Chloroacetonitrile-Induced Cytotoxicity and Oxidative Stress in Isolated Rat Hepatocytes

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Abstract: Chloroacetonitrile (CAN) is a disinfectant by-product of drinking water chlorination. The present work was designed to investigate the cytotoxic effects as well as the oxidative stress induced by CAN in isolated rat hepatocytes. Hepatocytes were exposed to different concentrations of CAN (5–40 μM) in a time-course experiment for up to 2 h. CAN exposure induced a significant decrease in cell viability and a significant increase in the leakage of hepatic enzymes in a concentration and time-related manner. In addition, CAN exposure results in a significant decrease in cellular GSH content as well as a significant enhancement of TBARS accumulation in a concentration and time-related manners. Also, a subsequent experiment was designed to evaluate the role of GSH modulation and oxidative stress in CAN toxicity in hepatocytes at 2 h. Pretreatment with the GSH-depleting agents enhanced the cytotoxicity of CAN. Conversely, pretreatment with GSH or sulfhydryl compounds attenuated CAN toxicity. Similarly, co-incubation with enzymatic antioxidants, or iron chelator, or hydroxyl radical scavengers exhibited significant protection against CAN cytotoxicity. In conclusion, our results suggest that CAN has a potential cytotoxic effect in isolated rat hepatocytes; and GSH modulation can play a critical role against CAN-induced cellular damage.
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1. Introduction:

Haloacetonitriles (HAN) have been identified in the environment as by-products of the chlorination of ground and surface water for disinfection of drinking-water supplies (Oliver, 1983; IARC, 1991). The only known route of environmental release of HAN is as a constituent of potable water supplies (IARC, 1991). However, residual chlorine in drinking water could also result in formation of HAN *in vivo* following consumption of chlorinated water (Mink *et al*, 1983; Mark *et al*., 2007). HAN is used as insecticides for stored grains (Cotton and Walkden, 1968), and as common laboratory chemicals (Barcelo *et al*., 1987).

An association between exposure to chlorinated water and the occurrence of gastrointestinal cancers has been reported in epidemiological studies (Flaten, 1992). Previous reports have indicated that levels of HAN in drinking water should be lower than those currently proposed by WHO (1994). It has been reported that HAN is metabolized *in vivo* to cyanide ion (CN^-), which is subsequently excreted in urine as thiocyanate (Silver *et al*., 1982; Tanii and Hashimoto, 1984). Animal studies indicate that HAN causes GSH depletion and inhibits glutathione-*S*-transferase (GST) in liver and gastrointestinal tract (Ahmed *et al*., 1991).

Chloroactonitrile (CAN, $Cl\text{-}CH_2\text{-}CN$), a member of the HAN group, possesses a chlorine substitution on the alpha carbon atom of the acetonitrile molecule. The potential mutagenic and carcinogenic activity of CAN was previously reported (IARC, 1991). It was shown that CAN increases the unscheduled DNA synthesis in cultured embryos after its maternal administration to animals (Shouman *et al*., 1993). Furthermore, CAN induced sister chromatid exchange in Chinese hamster ovary (CHO) cells and DNA strand breaks in human lymphoblast cell lines (Bull *et al*., 1985). Also, CAN showed mutagenic activity on Salmonella typhimurium strain TA100 and exhibited a clastogenic effect on the peripheral blood erythrocytes of Pleurodeles waltl larvae (Le Curieux *et al*., 1995). Also, CAN was shown to be mutagenic in the Ames fluctuation test and induced DNA damage in HeLa S3 cells (Muller-Pillet *et al*., 2000). Maternal exposure to CAN adversely affects mouse fetal livers as evidenced by the induction of oxidative

stress, apoptosis and histopathological changes (Abdel-Naim *et al*., 2009). CAN was shown to be toxic to rat gastric epithelial cells in vitro (Mohamadin and Abdel-Naim, 1999) and quantitative whole body autoradiographic studies in mice indicated that the gastrointestinal tract is a potential target site of CAN toxicity (Ahmed *et al*., 1991; Jacob *et al*., 1998).

Although, the exact mechanism of CAN toxicity is not fully explored (Abdel-Naim and Mohamadin, 2004). Free radicals derived from chemicals have been implicated in liver injury (Farber *et al*., 1990).

