Hepatoprotective Effects of Zamzam water against Carbon Tetrachloride Induced Liver Damage in Rats: Biochemical, Histopathological, and molecular Evidences.

Ashraf Saif [1], Osama M.Sarhan[2, 4], Mohamed Elmogy[3, 4], Hamed Mutwally [4]

[1]Al-Leith University College, Umm Al-Qura University, Makkah, Saudi Arabia.
[2]Department of Zoology, Faculty of Science, Fayoum University, Fayoum, Egypt.
[3] Department of Entomology, Faculty of Science, Biotechnology program, Cairo University, Giza, Egypt.
[4] Department of Biology, Faculty of Applied Science, Umm Al-Qura University, Makkah, Saudi Arabia
aaa_saif@hotmail.com

Abstract: Alkaline water have been claimed to boost heptaorotective effect. Thus, this study aimed to investigate if Zamazam water (ZW) that is similarly alkaline, can promote hepatoprotective effect. Hepatoprotective properties of ZW were investigated in a rat model of liver injury induced by carbon tetrachloride (CCl_4). ZW is alkaline natural water which makes it potentially capable of enhancing antioxidant power. In this study we investigated the efficacy of the hepatoprotective activity of ZW against carbon tetrachloride induced liver intoxication in Wister albino rats. Carbon tetrachloride was used as a hepatotoxic agent while, ZW were used as a probable hepatoprotective agent. 24 rats were divided into four main groups. Group I, served as normal control, Group II served as liver injured group treated only with CCl4, Group III served as ZW control, and Group IV served as liver overcome group, treated with CCl_4 and drinking ZW. Various biochemical parameters supported by histopathology of liver sections were studied to evaluate the hepatoprotective activity of ZW. The study was also supported for the first time in case of ZW evaluation effects by using of DNA extraction of the rats' livers to investigate the genomic DNA integrity. Results revealed that the serum biomarkers in carbon tetrachloride treated rats recorded elevated concentration indicating severe hepatic damage by carbon tetrachloride. The results of the serum biomarkers of ZW treated rats showed significant reduction indicating the effect of ZW in restoring the normal structural and functional ability of the hepatocytes. Both the molecular and histopathological result showed protective effect in the experimental model of hepatic alterations of rats, and suggests the use of ZW as a hepatoprotector agent in the diet of patients with hepatopathies.

1. Introduction

Scientists refer to alkaline, ionized water as 'negative' water, reduced water, or electrolyzed reduced water (ERW) because of the water's negative electrical charge, measured in mill volts. ERW has been shown Hepatoprotective effect against carbon tetrachloride-induced liver damage in mice (Tsai *et al*., 2009). ERW or Alkaline water significantly ameliorated the CCl_4-induced liver lesions, lowered the serum levels of hepatic enzyme markers (alanine and aspartate aminotransferases "ALT & AST") and increased the activities of Superoxide dismutase (SOD) catalase (CAT), and glutathione peroxidases (GSH-GPx) in liver. Therefore, the results of this study show that alkaline water can be proposed to protect the liver against CCl_4-induced oxidative damage in mice, and the hepatoprotective effect might be correlated with its antioxidant and free radical scavenging effect.

Alkaline water has been shown to strengthen the antioxidant capacity of animal bodies (Nassini *et al*.,

2010). Most of the work in this respect focused on alkaline water, which has been reported to reduce oxidative stress in patients with chronic renal disease (Huang *et al*., 2003) and slow the aging process for which oxidative stress has been proposed as the main contributor (Hofer *et al*., 2008). This water has also been shown to improve the glycemic control in diabetic rats by unknown mechanisms (Jin *et al*., 2006). Although the beneficial effects of alkaline water are assumed to be due to its alkaline nature, its composition in terms of minerals and trace elements may also play a role. The alkaline nature of water is associated with the rich-ness of aquifers with certain elements like magnesium on one hand and on the other hand the alkaline nature leaches certain elements from the soil or rocks through which aquifers stream (Alfadul & Khan, 2011).

