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Bax and CD68 Expression in Response to Liver Injury Induced by Acetaminophen: The Hepatoprotective Role of Thymoquinone and Curcumin

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ABSTRACT

Acetaminophen (APAP) overdose depleted glutathione (GSH) which lead to liver dysfunction and hence hepatotoxicity. N-acetylcysteine (NAC) is the best antidote for APAP but may induce a variety of side effects. This study was designed to compare the potential impact of NAC with that of Thymoquinone (THQ), and/or Curcumin (CUR) either alone or in combination on liver injury induced by inflammation, oxidative stress in response to APAP toxicity in rats. Serum aminotransferases (ALT and AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), total protein, total bilirubin, hepatic glutathione (GSH), nitric oxide (NO), superoxide dismutase (SOD) and lipid peroxides (LP) levels were estimated. Moreover these biochemical parameters were confirmed by histopathological examination using hematoxylin and eosin (H&E) and Mason trichrome stains (MTC). Immunohistochemical investigations for the expression of the proapoptotic protein (Bax) and the expression of macrophage cluster of differentiation (CD68). APAP elevated most of the previously measured parameters and decreased GSH, SOD, and total protein levels. Liver sections of H&E demonstrated liver injury characterized by centrilobular hepatocellular necrosis, CD68, and Bax expressions were also increased. Treatment with all the aforementioned antioxidants downregulated most of the elevated parameters compared to the APAP-treated group. Treatment with the combination of CUR and THQ was the most effective therapies in the attenuation of liver injury assessed by a decrease in ALT and ALP activities down-regulation of Bax and CD68 expressions. It was concluded that the combination strategy of THQ and/or CUR may be considered as a potential antidote in combating liver injury induced by APAP due to their antioxidant effects with fewer side effects compared to NAC.

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Authors' Contributions

LMF, NMA-R and NMA presented and designed the study concept. MA, AMA performed immunohistochemistry. LMF, IHH, NA, NQ and RK analysed and interpreted the data. LMF and HMA drafted the manuscript and revised it also.

Key words

Thymoquinone, Curcumin, Bax, CD68 Expression, Acetaminophen

INTRODUCTION

Oxidative stress arising from the imbalance between augmented free radical production and inadequate antioxidant defense has been implicated by a variety of factors such as ionizing radiation or exposure to drugs and xenobiotics (Jayakumar *et al.*, 2008). Acetaminophen (APAP) is an analgesic and antipyretic with weak anti-inflammatory properties. APAP was well-tolerated by human, hence became a common household analgesic (Hinson *et al.*, 2010). Metabolism of overdose of APAP by the cytochrome P450 system produced a metabolite. N-Acetyl-p-benzoquinone imine (NAPQI) which induces a hepatotoxic effect (Jollow *et al.*, 1973). When APAP was administered in therapeutic doses, this potent harmful metabolite was rendered harmless by reducing liver glutathione (GSH). However, when APAP was taken-in an overdose, the rate of formation of NAPQI was greatly increased and hepatic glutathione level was decreased which lead to liver dysfunction (Mitchell *et al.*, 1973).

APAP hepatotoxicity was characterized by highly increased oxidative stress which activate c-Jun N-terminal kinase (JNK) resulting in the induction of mitochondrial permeability then the generation of reactive oxygen species (ROS) (Hanawa *et al.*, 2008; Saito *et al.*, 2010). Moreover, the excessive ROS and reactive nitrogen species (RNS) production during inflammation contributed to the depletion of GSH (Bailey and McGuigan, 1998). Moreover, it was found that treatment of mice with APAP resulted in a marked accumulation of CD68 macrophage-positive inflammatory macrophages in the liver tissue 24 to 48 hs after treatments. Bax has been reported to be localized in the mitochondria during APAP toxicity (El-Hassan *et al.*, 2003) and induce apoptotic channels in the mitochondria with the release of apoptotic proteins including endonuclease G (Dejean *et al.*, 2005, 2006).

N-acetylcysteine (NAC) is derived from the sulfur-containing amino acid, cysteine along with glutamic acid and glycine, NAC is a precursor of glutathione, which is the most body's cellular antioxidant and ROS scavenger (Blouet *et al.*, 2007). NAC has beneficial effects in reducing many pathological events including APAP intoxication, respiratory distress syndrome, heavy metal toxicity, chemotherapy-induced toxicity and psychiatric disorders (Radomska and

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Skopinski, 2012). NAC is considered to be the most efficient antidote for APAP metabolism. It is used to protect hepatic GSH during detoxification of NAPQI. NAC administration might induce a variety of side effects, including anaphylactic reactions, headaches, nausea, vomiting and diarrhea (Corcoran *et al.*, 1985; Dawson *et al.*, 1989; Zyoud *et al.*, 2011). Therefore, it is important to discover therapeutically potent and safe compounds that can prevent APAP overdose-induced hepatotoxicity.