The objective of the present study was to investigate the potential cytotoxic effects and the oxidative stress induced by CAN in isolated rat hepatocytes as well as the role of glutathione modulation and free radical scavengers on CAN toxicity.

2. Materials and methods

1-Chemicals:

Bovine albumin, buthionine sulfoximine (BSO), bis(chloroethyl)-nitrosurea (BCNU), chloroactonitrile (CAN), chlorodinitrobenzene (CDNB), catalase (CAT), collagenase (type IV), cysteine (CYS), desferrioxamine (DFO), dimethylsulfoxide (DMSO), dithiothreitol (DTT), GSH, methionine (MT), superoxide dismutase (SOD), thiobarbituric acid (TBA) and tritonX-100 were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). All other chemicals were of the highest purity grade.

2-Animals:

Adult male Sprague Dawley rats (225-250 g) were obtained from the animal facility of Faculty of Veterinary Medicine, Cairo University, Egypt. Rats were housed in controlled- environment rooms, three per cage and were maintained on a 12 hour light/dark cycle at constant temperature (23-25 ^{0}C) and humidity (50-55%). Animals were fed standard laboratory chow (El-Nasr Co., Abo-Zaable, Egypt) and tap water *ad libitum* and quarantined for one week prior to use. They were maintained in accordance with NIH guidelines for the care and use of laboratory animals.

3-1 Isolation of Hepatocytes

Hepatocytes were isolated using a collagenase two-step perfusion technique as described by Berry and Friend (1969) with slight modifications as published by El-Tawil and Abdel-Rahman, (1997). The isolated liver cells were filtered through four layer of cotton gauze and centrifuged for two minutes at 600 rpm's. The cells were washed twice and suspended in HEPES-bicarbonate buffer (pH 7.4) containing 0.5% bovine albumin. All buffers were bubbled with carbogen (95% O_2, 5% CO_2) prior to use. The isolated hepatocytes were counted in a hemocytometer, while the viability of the cells was assessed by 0.4% trypan blue exclusion test (Baur *et al*., 1985). Freshly prepared cell suspension had 90% or greater viability prior to each experiment.

3-2 Incubation and treatment of hepatocytes

Freshly isolated hepatocytes (5 X 10^6 cells/ ml) were suspended in a HEPES-bicarbonate buffer (pH 7.4) and incubated at 37^{0}C in a shaking water bath at 30 oscillations per minute. Hepatocytes were incubated in plastic vials equipped with covers.

4-Two main experiments were performed:-

The first experiment involved determination of CAN cytotoxicity in isolated rat hepatocytes in a concentration- response as well as a time-course experiment. Different concentrations of CAN (5, 10, 20 and 40 µM) incubated with isolated hepatocytes at different time intervals (30, 60, and 120 min). Twelve replicates were used for each concentration. Cytotoxicity was determined by assessing of cell viability using trypan blue exclusion method, enzymes leakages percent {alanine aminotransferase (ALT), asparate aminotransferase (AST) and lactate dehydrogenase (LDH)}, GSH content and thiobarbituric acid reactive substances (TBARS) accumulation. Control replicates were carried out simultaneously under the same conditions and at the same time intervals.

The second experiment was designed to substantiate the role of oxidative stress in CAN (10 µM) toxicity in hepatocytes at 2 h. LDH release and TBARS generations were assessed in cultured hepatocytes as indicators of CAN–induced cytotoxicity and oxidative stress. The oxidative status of the cultured hepatocytes was enhanced by using SOD (100 U/ml), CAT (100 U/ml, DFO (20 mM), and dimethylsulfoxide (DMSO) (100 µM). GSH and non-protein sulfhydryl content was boosted using MT (0.5 mM), CYS (0.5 mM) and DTT (0.5 mM). On the other hand, GSH was depleted by using BCNU (0.5 mM), BSO (5 mM) and CDNB (0.25 mM). All the modulating chemicals were added 30 min before the addition of CAN except DFO, which was added 60 min before exposure to CAN. Concentrations as well as time of incubation of the aforementioned modulating chemicals were consistent with those published in the literature (Adamson and Harman, 1993; Wu *et al*., 1996 and

1997). Control replicates were carried out simultaneously under the same conditions.

