Mechanisms of hepatotoxicity of chloroacetonitrile: An end product of water chlorination

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Abstract: Chloroacetonitrile is a disinfectant by-product of chlorination of drinking water and is considered as a direct-acting mutagenic and carcinogenic agent. Time-course and dose-response studies were performed to examine the mechanism of chloroacetonitrile-induced hepatotoxicity. In the time-course study, animals were scarified at 2, 4, 6 and 12 h after a single oral dose of chloroacetonitrile (38 mg/kg, p.o.). In the dose-response study, rats were scarified at 2 h after a single oral dose of chloroacetonitrile (9, 19, 38, and 76 mg/kg). In the time-course study chloroacetonitrile induced a significant decrease of hepatic glutathione, and activities of glutathione-S-transferase, glutathione proxidase and superoxide dismutase accompanied with an increase of hepatic malondialdehyde, plasma cytokines (IL-6&10 and TNF-α), serum aminotransferases and total bilirubin after 2 h of administration. Maximal alteration of the estimated parameters was observed at 4 h and returned to normal value at 6 h and/or 12 h after chloroacetonitrile treatment. Moreover, the alterations in oxidant, antioxidant parameters, inflammatory cytokines and the liver function tests were dose dependant. Histopathological findings supported the biochemical results. These data indicate that the mechanism of chloroacetonitrile-induced hepatotoxicity may be mediated through depletion of antioxidants, induction of oxidative stress and inflammatory cytokines.

Keywords: Water-disinfectant, cytokine, hepatotoxicity, chloroacetonitrile, oxidative stress.

INTRODUCTION

The disinfection of the drinking water is one of the most important public health achievements of the last century. Chlorination of drinking water is essential for killing pathogenic microorganisms, but they are results in formation of a variety of toxic agents including haloacetonitriles (HAN) (Richardson *et al*., 2007). HAN are formed in the drinking water through the interaction of chlorine with the organic impurities (IARC, 1979) and in vivo following administration of the chlorine residual (Mink *et al*., 1983). Chloroacetonitrile (CAN) [$Cl(CH_2)CN$], a member of HAN group, possess a chlorine substitution on the alpha carbon atom of the acetonitrile molecule. CAN has been found in soft drinks, cigarette smoke, car exhaust and swimming pools (IARC, 1979). CAN has been used as a disinfectant in cooling towers, common laboratory chemical and insecticide for stored grains (Barcelo *et al*., 1987). Numerous toxicological effects of CAN have been reported such as; teratogenicity, mutagenicity and carcinogenicity (IARC, 1979) as well as adverse effects to the neurocytes (Ahmed *et al*., 2005), hepatocytes (Abdel-Naim *et al*., 2009), gastric mucosa (Ahmed *et al*., 1999) and skeletal system (Lipscomb *et al*., 2009). CAN induced oxidative stress through mitochondirial degradation and inhibition of ATP formation (Ahmed *et al*., 1999) as well as sister chromatid exchange and DNA strand breaks in Chinese hamster ovary cells and in human lymphoblast cell lines (Bull *et al*., 1985). Previous studies indicated that CAN and/or its metabolites can cross the placental barrier, concentrate in fetal brain and liver (Jacob *et al*., 1998) and covalently bind with the DNA under oxidative stress (Abdel-Aziz *et al*., 1993). The mechanisms of CAN-induced its toxicity are not fully understood. Many chemicals have been biotransformed to reactive metabolites in order to exert their toxic effects (Guengerich and Liebler, 1985). The most important enzyme involved in the biotransformation and mediated oxidation reactions of such chemicals (such as CAN) are hepatic cytochromes P450 (Gonzalez and Gelboin, 1994; Silver *et al*., 1982). The hydroxyl free radicals (•OH) are able to oxidize CAN to CN^- (Mohamadin, 2001) and the oxidative products of nitriles might produce toxic metabolites such as; hydrogen CN^-, formic acid, formaldehyde and formyl CN^- (Lipscomb *et al*., 2009). Therefore, the present work was designed to study the hepatotoxicity induced by CAN and its mechanism of hepatotoxicity.