Thymoquinone [2-Isopropyl-5-methylbenzo-1,4-quinone] (THQ) is the most potent component of *Nigella sativa*. It has been suggested that THQ may act as an antioxidant against APAP and prevent membrane lipid peroxidation in hepatocytes (Manik *et al.*, 2014). Curcumin (CUR) also was known as diferuloylmethane (1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione), a polyphenol, that is an active ingredient of turmeric (*Curcuma longa*) and is pharmacologically safe for animals. CUR has many biological activities, including anti-inflammatory and antioxidant. It significantly decreased serum transaminases and hepatic malondialdehyde (MDA) and increased GSH level (Fazel *et al.*, 2014).

The current study was designed to investigate the potential impact of CUR and/or THQ either alone or in combination on liver damage caused by APAP toxicity and to determine the hepatoprotective role of CUR and THQ compared to that of NAC which is the antidote of APAP.

MATERIALS AND METHODS

Chemicals

All chemicals used were of high analytical grade, a product of Sigma and Merck. APAP, NAC and THQ were purchased from Sigma Chemical Co. (Sigma, St. Louis, MO, USA) while CUR was purchased from Armal company.

Experimental animals

Thirty-six healthy male albino western rats (80–190 g) were obtained from the Experimental Animal Center, College of Pharmacy, King Saud University. Animals were kept in special cages and maintained on a constant 12-h light/12-h dark cycle with air conditioning and a controlled temperature of 20–22° C and humidity of 60%. Rats were fed a standard rat pellet chow with free access to tap water *ad libitum* for 1 week before the experiment for acclimatization. Animal utilization protocols were performed in accordance with the guidelines provided by the Experimental Animal Laboratory and approved by the Animal Care and Use Committee of the College of Pharmacy, King Saud University.

Experimental design

Thirty-six male rats were divided into six groups, each of six rats. Group 1 served as control and received normal saline orally. Groups 2 received single oral dose of APAP

(750mg/kg) (Sener *et al.*, 2003; Abraham, 2005). Group 3 was treated with NAC (20mg/kg dissolved in normal saline) (Samuhasaneeto *et al.*, 2007) after a single oral dose of APAP at 750mg/kg. Group 4 received CUR (200mg/kg dissolved in corn oil) (Chuang *et al.*, 2000) after a single oral dose of APAP at 750mg/kg. Group 5 was administered THQ (15mg/kg dissolved in corn oil) (Aycan *et al.*, 2014) after a single oral dose of APAP at 750mg/kg. Group 6 received CUR+THQ after a single oral dose of APAP at 750mg/kg.

Three doses of NAC, CUR and or THQ were administered orally. The first one was 24h before APAP then the second dose was 2h after APAP administration, whereas the third dose was 12h after APAP administration.

All rats were sacrificed under ether anesthesia 24h after APAP treatment. Blood samples were collected from each animal in all groups in sterilized tubes for serum separation by centrifugation at 3,000 rpm for 20 min and used for biochemical analysis. After blood collection, the livers were collected, washed using chilled saline solution. The livers were minced and homogenized in ice-cold bi-distilled water to yield 20% homogenates. The homogenates were centrifuged for 20 min at 3,000 rpm at 5°C, and the supernatants were used for biochemical analyses. Three livers from each group were kept in 10% formaline for histopathological and immunohistochemistry examinations.

Biochemical serum analysis

Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), total protein and total bilirubin levels were estimated using the commercially available kit (Randox Laboratories) according to the manufacturers' instructions.

The lipid peroxidation (MDA) level in hepatic tissues was determined by measuring thiobarbituric acid reactive substances (TBARS) in the liver homogenate (Uchiyama and Mihara, 1978). The absorbance was measured spectrophotometrically at 532nm and the concentrations were expressed as $\mu\text{mol TBARS /g wet tissue}$. The hepatic superoxide dismutase (SOD) was measured using EDTA and Pyrogallol according to McCord (1994). The absorbance was measured spectrophotometrically at 430nm. The hepatic reduced glutathione (GSH) was determined using the method of Ellman (1959) based on its reaction with 5,5'-dithiobis (2-nitrobenzoic acid) to yield the yellow chromophore, 5-thio-2-nitrobenzoic acid. The hepatic total nitrate/nitrite were measured according to the method described by Moshag *et al.* (1995) using Griess reagent (sulfanilamide and N-1-naphthylethylenediamine dihydrochloride) in acid medium.

Histological examination

Liver specimens were excised and stored in 4% paraformaldehyde in phosphate buffer over-night, and embedded in paraffin wax at 57°C. Serial sections were

cut at 4 μ m using a Spencer 820 microtome. These sections were used for histopathological examination using H and E and Mason trichrome stains.