5-Sample preparation for enzyme leakage :

Enzymes activity (ALT), (AST) and (LDH) was monitored using Sigma–Aldrich ready made kits (Sigma Chemical Co., St. Louis, MO, USA) in an aliquot of cell-free medium and compared to the total activity achieved after lyses of the cells (Moldeus *et al*., 1978). The cell-free medium was obtained by centrifugation of the aliquots at 2200 rpm's for 15 min. Lysate was obtained by addition of 1% triton X-100 and shaking for 15 min followed by centrifugation at 2200 rpm's. The leakage was expressed as percentage of total lysate activity at each time point.

6-Glutathione (GSH) assay

Reduced GSH levels in hepatocytes were determined by measuring total soluble-reduced sulfhydryl content. Aliquots were collected at specified time points and centrifuged with phosphate buffer saline (PBS) at 3000 g for 5 min. The obtained precipitate was mixed with 0.7 ml of 0.2% triton X-100 and 2.5% sulfosalicylic acid. Solutions were centrifuged at 3000 g for 5 min. A 0.5 ml aliquot of the acid-soluble supernatant medium was then added to 1.0 ml of 0.3 M Na2HPO4 solution. Spectrophotometric determinations were performed at 412 nm immediately after the addition of 0.125 ml of 5,5`-dithiobis-(2-nitrobenzoic acid) (Beutler *et al*., 1963).

7-Lipid peroxidation assay

Lipid peroxidation was assessed by determining thiobarbituric acid reactive substances (TBARS) in hepatocyte culture media by the method of Uchiyama and Mihara (1978). Protein concentration was determined by the method of Lowry *et al*. (1951) using bovine serum albumin as standard.

8-Data Analysis

The GRAPHPAD (ISI Software, Philadelphia, PA, USA) computer program was used to conduct regression analysis and to plot collected data. Data were expressed as means ± standard error of means (SEM). Assessment of the results was performed using one-way analysis of variance (ANOVA) procedure followed by Tukey-Kramer multiple comparison post-tests. Statistical analysis were performed using Software GRAPHPAD INSTAT (Version 2). The 0.05 level of probability was used as the criterion for significance.

3. Results

Assessment of CAN cytotoxicity

Cell survival was assessed by trypan blue exclusion method after exposing rat hepatocytes to different concentrations of CAN (5–40 μM). Incubation of hepatocytes for 120 min with CAN showed significant decreases ($p<0.05$) in cell viability in a concentration and time-related manner (Fig.1). The decreased in cell viability started as early as 30 min after incubation with the highest concentrations (10, 20 and 40 μM) and reached the maximum cytotoxicity at 120 min. However, exposing hepatocytes to of 5 μM caused a significant decrease($p<0.05$) in cell viability at 60 min and 120 min of incubation.

Plasma membrane damage was assessed by monitoring LDH, ALT and AST enzyme leakages from intracellular compartment of hepatocytes into the extracellular space. Incubation of hepatocytes with different concentrations of CAN (5–40 μM) for 120 min showed a significant increase($p<0.05$) in LDH leakage compared to the control incubations. This effect was concentration and time-dependent (Fig. 2). Throughout the time course studied, a significant increase in LDH leakage was observed after incubation with the concentrations 10, 20 and 40 μM. However, the significant effect of concentrations 5 μM on LDH leakage started to appear at 120 min of incubation.

The data in Fig (3) illustrate the time course of ALT leakage from hepatocytes exposed to CAN (5- 40 μM). Incubation of hepatocytes with the highest concentrations of CAN (10, 20, and 40 μM) caused significant increase($p<0.05$) in ALT leakage during all the time points studied. However, the 5 μM concentration significantly increased ALT leakage only at 120 min of incubation. Elevation in AST leakage has been also associated with hepatocellular injury. The effect of CAN on AST leakage from hepatocytes is illustrated in Fig (4). As early as 30 min of incubation with 40 μM CAN a significant increase ($p<0.05$) in AST leakage was observed. This effect continued throughout the experiment. The concentrations of 10 μM and 20 μM significantly increased AST leakage at 60 min and 120 min. However, the 5 μM concentrations had significant effect ($p<0.05$) on AST leakage only at 120 min of incubation.

Assessment of oxidative stress-induced by CAN

Incubating hepatocytes in the presence of different CAN concentrations (5–40 μM) resulted in an observable concentration and time-dependent loss of cellular GSH (Fig. 5). In the time–course experiment, cellular GSH levels were significantly depleted after incubation with all concentrations at all

time intervals compared to the corresponding control values. GSH depletion was maximal at 120 min after CAN addition.