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MATERIALS AND METHODS

Chemicals

CAN (99%), reduced glutathione (GSH), superoxide dismutase (SOD), Ellman's reagent [5,5-dithio-bis(2-nitrobenzoic acid)], 2-thiobarbituric acid (TBA) and crystalline bovine albumin were obtained from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals were of the highest purity commercially available.

Animals and treatment

Adult male albino rats (130-150 g) of Wistar strain were used in the current study. Animals were maintained on a

regular diet and water *ad libitum* as well as a 12 h light-dark cycle and 23±2ºC room temperature without any stressful stimuli. All animal manipulations were performed between 8.00 and 10.00 a.m. All animal experiments were performed according to a protocol approved by the Committee on Bioethics for Animal Experiments of Taif University, Taif, Saudi Arabia.

For dose-response study
Rats were randomly assigned eight/group and fasted over night (18 h). One group served as control and received bi-distilled water. Each of the other 4 groups treated with a single oral dose of CAN (9, 19, 38, or 76 mg/kg). Rats were sacrificed at 2 h after CAN administration; their blood and liver were taken out and used for biochemical analysis. CAN doses were selected in accordance to the previous study carried out by Ahmed *et al*. (1999).

For the time-course study
Forty rats were used and divided into 5 groups (8 rats/ group). Rats were fasted over night (18 h) prior to receiving a single oral dose of either bi-distilled water (10 ml/kg) or CAN (38 mg/kg) dissolved in bi-distilled water (Ahmed *et al*., 1999). At 2, 4, 6, and 12 h after treatment, rats were anesthetized with ether, blood and liver were removed and used for biochemical analysis.

Sample preparations
The blood sample of each rat was collected in separated, sterilized, dry tube, coagulates and centrifuged at 3000 rpm/15 min for serum separation. Another portion of blood was collected in heparin containing tubes and used for plasma separation by centrifugation at 600 rpm/15 min. Livers were homogenized in phosphate buffer saline (pH 7.0), centrifuged at 3000 rpm for 15 min at 4ºC and the separated supernatant was used for assay of the hepatic parameters.

Blood parameters
Serum aspartate aminotransferase (AST), alanine amiotransferase (ALT) and total bilirubin were determined spectrophotometrically as described by Gambino (1965) and Henry *et al*. (1974) using a biological kits. Plasma was used for estimation of interleukin-6 (IL-6) using rat ELISA Kit (raIL-6, IBL Co., Ltd. Hamburg, Germany) (Safieh-Garabedian *et al*., 1997), tumor necrosis factor-alpha (TNF-α) using rat ELISA kit (raTNF-α kit, BioSource International Inc., CA, USA) (Wolters *et al*., 1977) and interleukin-10 (IL-10) using rat ELISA (raIL-10, Immunotech; Beckman Coulter Company, Marseille Cedex, France) (Drimal *et al*., 2008). Results were calculated by ELISA reader ELx800 using standard curve.

Hepatic parameters
Hepatic supernatant was used for estimation of GSH (Sedlak and Lindsay, 1968), malondialdehyde (MDA) (Uchiyama and Mihara, 1978), SOD (Marklund, 1985), glutathione peroxidase (GSHpx) (Paglia and Valentine, 1976), Glutathione S-transferase (GST) (Habig *et al*., 1974) and total protein (Lowry *et al*., 1951).

Histopathological examination
Livers of two rats were used for histopathological examination and fixed in neutral buffered 10% formalin solution and then embedded in paraffin. Sections (5µm thickness) were made and stained with hematoxylin-eosin (H & E) (Banchroft *et al*., 1996).

STATISTICAL ANALYSIS

Data are expressed as the means ± SEM and statistically analyzed by Student's *t*-test or ANOVA followed by Student-Tukey-Kramer multiple comparison tests using Excel or GraphPad InStat 3 (ISI Software, Philadelphia, PA) software respectively. The following equation was used for determination of the percentage to control (treated/control × 100). Statistical significance was accepted at $p \leq 0.05$.