Immuno-staining for localization of CD68 and Bax proteins

Immuno-staining of the liver sections for detection of CD68 and Bax was performed using streptavidin-biotinylated horseradish peroxidase method (Novolink Max Polymer detection system; Novocastra Laboratories, Newcastle, UK). The procedure involved the following steps: endogenous peroxidase activity was inhibited by 3% H_2O_2 in distilled water for 5 min., and then the sections were washed twice in Tris-buffered saline (pH 7.6) (Sigma-Aldrich) for 10 min. Nonspecific binding of antibodies was blocked by incubation with protein block for 10 min. (Novocastra). Sections were incubated with mouse monoclonal anti-CD68 [ED1] ab31630 and rabbit polyclonal anti-Bax-ab7977-antibodies, diluted 1:400 and 1:100, respectively, for 1h at room temperature. Sections were washed three times in Tris-buffered saline and then incubated with biotinylated IgG (Novocastra) for 30 min., followed by washing in Tris-buffered saline and incubation with Novolink polymer (Novocastra) for 30 min. Peroxidase was detected with working solution of diaminobenzidine (DAB) chromogen (Novocastra) for 10 min. Sections were then washed in distilled water for 10 min., counterstaining was carried out with Mayer's hematoxylin to stain the nuclei, and sections were mounted in DPX. For negative control sections, the same procedure was followed with the

omission of incubation in primary antibodies.

Statistical analysis

The experimental data were statistically analyzed using one-way analysis of variance (ANOVA) followed by Bonferroni test for multiple comparisons. Data were expressed as mean $\pm$ S.D. Differences were considered significant at a p-value of less than 0.05.

RESULTS

The current investigation revealed that APAP intoxication resulted in significant elevation of serum ALT, AST, LDH, ALP and total bilirubin levels compared with the control group ($P \leq 0.01$) (Fig. 1, Table I). NAC alone, CUR and/or THQ either alone or in combination treatments successively alleviated the changes in the previous measured serum biochemical parameters. On the other hand, the present investigation revealed that APAP intoxication significantly elevated lipid peroxides (MDA) and NO levels with a concomitant depletion of hepatic non-enzymatic antioxidant GSH content and hepatic SOD as well as serum total protein level compared to normal control group ($P < 0.001$) (Fig. 1). NAC alone, CUR and/or THQ either alone or in combination treatments alleviated the changes in oxidative stress and antioxidant biomarkers (Fig. 1). CUR and THQ combination treatment revealed the best results compared to both NAC and APAP groups.

Microscopic investigation of H&E-stained liver sections revealed that, control rats demonstrated typical