The effect of various concentrations of CAN (5–40 μM) on lipid peroxidation, as indicated by TBARS formation, was estimated. Fig (6) shows a significant ($p<0.05$) and concentration –related increase of TBARS production in hepatocytes as compared with the control value. In the time–course experiment, CAN (10, 20 and 40 μM) resulted in a significant increase in the production of TBARS in hepatocytes, which occurred early at 30 min of incubation and reached its maximum level after 120 min. However, the 5μM CAN concentrations induced significant increase (0.05) in the production of TBARS only at 60 min and 120 min of incubation. From all the studied parameters, it was observed that the effects of CAN at a concentration of 10 μM at 2 h of incubation were significantly different from corresponding control and were not maximal. In addition, a period of 2 h was practically convenient. Therefore, subsequent mechanistic experiments were performed using the submaximal CAN concentration (10 μM) and 120 min incubation time point.

Assessment of potential protective effects of different antioxidants

The protective effects of different thiol-containing compounds on CAN -induced LDH release and lipid peroxidation in hepatocytes are illustrated in Table (1). All of the tested compounds (GSH, MT, NAC and DTT) could significantly reduce LDH release by about 29, 20, 37 and 34 %, respectively, as compared with CAN alone-treated incubations. Similar protective effects offered by these compounds were also observed on CAN -induced TBARS production (33, 50, 57 and 37 % for GSH, MT, NAC and DTT, respectively). However, pretreatment with thiol-containing compounds did not restore the basal levels of LDH release or lipid peroxidation. On the other hand, the GSH-depleting agents BSO, CDNB and BCNU significantly ($p<0.05$) enhanced CAN cytotoxicity by increasing LDH release by 140, 129 and 155 % respectively. The TBARS generation was also elevated by about 153, 169 and 184% respectively compared to the CAN alone incubations (Table 2).

The potential protective effects of antioxidant enzymes (SOD and CAT) as well as the hydroxyl radical scavenger DMSO and the iron chelator DFO against LDH leakage and lipid peroxidation in hepatocytes exposed to 10 mM CAN for 120 min were also evaluated (Table 3). It was found that pretreatment of hepatocytes with either SOD (100 U/ml) or CAT (100 U/ml) significantly ($p<0.05$) inhibited LDH leakage by approximately 25 and 34 %, respectively as compared with CAN alone-treated cells. Also, both of two antioxidant enzymes significantly diminished the TBARS production-induced by CAN by 30 and 49%, respectively. Pretreatment of hepatocytes with DMSO or DFO diminished CAN -induced LDH leakage by 20 and 30% of total leakage, respectively, and TBARS production by 25 and 34%, respectively. However, none of the used protectors could restore LDH leakage or TBARS to control values.

Table (1): Effects of thiol-containing compounds on CAN-induced LDH leakage and lipid peroxidation in isolated rat hepatocytes

Addition	LDH leakage (% of total)	TBARS (nmol/mg protein)
None (control)	24.8 ± 1.40	0.62 ± 0.04
CAN (10 μM)	56.4 ± 3.80 [a]	2.79 ± 0.13 [a]
CAN + GSH (0.5 mM)	40.1 ± 2.10 [a, b]	1.88 ± 0.09 [a, b]
CAN + MT (0.5 mM	45.3 ± 3.20 [a, b]	1.40 ± 0.10 [a, b]
CAN + NAC (0.5 mM	35.8 ± 1.80 [a, b]	1.21 ± 0.09 [a, b]
CAN + DTT (0.5 mM)	37.2 ± 2.70 [a, b]	1.76 ± 0.14 [a, b]

- Data are presented as mean ± SEM of twelve replicates.
- GSH, reduced glutathione; MT, methionine; NAC, N-acetyl-L cystiene; DTT, dithiothreitol. All thiol compounds were added 30min before the addition of CAN.
- LDH and TBARS were determined 120min after the addition of CAN.
(a) Significantly different from corresponding control group at $p < 0.05$
(b) Significantly different from CAN alone-treated group at $p < 0.05$.