RESULTS

Effect of CAN on hepatic levels of GSH, GST, GSHpx, SOD and MDA in rats
In the time-course study (table 1), administration of a single oral dose of CAN (38 mg/kg) induced a significant decrease in GSH, GST, GSHpx and SOD activities accompanied with an increase in MDA contents in hepatic tissue. Depletion of hepatic GSH, GST, GSHpx and SOD as well as the elevation of MDA were maximum at 4 h and minimum at 6 h after CAN treatment. Twelve hours after CAN administration; the hepatic levels of GSH, GST, GSHpx, SOD and MDA were returned to control values. After 2 h of CAN administration, hepatic GSH, GST, GSHpx, SOD and MDA levels were 46%, 60%, 67%, 45% and 246% respectively of the control. While after 4 h of CAN treatment, hepatic GSH, GST, GSHpx, SOD and MDA levels were 25%, 32%, 53%, 34% and 321%, respectively of the control.

In the dose-response experiment (table 2), data showed that oral administration of 19, 38 and 76 mg/kg of CAN induced a significant decrease in hepatic GSH, GST, GSHpx, SOD activities associated with an increase in MDA levels at 2 h after treatment. The alteration in the hepatic oxidant and antioxidant parameters were directly proportional to the dose value.

Effect of CAN on plasma levels of pro-inflammatory cytokines (TNF-α, IL-6 and IL-10) in rats
Oral administration of a single dose of CAN (38 mg/kg) induced a significant increase in the plasma inflammatory cytokines (IL-6, TNF-α and IL-10) compared to control values. IL-6, TNF-α and IL-10 were significantly elevated at 2 h (443.5 pg/ml, 1.3 ng/ml and 58.9 pg/ml, respectively) after administration of CAN. The maximal

Table 1: Time-course study for the effect of CAN on GSH, GST, GSHpx, SOD and MDA levels in hepatic tissue of rats

Parameters	Time (h)				
	Control	2	4	6	12
GSH (µg/g liver tissue)	15.78 ± 0.83	7.26 ± 0.50[a]	4.00 ± 0.12[a,b]	14.23 ± 0.94[b,c]	15.53 ± 0.69[b,c]
GST (nmol/min/mg protein)	658.33 ± 20.89	397.50± 19.09[a]	213.67±10.56[a,b]	594.83±30.98[b,c]	655.50±18.44[b,c]
GSHpx (nmol/min/ mg protein)	1201.50±72.36	815.00± 65.65[a]	644.67±36.04[a]	1212.17±72.36[b,c]	1205.00±77.93[b,c]
SOD (U/mg protein)	13.58 ± 1.28	6.10 ± 0.60[a]	4.72 ± 0.39[a]	10.87± 1.02[b,c]	12.10 ± 1.11[b,c]
MDA (nmol/mg protein)	3.10 ± 0.29	7.65 ± 0.51[a]	9.95 ± 0.86[a,b]	4.23 ± 0.43[b,c]	3.61 ± 0.32[b,c]

Values are expressed as mean ± SEM (n = 6). Rats were treated with single oral dose of CAN (38 mg/kg) and sacrificed at various times as indicated. Control rats received only solvent (values at time zero). a: significantly different from control group; b: significantly different from group sacrificed after 2 h of treatment. c: significantly different from group sacrificed after 4 h of treatment at $P \leq 0.05$ using one-way ANOVA with Tukey-Kramer multiple comparison test.