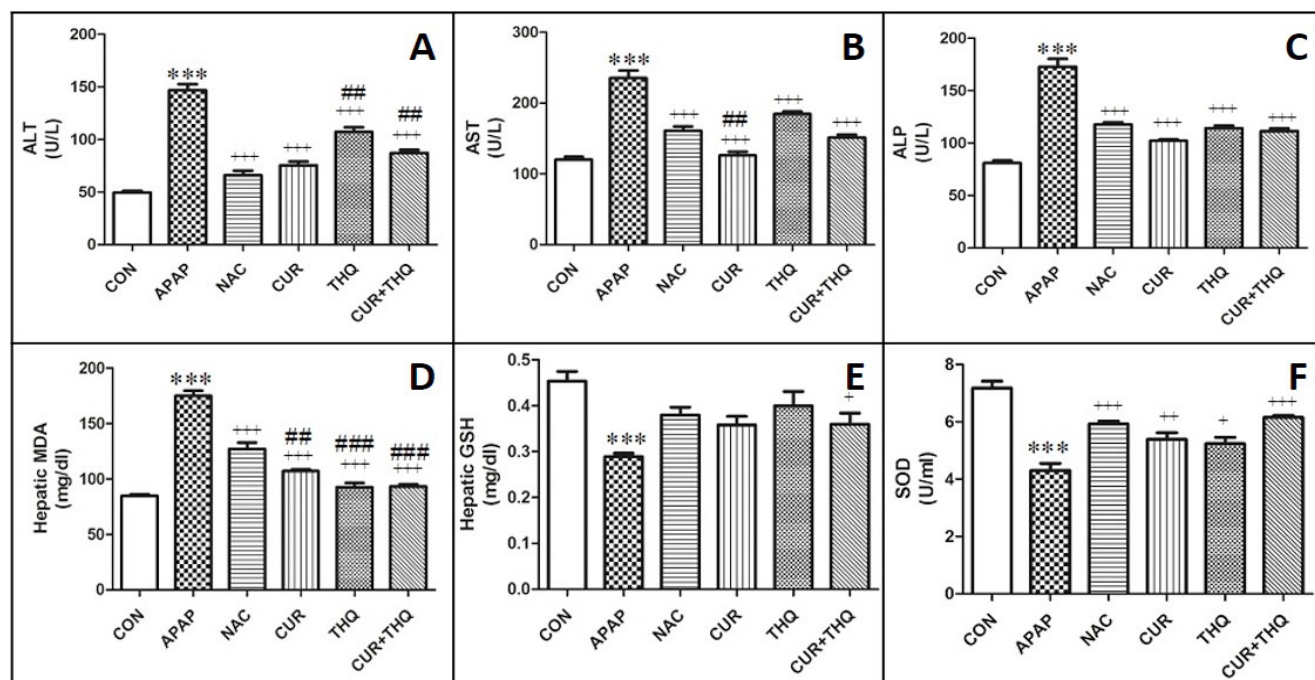


Fig. 1. Serum level of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) and hepatic malondialdehyde (MDA), reduced glutathione (GSH) and superoxide (SOD) in control (CON) and all experimental groups. The experimental groups are as follows: acetaminophen-treated; NAC, N-acetylcysteine-treated group; CUR, curcumin-treated; THQ, thymoquinone-treated; CUR + THQ, curcumin and thymoquinone-treated group.

Notes: Data are mean $\pm$ SEM (n=6). ***, $P \leq 0.001$ vs control; +, $P \leq 0.001$ vs APAP group; ###, $P \leq 0.001$ vs NAC group.

Table I.- Serum level of total protein, total bilirubin and LDH as well as hepatic NO in control and all experimental groups.

Parameters	CON	APAP	NAC	CUR	THQ	CUR+THQ
Total Protein	6.34±0.26	4.28±0.09***	6.03±0.3***	6.23±0.26***	6.14±0.1***	6.29±0.24***
LDH	144.2±10.82	396.2±21.93***	192.3±11.56 ***	181.3±12.92***	221.8±5.047***	181.8±10.21***
Total bilirubin	1.63±0.087	2.4±0.07***	1.87±0.082 ***	1.94±0.03***	1.98±0.03***	1.89±0.04***
Hepatic NO	0.80±0.04	1.3±0.07***	0.97±0.032***	0.76±0.03***#	1.07±0.04 ⁺	0.99±0.041***

Note: Data are mean ± SEM (n=6).

For abbreviations see legends of Figure 1. NO, Nitric Oxide.