Table (2): Effects of GSH depleting agents on CAN-induced LDH leakage and Lipid peroxidation in isolated rat hepatocytes

Addition	LDH leakage (% of total)	TBARS (nmol/mg protein)
None (control)	24.8 ± 1.40	0.62 ± 0.04
CAN (10 µM)	56.4 ± 3.80 [a]	2.79 ± 0.13 [a]
CAN + BCNU (0.5 mM)	79.1 ± 4.20 [a, b]	4.28 ± 2.80 [a, b]
CAN + BSO (5 mM)	72.8 ± 3.80 [a, b]	4.73 ± 3.20 [a, b]
CAN + CDNB (0.25 mM)	87.4 ± 5.10 [a, b]	5.12 ± 3.80 [a, b]

- Data are presented as mean ± SEM of twelve replicates.
- BCNU, bis(chloroethyl)-nitrosurea; BSO, buthionine sulfoximine; CDNB, chlorodinitrobenzene. All compounds were added 30 min before the addition of CAN.
- LDH and TBARS were determined 120min after the addition of CAN.
(a) Significantly different from corresponding control group at $p < 0.05$
(b) Significantly different from CAN alone-treated group at $p < 0.05$.

Table (3): Effects of SOD, CAT, DMSO and DFO on CAN-induced LDH leakage and lipid peroxidation in isolated rat hepatocytes

Addition	LDH leakage (% of total)	TBARS (nmol/mg protein)
None (control)	24.8 ± 1.40	0.62 ± 0.04
CAN (10 µM)	56.4 ± 3.80 [a]	2.79 ± 0.13 [a]
CAN + SOD (100 U/ml)	42.4 ± 3.40 [a, b]	1.95 ± 0.11 [a, b]
CAN + CAT (100 U/ml)	37.2 ± 2.40 [a, b]	1.42 ± 0.11 [a, b]
CAN + DMSO (100 µM)	45.1 ± 3.20 [a, b]	2.10 ± 0.16 [a, b]
CAN + DFO (20 mM)	39.6 ± 2.80 [a, b]	1.84 ± 0.12 [a, b]

- Data are presented as mean ± SEM of twelve replicates.
- SOD, superoxide dismutase; CAT, catalase; DMSO, dimethyl sulfoxide; DFO, desferrioxamine. All antioxidants were added 30 min before the addition of CAN.
- LDH and TBARS were determined 120min after the addition of CAN.
(a) Significantly different from corresponding control group at $p < 0.05$
(b) Significantly different from CAN alone-treated group at $p < 0.05$