Table 2: Effect of various doses of CAN on GSH, GST, GSHpx, SOD and MDA levels in hepatic tissue of rats

Parameters	Doses of CAN (mg/kg bwt)				
	Control	9	19	38	76
GSH (µg/g liver tissue)	16.10 ± 1.06	15.54 ± 0.69	12.00 ± 0.89[a,b]	8.00 ± 0.71[a,b,c]	5.00 ± 0.19[a,b,c]
GST (nmol/min/mg protein)	656.33 ± 19.59	642.83± 26.59	499.83 ± 9.47[a,b]	393.83±13.31[a,b,c]	249.67±12.90[a,b,c,d]
GSHpx (nmol/ min/mg protein)	1192.50±76.47	1196.67±75.23	1049.83±50.78[a,b]	797.00±32.36[a,b,c]	522.67±33.75[a,b,c,d]
SOD (U/mg protein)	11.75 ± 1.20	10.87 ± 1.02	8.50 ± 0.38[a]	5.68 ± 0.47[a,b]	4.63 ± 0.41[a,b,c]
MDA (nmol/mg protein)	3.20 ± 0.11	3.61 ± 0.32	6.50 ± 0.10[a,b]	8.00 ± 0.68[a,b]	12.07±1.07[a,b,c,d]

All values represent the mean ± SEM (n = 6). Animals were treated orally with a single dose of CAN and killed 2h after treatment. Control animals received only solvent. a: significantly different from control group; b: significantly different from group treated with 9 mg/kg of CAN; c: significantly different from group treated with 19 mg/kg of CAN; d: significantly different from group treated with 38 mg/kg of CAN at $P \leq 0.05$ using one-way ANOVA with Tukey-Kramer multiple comparison test.

elevation of the plasma levels of IL-6, TNF-α and IL-10 (713.8 pg/ml, 1.9 ng/ml and 82.0 pg/ml, respectively) was identified at 4 h after CAN treatment compared to control group and rebound to control level by 6 h (fig. 1).

In dose-response study rats were treated with a different dose of CAN and killed 2 h later. Fig. 2 indicated that there is a dose-response relationship in CAN-induced elevation in plasma levels of IL-6, TNF-α and IL-10. Treatment of rats with a single oral dose of CAN (19, 38 or 76 mg/mg) significantly elevated plasma levels of IL-6, TNF-α and IL-10. Increasing CAN doses (76 mg/kg) induced further increase in plasma levels of IL-6, TNF-α and IL-10 (680.3 pg/ml, 2.8 ng/ml, 80.1 pg/ml respectively). Moreover, CAN (76 mg/kg), significantly elevated plasma levels of IL-6, TNF-α and IL-10 compared to control and groups treated with doses 9, 19 and 38 mg/kg of CAN. While CAN at 9 mg/kg insignificantly affected plasma levels of inflammatory cytokines.

Effect of CAN on liver function tests in rats

Table 3 revealed that serum activities of AST, ALT and total bilirubin were significantly increased at 2 h (212%, 226% and 249% respectively) and at 6 h (165%, 157% and 166% respectively) after CAN (38 mg/kg) administration compared to control group. The maximal elevation in the serum levels of AST (266%), ALT (290%) and total bilirubin (386%) was observed at 4 h after CAN treatment of the control levels. Interestingly, liver function testes returned to normal values after 12 h, while plasma cytokines and hepatic parameters at 6 h after CAN administration.

CAN treatment significantly increased serum levels of AST, ALT and total bilirubin in a dose dependant manner. CAN at dose 19 mg/kg caused a 72%, 88% and 66% increase in serum levels of AST, ALT and total bilirubin respectively, while it insignificantly altered these parameters at dose 9 mg/kg. Furthermore, 38 mg/kg of CAN caused an increase in AST (119%), ALT (126%) and total bilirubin (155%); while maximal elevation in serum levels of AST (224%), ALT (213%) and total bilirubin (407%) was observed at dose 76 mg/kg (table 4).

Table 3: Time-course study for the effect of CAN on the serum levels of AST, ALT and total bilirubin in rats

Serum Parameters	Time (h)				
	Control	2	4	6	12
AST (IU/L)	41.83 ± 3.79	88.67 ± 4.69[a]	111.33±5.63[a,b]	69.17±3.38[a,b,c]	46.33±3.78[b,c,d]
ALT IU/L)	55.17 ± 5.82	125.00 ± 9.26[a]	160.17±8.38[a,b]	86.67±5.10[a,b,c]	57.67±5.08[b,c,d]
Total Bilirubin mg/dl	0.117 ± 0.005	0.292 ± 0.021[a]	0.452±0.030[a,b]	0.195±0.015[a,c]	0.138±0.010[b,c,d]