***, $P \leq 0.001$ vs control; ***, $P \leq 0.001$ vs APAP group; #, $P \leq 0.05$ vs NAC group.

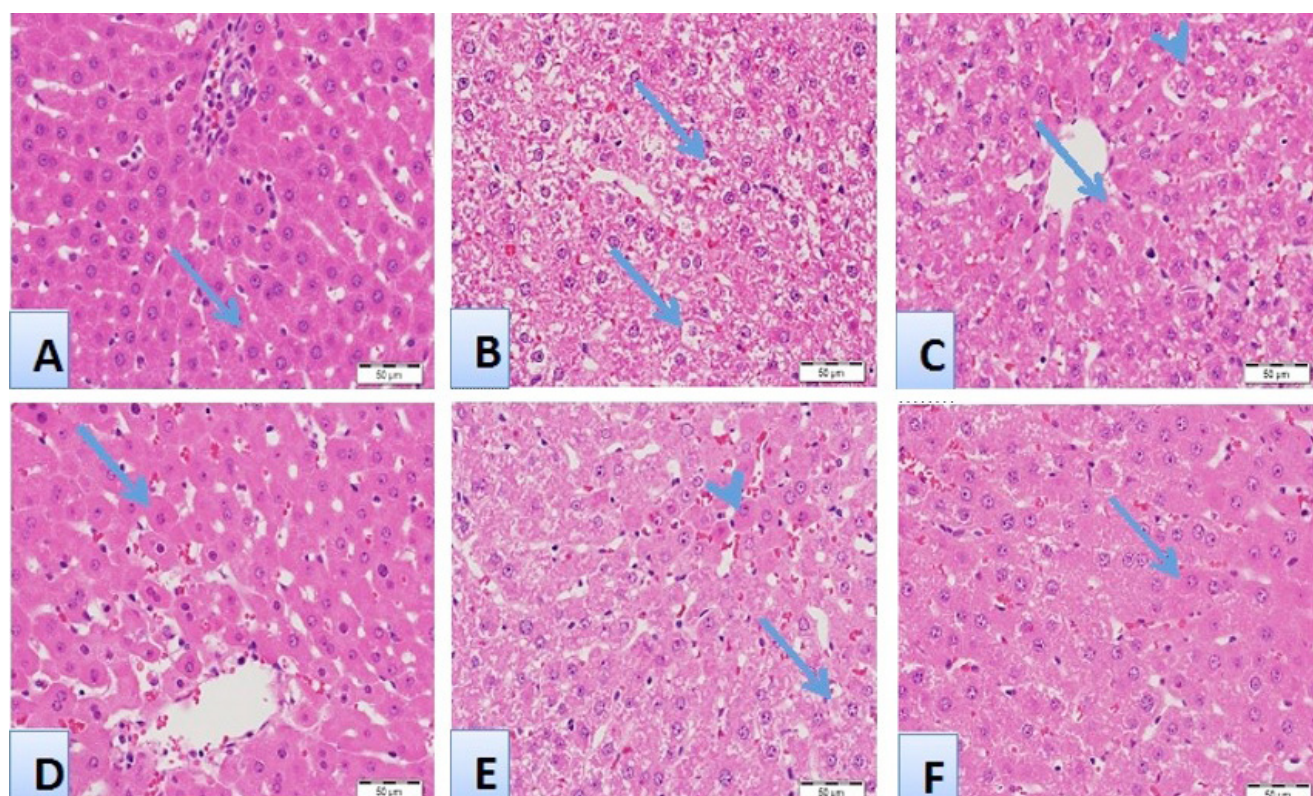


Fig. 2. Histological investigation of rat's liver stained at H&E.

A, liver of control rat showing normal hepatic architecture with normal hepatocytes (arrow) and blood sinusoids; **B**, Liver from rat receiving APAP showing massive cytoplasmic degeneration of the hepatocytes that appeared vacuolated (arrows) with the loss of hepatic lobular architecture; **C**, Liver from rat receiving APAP and NAC showing marked improvement of hepatocellular degeneration (arrow) with the restoration of normal lobular architecture. The arrowhead indicates hepatocyte with cytoplasmic degeneration; **D**, Liver from rat receiving APAP and THQ showing marked improvement of most of the degenerated hepatocytes (arrow) and lobular architecture; **E**, Liver from rat receiving APAP and CUR showing the moderate renewal of the degenerated hepatocytes (arrow) with the appearance of apparently normal hepatocytes (arrowhead); **F**, Liver from rat receiving APAP and combination of THQ and CUR showing normal hepatic lobular architecture and hepatocytes (arrow). Scale bar, 50µm.

normal hepatic architecture, and the normal amount and distribution of the vascular endomysium between hepatic cells (Fig. 2A). Hepatic sections of rats treated with APAP showed multiple large foci of degenerated hepatocytes with vacuolated cytoplasm and pyknotic nuclei, in addition to congestion of hepatic blood sinusoids (Fig. 2B).

While liver sections from rats receiving APAP and NAC showed apparently normal hepatic architecture with very few cells with minimal cytoplasmic degeneration (Fig. 2C). Liver

sections from rats receiving APAP and CUR showed also marked improvement of hepatic cell degeneration with few cells with minimal cytoplasmic degeneration. Liver sections from rats receiving APAP and THQ showed small foci of hepatic cells with moderate cellular degeneration. Whereas sections of livers from rats receiving APAP, CUR and THQ showed marked improvement of hepatic degeneration which was restricted to few hepatocytes with mild vacuolation of their cytoplasm while the nuclei appear normal (Fig. 2F).

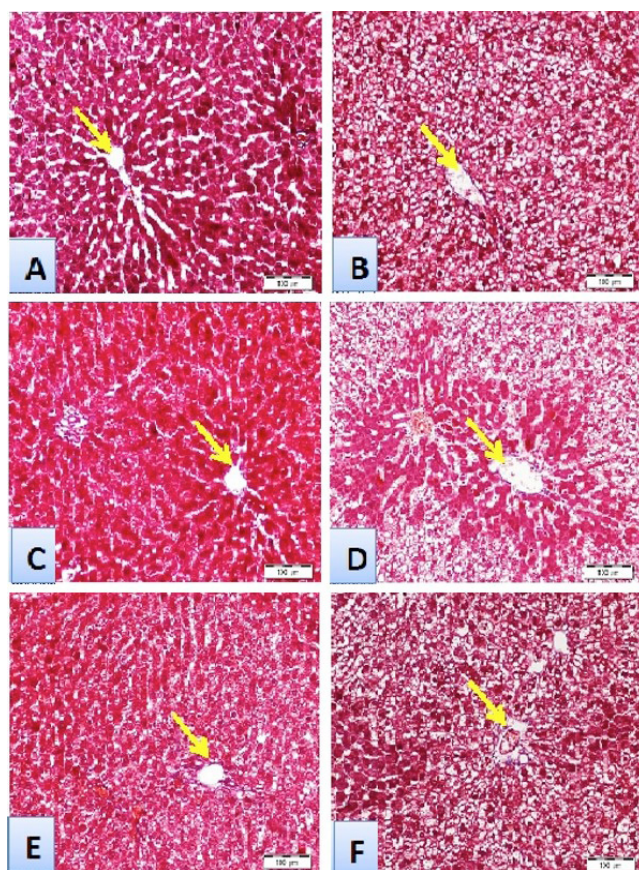


Fig. 3. Histological examination of rat's liver stained by Masson's Trichrome. **A**, Liver from control rat showing normal fibrous tissue deposition which restricted to the wall of the blood vessels in portal areas and central veins of the hepatic lobules (arrow). There is no abnormal change of fibrous tissue distribution in any of experimental groups; **B**, Liver from rat receiving APAP; **C**, Liver from rat received APAP and NAC; **D**, Liver from rat receiving APAP and THQ; **E**, Liver from rat receiving APAP and CUR; **F**, Liver from rat exposed to APAP and mixture of THQ and CUR (arrows). Scale bar: 100µm.