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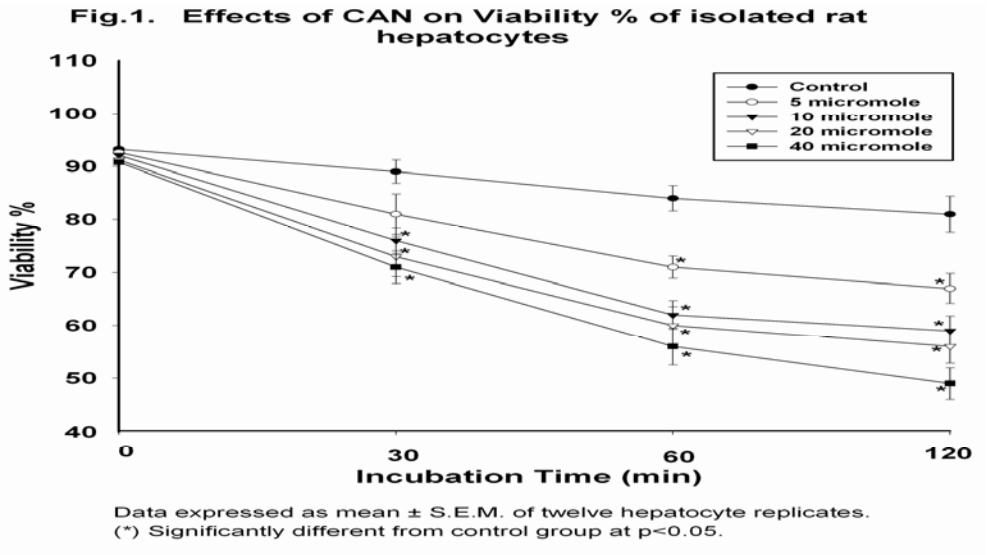
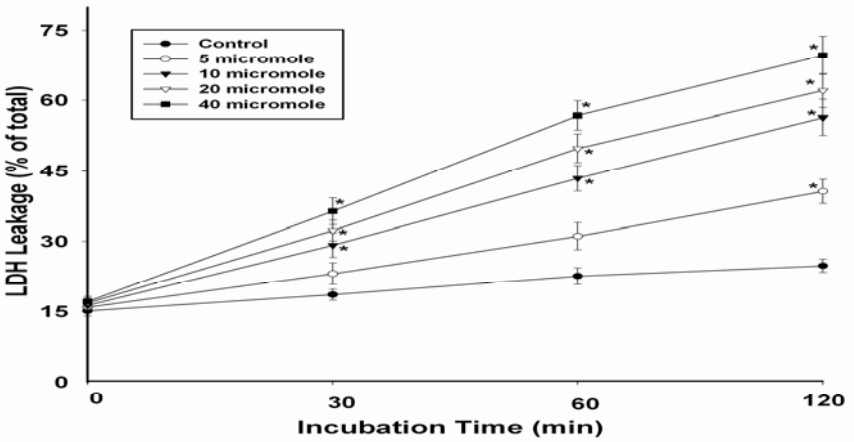
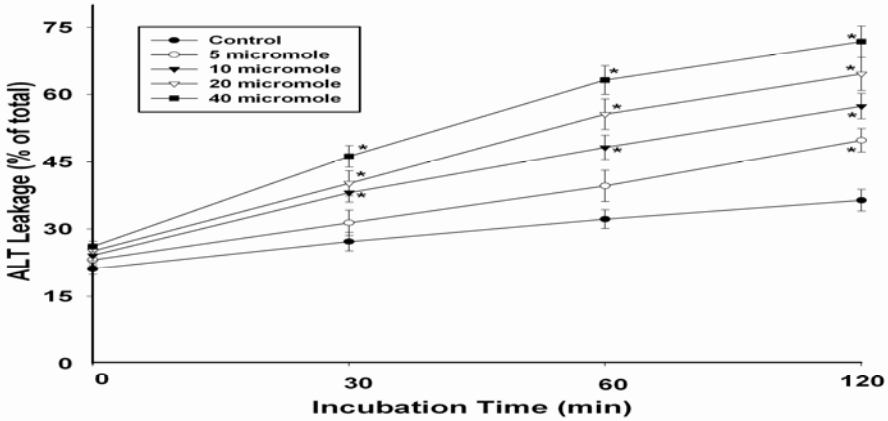


Fig.2. Effects of CAN on LDH leakage % of isolated rat hepatocytes



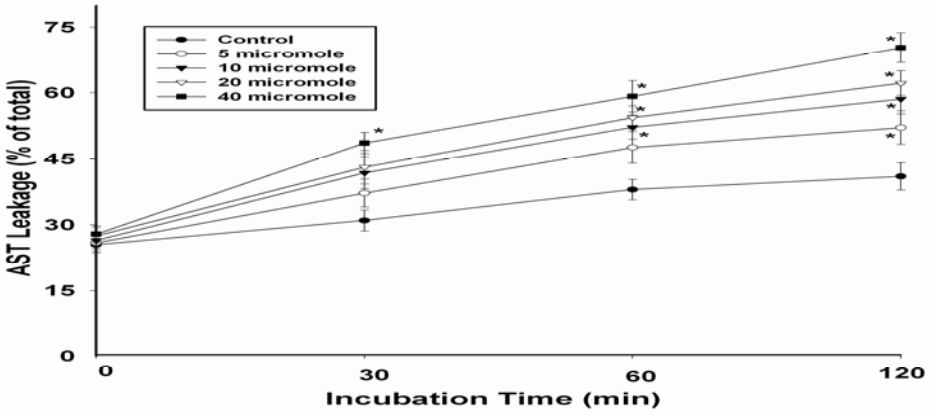
Data expressed as mean ± S.E.M. of twelve hepatocyte replicates.
(*) Significantly different from control group at p<0.05.