Values are expressed as mean ± SEM (n = 6). Rats were treated with single oral dose of CAN (38 mg/kg) and sacrificed at various times as indicated. Control rats received only solvent (values at time zero). a: significantly different from control group; b: significantly different from group sacrificed after 2 h of treatment. c: significantly different from group sacrificed after 4 h of treatment; d: significantly different from group sacrificed after 6h of treatment at $P \leq 0.05$ using one-way ANOVA with Tukey-Kramer multiple comparison test.

Table 4: Effect of various doses of CAN on serum levels of AST, ALT and total bilirubin in rats

Serum Parameters	Doses of CAN (mg/kg bwt)				
	Control	9	19	38	76
AST (IU/L)	41.00 ± 3.74	43.83 ± 2.89	70.50 ± 4.99[a,b]	89.87±4.44[a,b,c]	132.83±5.28[a,b,c,d]
ALT (IU/L)	54.33 ± 5.44	68.33 ± 4.26	102.17±7.92[a,b]	122.83±9.71[a,b]	170.00±3.94[a,b,c,d]
Total Bilirubin (mg/dl)	0.115 ± 0.005	0.128 ± 0.008	0.191 ± 0.004[a]	0.294±0.025[a,b,c]	0.583±0.009[a,b,c,d]

All values represent the mean ± SEM (n = 6). Animals were treated orally with a single oral dose of CAN and killed 2h after treatment. Control animals received only solvent. a: significantly different from control group; b: significantly different from group treated with 9 mg/kg of CAN; c: significantly different from group treated with 19 mg/kg of CAN; d: significantly different from group treated with 38 mg/kg of CAN at $P \leq 0.05$ using one-way ANOVA with Tukey-Kramer multiple comparison test.

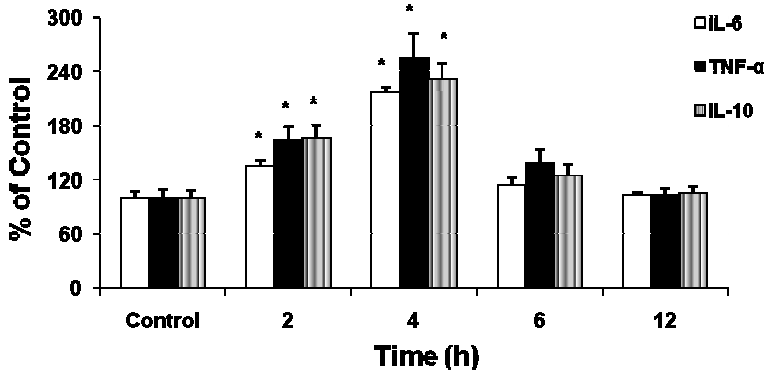

Fig. 1: Time-course study for the effect of CAN on the plasma levels of Il-6, TNF-α and IL-10 in rats.
Values are expressed as mean ± SEM (n = 6). Rats were treated with single oral dose of CAN (38 mg/kg) and killed at indicated time after treatment. Control rats received only solvent (values at time zero). * significantly different from control ($P \leq 0.05$, using Student's *t*-test) for IL-6, TNF-α and IL-10 respectively.