Liver sections stained with Mason trichrome show no differences between all groups indicating no fibrosis in APAP toxicity model as well as all treated groups (Fig. 3A-F).

The immunohistochemical staining of the liver with anti CD68 antibody shows a normal number, distribution and immune density of CD68 positive phagocytic, characteristic Kupffer cells in the wall of the hepatic blood sinusoids. Whereas sections from rat receiving APAP showing the appearance of scattered aggregations of intensely immuno-stained cells especially around the central vein (center-lobular zone). While liver from rat receiving APAP and NAC, or THQ or CUR alone showed moderate decrease in the foci of aggregated phagocyte cells, but still they are much more than the control. Liver section from rats receiving APAP and combination of THQ and CUR showed a mild increase of immune positive cells that were scattered and did not aggregate (Fig. 4A-F).

Liver section from control rat immunostained with anti-

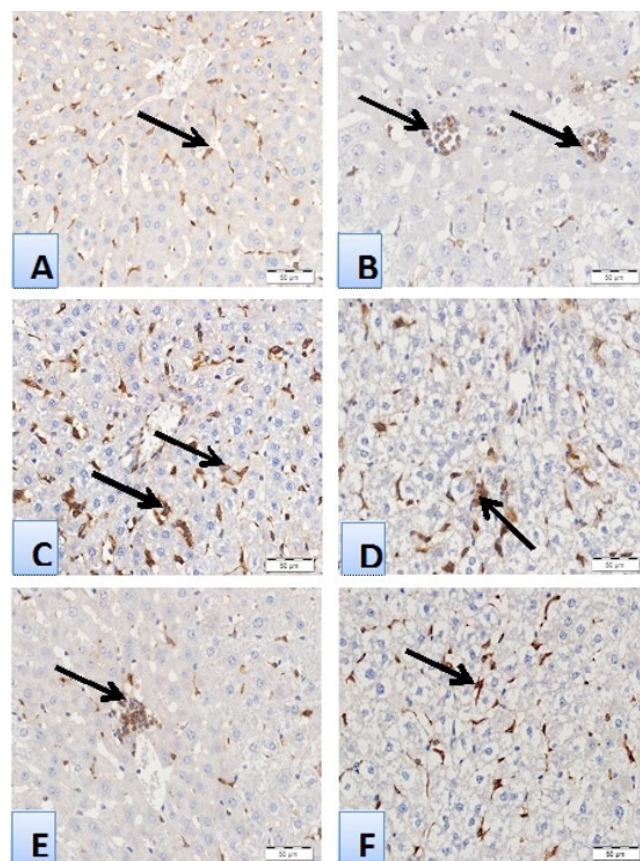


Fig. 4. Histological investigation of rat's liver stained by Immuno-stain with the anti CD68 antibody. **A**, liver from control rat showing a normal number, distribution and immune density of CD68 positive phagocytic, characteristic Kupffer cells (arrows) in the wall of the hepatic blood sinusoids; **B**, Liver from rat received APAP showing the appearance of scattered aggregations of intensely immuno-stained cells (arrow) especially around the central vein (center-lobular zone); **C**, **D**, **F**, Liver from rat received APAP and NAC, or THQ or CUR showing moderate decrease the foci of aggregated phagocytic cells (arrows), but still they are much more than control one. Liver section from rat received APAP and combination of THQ and CUR; **E**, shows a mild increase of immune positive cells that scattered not forming aggregations. Scale bar 50µm.

Bax antibody showed absence of Bax immuno-stained cells. While livers from rats receiving APAP showed patches of hepatic tissue with strong positive immunostaining especially in center-lobular zones. The livers of rats receiving APAP and NAC or THQ or combination of CUR and THQ showed marked decrease of the immunostained hepatic tissue, while livers from rats receiving APAP and CUR alone showed few of strong immune positive cells surrounding the central vein (Fig. 5A-F).

