis was due to a reduction in disease activity as reflected by serum aminotransferase levels. However, most studies were population surveys, with no evaluation of liver histology. So, the possible antifibrotic effects of coffee/caffeine on liver were indirectly assessed. The distinction between anti-fibrogenic effects and protection against decompensation is important in understanding the underlying beneficial mechanism.[44] With respect to caffeine specifically, Gressner et al.

reported that caffeine interferes with transforming growth factor beta (TGF-β) thus inhibiting expression of connective tissue growth factor (CTGF).[45] Caffeine was also found to upregulate peroxisome proliferator-activated receptor gamma (PPARγ) levels, which further reduce CTGF expression.[44]

Evidence has also shown that MMPs expression by HSCs is regulated in a cytokine-specific pattern.[46] Our work supported this theory as the levels of MMP-9 were decreased together with decreased serum levels of inflammatory cytokines TNF-α, IL-1β, and IL-6 in combined caffeine and TAA-treated rats as compared with that of the TAA alone group. Previous research reported marked stimulation of MMPs caused by TNF-α.[39,46] Thus, the reduced MMPs activity may be linked to attenuation of the inflammatory and fibrotic processes.[47]

In this work, the antioxidant properties of caffeine were evidenced by increased liver content of reduced glutathione, GH-Px activity, and immunohistochemical expression of SOD together with decreased MDA, PCO, and TBARS levels, thus showing a reduction of membrane oxidative stress. It was reported that liver fibrosis/cirrhosis resulted from different factors that are associated with increased reactive oxygen species (ROS) production. Further, ROS are involved in the development of liver fibrosis/cirrhosis under these pathologic conditions.[48,49] Supporting our results, previous research confirmed the antioxidant effects of coffee, especially its caffeine content, that prevent free radical tissue damage by reducing or eliminating ROS.[47,50] Caffeine is rich in phytochemical derivatives such as triterpenes, flavonoids, or polyphenols. The preventive effects of caffeine could be attributed to their protective activity as kahweol and cafestol phenolic diterpenes of caffeine inhibit lipid peroxidation.[51,52]

In the current study, a significant increase in reduced glutathione and GH-Px in the liver tissue of the caffeine-treated group was detected without significant changes regarding MDA and SOD in this group in comparison to control group. Overall, the results obtained from previous studies about the role of coffee in the modulation of antioxidant enzymes in healthy individuals are conflicting. After coffee intake, healthy individuals reported an increase in GST activity in erythrocytes[53] but not in saliva.[54] Different types of coffee, doses, bioactive compounds, and duration of the study together with the chosen biomarker and the

methods used for their evaluation could partially explain the observed variability among findings. More studies are necessary to understand the effect of coffee on oxidative stress markers in healthy individuals.[55]

In conclusion, the results presented here provide basic information that may explain the beneficial effects of caffeine on liver diseases observed in clinical trials. Caffeine provided an anti-fibrogenic, anti-inflammatory, and antioxidant effect that was associated with the recovery of hepatic histological and functional alterations from TAA-induced hepatotoxicity.

Declaration of conflicting interests

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