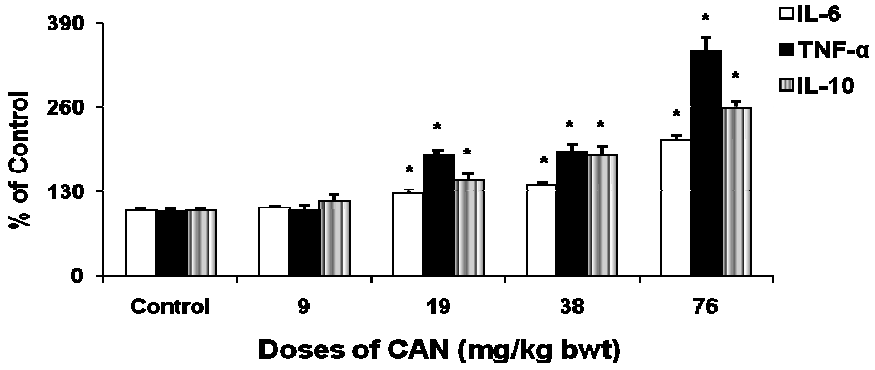

Fig. 2: Effect of various doses of CAN on the plasma levels of Il-6, TNF-α and IL-10 in rats.
Values are expressed as mean ± SEM (n = 6). Rats were treated with single oral dose of CAN (9, 19, 38 and 76 mg/kg) and killed at 2 h after treatment. Control rats received only solvent (values at time zero). * significantly different from control ($P \leq 0.005$, using Student's *t*-test) for IL-6, TNF-α and IL-10 respectively.

Histological examination of rat liver after short-term treatment with CAN

Histopathological examination showed normal histological structure in the control group. In the dose-response study, the histopathological changes increased in a dose dependent manner. In the time-course study, maximal tissue degeneration was observed in groups treated with 38 mg/kg and killed at 2 h and 4 h later, while groups killed at 6 and 12 h later had no histpathological modification (non-showed photo). Histopathological changes such as; fatty change, congestion in the central vein, inflammatory cell infiltration, fibrosis, cystic bile ducts in the portal area, apoptosis and necrosis of hepatocytes (fig. 3).

DISCUSSION

HAN has been shown to possess a potential carcinogenic, teratogenic and embryotoxic properties (IARC, 1979; Lipscomb *et al*., 2009). Oxidative bioactivation of HAN, such as CAN by biological reactions that utilize reactive oxygen intermediate (ROI) (Ahmed *et al*., 1999). Interactions of CAN or its metabolites with animal tissues induce several adverse effects (Ahmed *et al*., 1999; Ahmed *et al*., 2005; Abdel-Naim *et al*., 2009; Lipscomb *et al*., 2009). In the present study, CAN was selected as a model compound for HAN, because it was reported that it has the highest acute toxicities and highly conjugates with GSH (Lipscomb *et al*., 2009). The present study was aimed to investigate the potential hepatotoxicity of CAN