DISCUSSION

APAP is a widely used over the counter analgesic and antipyretic drug (Bessemis and Vermeulen, 2001; James *et al.*, 2003). An overproduction of NAPQI which causes depletion of glutathione by reaction with glutathione to form 3-glu-

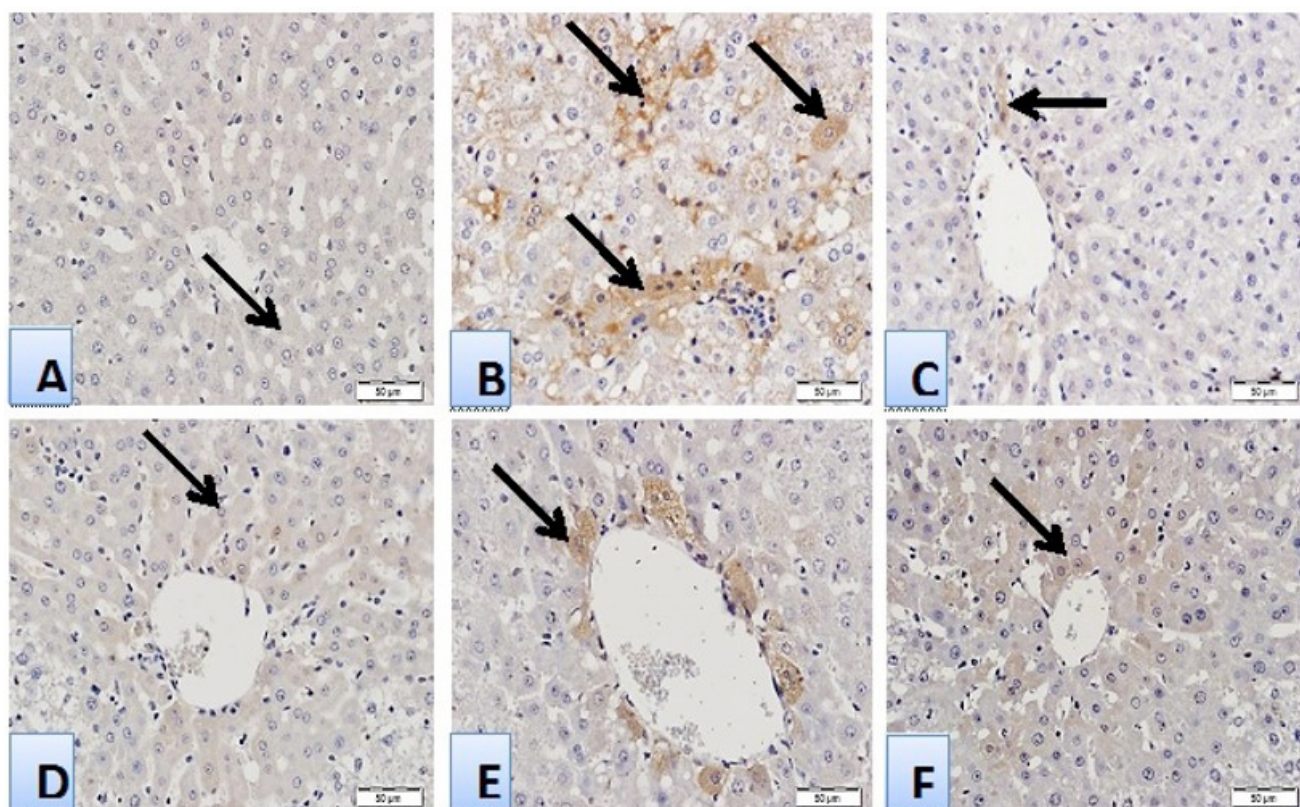


Fig. 5. Histological investigation of rat's liver stained by Immuno-stain with the anti-Bax antibody. **A**, Liver from control rat showing the normal absence of Bax immuno-stained cells (arrow); **B**, Liver from rat received APAP showing patches of hepatic tissue with strong positive immunostaining especially in center-lobular zones (arrows); **C**, **D**, **F**, Liver from rat received APAP and NAC, or THQ or combination of CUR and THQ showing marked decrease of the immunostained hepatic tissue (arrows), while liver from rat received APAP and CUR alone; **E**, shows few of strong immune positive cells surrounding the central vein (center-lobular zone). Scale bar 50µm.

tathion-*S*-yl-acetaminophen is a major problem worldwide and is produced by an overdose of APAP resulting in hepatotoxicity (Dahlin and Nelson, 1982; Hinson *et al.*, 1982). Cell injury is caused by NAPQI by binding covalently to proteins within the cells (Databases, 2007). One cascade of the intracellular events is glutathione depletion that includes the oxidative stress of mitochondria, nitrogen species and reactive oxygen generation, stress proteins and gene transcription mediators' activation, and then the liver innate immune system mobilization. The recovery or cell death is determined by whether there is balance between these numerous pathways ultimately (Dambach, 2005).

Mitochondrial failure seems to be the terminal event leading to cell death (Ono and Han, 2000). Once mitochondrial failure precludes ordered cell death, it is typically necrotic even the apoptotic pathways were activated (Dambach, 2005; Korb *et al.*, 2006). Prescott (2000) reported that a dramatic increase in serum ALT and AST levels, mild hyperbilirubinemia, and increased prothrombin time result from the biochemical changes after APAP.