Fig.3. Effects of CAN on ALT leakage % of isolated rat hepatocytes

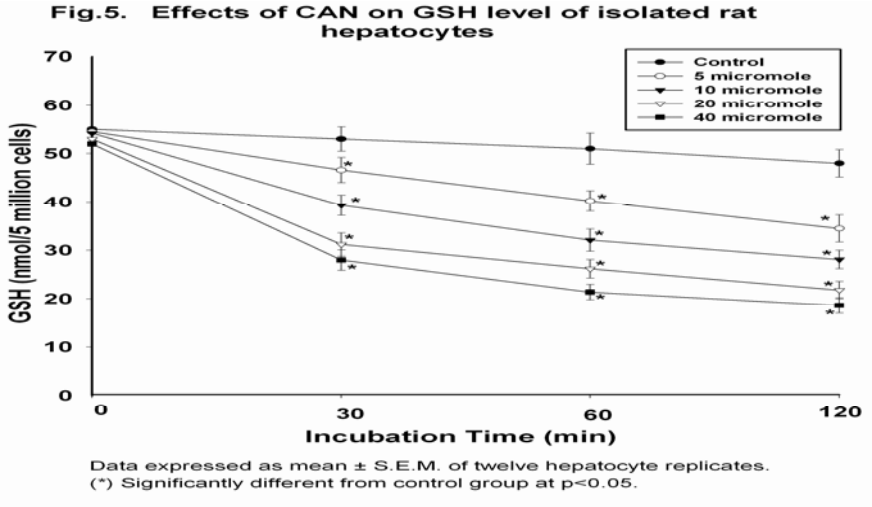


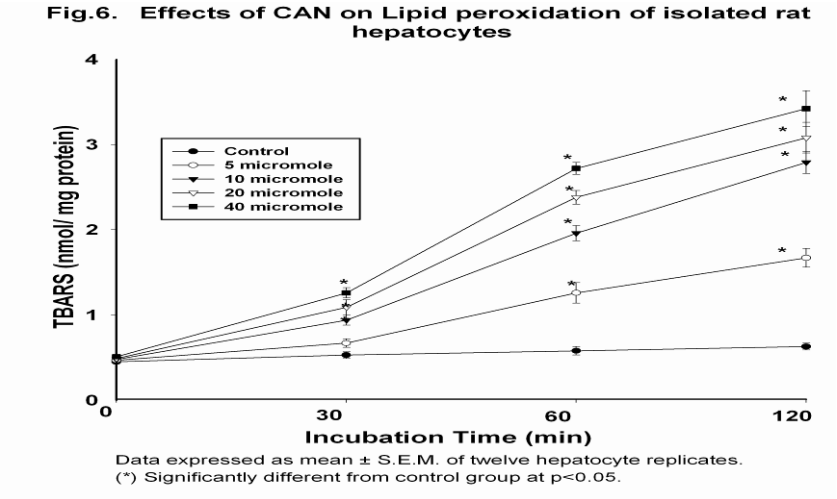
Data expressed as mean ± S.E.M. of twelve hepatocyte replicates.
(*) Significantly different from control group at p<0.05.

Fig.4. Effects of CAN on AST leakage % of isolated rat hepatocytes



Data expressed as mean ± S.E.M. of twelve hepatocyte replicates.
(*) Significantly different from control group at p<0.05.





4. Discussions:

The data presented reflect the utilization of isolated liver cells to investigate the toxic effects of CAN using different parameters. As membrane damage occurs, hepatocytes release the cytosolic enzymes into incubation media and lose the ability to exclude trypan blue. In this study, trypan blue exclusion was used to assess cell viability. Staining of the cells by trypan blue indicates severe irreversible damage and reflects the end point to evaluate the effect of CAN (Baur *et al.*, 1985). Consequently, cell damage exhibits a good correlation with enzyme leakage (Berg and Aune, 1987). ALT and LDH leakage and cell viability parameters are indices to measure the degree of the cell membrane damage produced by toxicant, while mitochondrial damage is responsible for the major portion of AST leakage (Story *et al*., 1983).

Although the gastrointestinal tract is one of the most important target organs for CAN (Farooqui and Ahmed, 1983; Jacob and Ahmed, 2003), there is a scanty of information regarding CAN hepatotoxicity. Therefore, the present study aimed to investigate the potential cytotoxic effects as well as the oxidative stress induced by CAN in isolated primary rat hepatocytes.