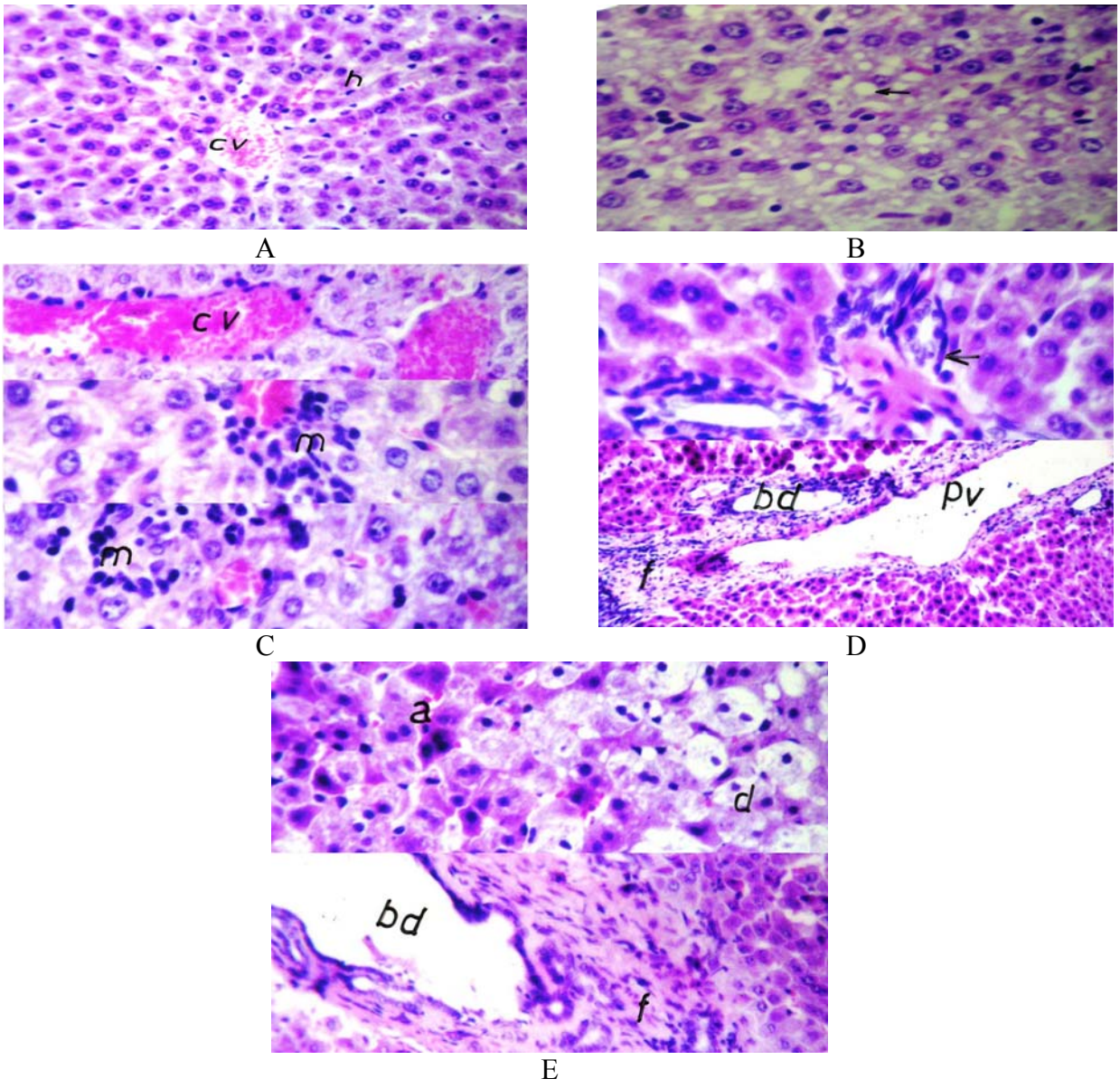

Fig. 3: Light microscopy of rat liver after short-term treatment with CAN.

and the possible mechanisms underlying its toxicity. This was achieved by assessment of hepatic oxidant and antioxidant as well as plasma cytokines, serum aminotransferases and total bilirubin using dose-response and time-course experiments. In the dose-response study, CAN at doses 19, 38 and 76 mg/kg significantly induced hepatic injury at 2 h after administration. Based on these data, a dose of 38 mg/kg was chosen for the time-course study.

The present findings demonstrated that increased hepatic MDA an end product of lipid peroxidation and decreased antioxidative enzyme activities (GST, GSHpx and SOD) as well as non-enzymatic antioxidant (GSH) content in animals treated with CAN. Our data is agree with other study reported that CAN-induced changes in lipid peroxidation and antioxidant profiles in rats (Abdel-Naim *et al*., 2009). These results illustrate the protective role of GSH and the antioxidant enzymes (GST, GSHpx and SOD) against oxidative damage in hepatic tissue. Chassaud, (1979) reported that GSH and the antioxidant enzymes represents the protective mechanism of the cell against electrophilic, carcinogenic or necrotic agents. Depletion of the GSH may be through one or more of the following: a) an increased rate of GSH conjugation (catalyzed by GST); b) an increased rate of GSH oxidation (catalyzed by GSHpx); c) a decreased rate of GSH biosynthesis (Chassaud, 1979). Our findings suggested that GSH depletion is due to conjugation of GSH with CAN or its metabolites. Previous study indicated that HAN group (such as CAN) producing an inhibition of rat hepatic and brain GST, GSHpx and SOD activities as well as depletion of GSH and increase in lipid peroxidation (Abdel-Naim *et al*., 2009). In view of the current and previous results, CAN-induced liver damage through the immediate and/or prolong inhibition of GST, GSHpx, SOD and GSH defense mechanism concomitant with an increased of the MDA in the liver (Abdel-Naim *et al*., 2009).