Formation of superoxide and nitric oxide that react together to produce peroxynitrite, which again exhibits hydroxyl radical-like activity causing hepatotoxicity which is contributed by hepatic macrophages via different mechanisms (Michael *et al.*, 1999). The mechanism by

which N-acetylcysteine inhibits acetaminophen toxicity has been postulated to be mediated detoxification of NAPQI by a direct conjugation or increased glutathione synthesis (Corcoran *et al.*, 1985). The previous results were in accordance with that of the current work as APAP increase ALT, ALP, bilirubin, NO and lipid peroxide levels, whereas GSH and SOD protein level was decreased.

Co-administration with THQ, and/or CUR either alone or in combination modulate the above-mentioned parameters compared with NAC group. Increasing oxidative stress after APAP treatment induces both JNK activation (Matsumaru *et al.*, 2003) and mitochondrial permeability transition (mPT) (Lemasters *et al.*, 1998). Loss of the ability of the hepatocyte to synthesize ATP, GSH depletion, leading to increased toxicity and oxidative stress with the loss of mitochondrial membrane potential are the results of APAP administration.

Bax had been localized in the mitochondria in APAP toxicity model (El-Hassan *et al.*, 2003) and is well recognized to induce apoptosis-induced channels in the mitochondria with the release of apoptotic proteins including endonuclease G (Antonsson *et al.*, 2000; Dejean *et al.*, 2005, 2006). Permeabilization of the mitochondrial outer membrane with the apoptosis-induced channels of mitochondria and intermembrane proteins release are strongly affected by Bax level (Bajt *et al.*, 2008). Pourbakhsh *et al.* (2014) reported

that Western blot analysis and quantitative real-time RT-PCR showed that *Nigella sativa* oil treatment inhibited apoptosis stimulated by ethanol through decreased Bax/Bcl-2 ratio (both protein and mRNA levels), and the level of cleaved caspase-3, caspase-8 and caspase-9 in liver and kidney. It was documented that CUR treatment efficiently protected the liver against APAP-induced apoptosis via increasing Bcl-2/Bax ratio. It was also found that treatment of mice with APAP resulted in marked accumulation of CD68 macrophages in the liver 24 to 48h after treatment. The number of CD68(+) iNOS(+) double positive macrophages was increased obviously in experimental autoimmune myocarditis group but decreased markedly after CUR treatment (Gao *et al.*, 2015).

Histologically APAP toxicity was marked by vacuolization of centrilobular hepatocyte and loss of glycogen which resulted in a clear demarcation of the centrilobular areas from the rest of the liver. The nuclear changes were observed in centrilobular hepatocytes and single cell necrosis with pyknotic cells leading to gross necrosis of the entire centrilobular areas. Mitchell *et al.* (1973) reported that toxicity was associated with hepatic congestion. The role of apoptosis in APAP liver injury is controversial. Ray *et al.* (1996) reported that as many as 40% of the dead hepatocytes were apoptotic and 60% necrotic as a result of APAP toxicity in mice. In the present study liver histological sections stained with H&E from rats receiving APAP showed multiple large foci of degenerated hepatocytes with vacuolated cytoplasm and pyknotic nuclei in addition to congestion of hepatic blood sinusoids. The rats that received APAP and NAC showed apparently normal hepatic architecture with very few cells with minimal cytoplasmic degeneration. Rats receiving APAP and CUR showed also marked improvement in hepatic cell degeneration with few cells with minimal cytoplasmic degeneration. Rats receiving APAP and THQ showed small foci of hepatic cells with moderate cellular degeneration. Rats received APAP, CUR and THQ showed a marked improvement in hepatic degeneration which was restricted to few hepatocytes with mild vacuolation of their cytoplasm while the nuclei appear normal. Light photomicrograph of a liver section stained with Masson's Trichrome showed control rats with normal fibrous tissue deposition which were restricted to the wall of the blood vessels in the portal areas and central veins of the hepatic lobules. There are no abnormal changes in fibrous tissue distribution in all the experimental groups.

Rats receiving APAP showed scattered aggregations of intensely immuno-stained cells especially around the central vein (center-lobular zone) in liver sections immuno-stained with the anti CD68 antibody. The administration of NAC, THQ or CUR showed moderate decrease in the foci of aggregated phagocytic cells but still they are much more than the control. On the other hand, the combined administration of THQ and CUR showed mild increase in the immune positive cells which remain scattered and do not aggregate.

Liver histological sections immunostained with the anti-Bax antibody revealed that control rats showed normal

absence of Bax immuno-stained cells, while that of APAP showed patches of hepatic tissue with strong positive immunostaining especially in center-lobular zones. The rats receiving APAP and NAC, or THQ or combination of CUR and THQ showed marked decrease in the immunostained hepatic tissue while rats receiving APAP and CUR alone showed few strong immune positive cells surrounding the central vein.

CONCLUSION

It was concluded that co-administration of the natural antioxidants such as THQ and CUR were good candidates for amelioration of APAP hepatotoxicity with fewer side effects compared to NAC.

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Conflict of interest statement

We declare that we have no conflict of interest.

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