Our data indicated that CAN had a potent cytotoxic effect on isolated rat hepatocytes as evidenced by decreased hepatocytes viability and increased enzymes leakages. This effect was concentration and time-related. The cytotoxic effect of CAN was accompanied by observed GSH depletion and significant elevation of lipid peroxides generation as determined by TBARS formation. This finding is in agreement with the work of Ivanov and Alyshanskii (1982) who reported that the barrier and regulatory functions of the hepatocytes membrane were impaired during acute CAN poisoning. In addition, Geigar *et al*. (1983) reported that CAN deplete reduced glutathione and decreases the

viability of hepatocyte in a concentration-dependent manner.

One of our main objectives was to investigate the effect of oxidative stress and/or GSH depletion on the cytotoxicity induced by CAN in hepatocyte cultures. Glutathione is an important cellular antioxidant and plays a major role in protecting cells against oxidative stress. Several studies have shown that the rapid depletion of GSH in the liver *in vivo* (Younes and Siegers, 1981; Maellaro *et al*., 1990) and in freshly isolated hepatocytes (Stacey and Klaassen, 1982; Meredith and Reed, 1983) is associated with lipid peroxidation and cell death. To investigate the role of GSH depletion on CAN cytotoxicioty, the inhibition of GSH synthesis was accomplished by BSO, a selective inhibitor of γ-glutamylcysteine synthetase (Kera *et al*., 1989), CDNB, a glutathione-S-transferase inhibitor (Summer and Wiebel, 1981) or BCNU a GSSG reductase inhibitor (Eklow *et al*., 1981). Pretreatment of hepatocytes with BSO, CDNB or BCNU significantly potentiated the toxicity of CAN as evidenced by enhanced LDH leakage and TBARS accumulation.

Lipid peroxidation and leakage of cytosolic enzymes are markers of cellular oxidative damage initiated by ROS (Farber *et al*., 1990). Thus, factors interfering with the generation or effects of ROS are anticipated to protect against cell injury. The observed protective effects of GSH, DTT and NAC can be attributed to direct interaction with ROS, direct binding to toxic metabolites and/or enhancement of cellular GSH synthesis (Maxwell, 1995). Superoxide anion and hydrogen peroxide, as precursors of hydroxyl radical can exhibit similar deleterious effects (Maxwell, 1995). Thus, in the present study, the effectiveness of antioxidant enzymes (SOD and CAT) and the hydroxyl radical scavenger (DMSO) against CAN -induced lipid peroxidation and enzyme leakage can be explained by their ability to remove the generated hydrogen peroxide, superoxide anions and hydroxyl radicals, respectively. Superoxide anion can act as a reducing agent for ferric ions to give ferrous ions, which facilitate hydroxyl radical generation through the Fenton reaction (Ito *et al*., 1992). Therefore, it is believed that the addition of SOD or CAT reduces the availability of ferrous ions and hydrogen peroxide and so inhibits the production of hydroxyl radical. This is based on the known role of iron in the generation of free radicals and induction of oxidative damage (Halliwell and Gutteridge, 1990). Thus, depletion of intracellular iron by DFO could indirectly prevent cell damage by inhibiting the generation of hydroxyl radical through the Fenton reaction. Collectively, the protective effects afforded by the thiol-group donors, SOD, CAT, DMSO and DFO against CAN -induced cell injury highlights the role of ROS in CAN -induced oxidative damage. Our results are in agreement with the work of (Jiang *et al*., 1998) who previously confirmed the participation of oxidative stress in the cytotoxicity of CAN in mouse fetal liver (Abdel-Naim *et al*., 2009).and in other target organs as brain. Also, Ahmed *et al*. (1996) reported that CAN-induced changes in the homeostasis of tissue GSH might play a major role in the initial processes underlying CAN toxicity.

However, despite that all the examined interventions could significantly inhibit CAN -induced cytotoxicity, they failed to restore the normal level of LDH leakage or TBARS production. This suggests that, in addition to lipid peroxidation, other causes contribute to CAN -induced loss of viability.

In conclusion, exposing hepatocytes to CAN inhibit cell viability, enhances LDH leakage, depletes GSH and induces lipid peroxidation. CAN-induced oxidative stress and GSH depletion are at least partly responsible for its cytotoxicity. Thiol-group donors, antioxidant enzymes, hydroxyl radical scavengers and iron chelators can play an important role against CAN -induced hepatotoxicity.

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