The possibility of involvement of the pro-inflammatory cytokines as a mechanism of CAN-induced hepatotoxicity was studied. Plasma levels of IL-6, TNF-α and IL-10 were evaluated as a biomarker for such hepatic toxicity following different doses of CAN. Data of the present study indicated that plasma cytokines significantly increased at 2 h after CAN administration in a dose dependent manner. This results is in harmony with previous results illustrates that pro-inflammatory cytokines (Il-6, TNF-α & IL-10) are increased in rats exposed to HAN and under oxidative stress (OS) (Ahmed *et al*., 2000). Moreover, HAN induced OS, and produced ROI (superoxide anion, H_2O_2 and $^{\cdot}OH$) and inflammatory cytokines resulting in DNA degradation, cellular apoptosis and necrosis (Ahmed *et al*., 2000). Also, our results are parallel with a pervious study which reported that cycloheximide increased expression of mRNA of pro-inflammatory cytokines in the liver (IL-6, TNF-α and IL-1B) and an anti-inflammatory cytokine (IL-10) either before or concomitant with the induction of apoptosis (Ito *et al*. 2006). Previous data have implicated molecular mediators of the immune response such as TNF-α and IL-6 in acute and chronic liver damage induced by acetaminophen and phenobarbitone (Lacour *et al*., 2005). IL-10 can act as a pro-inflammatory or anti-inflammatory cytokine. Law *et al*. (2002) reported that IL-10 administration induced activation of the cytotoxic natural killer cells and T-lymphocytes in human previously injected with lipopolysacharide. While, Schopf *et al*., (2002) reported that IL-10 treatment inhibited lymphocyte activation-induced apoptosis by induction of the anti-apoptotic factor Bcl2 proteins in human. Further, anti-IL-10 increased lymphocytes apoptosis (Ismail *et al*., 2006) as well as increased liver apoptosis induced by lipopolysaccharide in IL-10 knockout mice (Zhong *et al*., 2006). IL-10 produced by macrophages enhanced intracellular bacterial growth through inhibition macrophage activity (de Waal Malefyt *et al*., 1991). Furthermore, TNF-α induced intracellular ROI by inhibition of cellular mitochondrial electron transport system (Larrick and Wright, 1990; Zhang *et al*., 1990), cellular injury (Imanishi *et al*., 1997) and oxidative damage in various cells (Zimmerman *et al*., 1989). Mitochondria are the main source of ROI and the key target sites for ROI activity. GSH is the main defense mechanism against ROI for mitochondria (and other organelles) and the cell proliferation can be shifted to apoptosis and/or necrosis by increasing intracellular levels of ROI (Buttke and Sandstorm, 1994).

Biochemical liver function tests related to the hepatocellular integrity were checked to assess liver injury. The obtained results indicated that CAN significantly elevated serum levels of AST, ALT and total bilirubin at doses of 19, 38 and 76 mg/kg after 2 h of treatment. Furthermore, CAN (38 mg/kg) significantly elevated AST, ALT and total bilirubiin at 2, 4 and 6 h after administration. These results are in agreement with previous findings which showed that aminotrnsferases enzymes and bilirubin were elevated in rats treated with chemicals or drugs-induced hepatotoxicity (Kebieche *et al.,* 2008; Abo-Salem *et al*., 2011). These parameters have been reported to be sensitive indicators of liver injury (Recnage, 1983). Histopathological examination confirmed the biochemical results in which the histopathological changes increased in a dose dependent manner and returned to nearly normal by increasing the time after treatment.

CONCLUSION

The current work demonstrated that the cellular defense mechanisms (GSH), the antioxidant enzymes (GST, GSHpx and SOD) and lipid peroxidation, as well as pro-

inflammatory cytokines (IL-6, TNF-α and IL-10) are significantly altered by CAN in liver tissues of rats. CAN-induced hepatotoxicity may be due to disturbance in the intracellular redox status, induction of the oxidative damage and pro-inflammatory cytokines.

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