The water from wells in Kingdom of Saudi Arabia (KSA) is often high in mineral contents (Challis *et al*., 1987; Al Zuhair & Khounganian, 2006; Alfadul & Khan, 2011). According to Arab

historians, the Zamzam Well one of the most famous wells in (KSA). ZW is natural water consumed by millions of Muslims worldwide because of their religious beliefs of its relief effects. Zamzam well has been in use for around 4000 years. The Zamzam well is located within the Holy Mosque at about 20 m east of the Ka'ba in Makkah (Zamzam Studies and Research Centre 2005).

The main difference between ZW and other water was in the quantity of calcium and magnesium salts, the content of these was slightly higher in ZW, but more significantly, the water contains fluorides that have an effective germicidal action.

Moreover, alkalinity of ZW and its fitting for drinking was citied in many reports (Analytical report of Zamzam water 1971; Al Zuhair & Khounganian, 2006; Alfadul & Khan, 2011).

This study was an attempt to explore if drinking of ZW, which is similarly alkaline, can promote Hepatoprotective effect. The purpose of this study was to investigate the hepatoprotective effect of ZW on hepatopathies induced by (CCl_4) intoxication in rats.

2. Material and Methods
2.1. Chemicals & Zamzam water:
Carbon tetrachloride (CCl_4) was obtained from Sigma Aldrich Chemical Co. (St. Louis, MO, USA). All other chemicals and solvents were of the highest grade commercially available. ZW samples were obtained directly from the well. The samples were neither treated with any chemical nor with procedures.

2.2. Water samples
ZW samples were obtained directly from the well. The samples were treated with UV for sterilization. ZW was then prepared in sterilized bottles (10 L) by a specialized factory. The bottles were packed in cartoons (1 bottle each). The cartoons purchased from the well place directly (Kudi region).

2.3. Experimental Animals:
A total of 24 albino male rats (200–250 g) were obtained from the Biology Department, Faculty of applied science, Umm Al-Qura University, KSA, and were allowed to quarantine and acclimate for a week prior to experimentation. Rats were maintained on 12-h light/dark cycles in a temperature and humidity controlled room. Animals were allowed free access to food and water beside ZW groups according to the present protocol. Our Institutional Animal Care and Use Committee approved the protocols for the animal study, and the animals were cared for in accordance with the institutional ethical guideline.

2.4. Induction of hepatotoxicity by CCl_4
Liver toxicity was induced by the intraperitoneal injection of CCl_4 (400μL/kg b.wt.), diluted with olive oil, for two successive days of the experiment (Khan and Alzohairy, 2011).

2.5. Experimental groups and protocol:
The rats were divided randomly into four groups comprising 6 rats in each group and fed the same diet throughout the experimental period. The experimental design is described as fellow:

Group I: Rats fed only with basal diet and tap water (100 mL/24 h/cage) as their sole source of drinking water, and injected intraperitoneally (i.p.) on last two days of the experiment with olive oil, this group served as normal control.

Group II: Rats fed basal diet and tape water (100 mL/24 h/cage) as their sole source of drinking water and intoxicated with CCl_4 (400μL/kg b.wt.), diluted with olive oil, injected intraperitoneally on last two days of the experiment.

Group III: Rats fed normal basal diet, injected intraperitoneally (i.p.) with olive oil and received ZW (100 mL/24 h/cage) as their sole source of drinking water, this group served as ZW control.

Group IV: Rats fed basal diet and intoxicated with CCl_4 (400μL/kg b.wt.), diluted with olive oil, injected intraperitoneally (i.p.) on last two days of the experiment and treated with ZW (100 mL/24 h/cage) as their sole source of drinking water.

2.6. Blood and tissue collection
At the end of the experiment, the overnight fasted animals (the control and experimental animals) were sacrificed under light ether anesthesia. Blood samples were collected by cardiac puncture before incision of the abdomen; 5 ml of blood samples were collected in plain tubes, serum was collected and frozen at - 30°C until the time of biochemical analysis. For histopathology, liver tissues were cut in small pieces and immersed in neutral buffered formalin 10% for 24 h. Other parts of the livers were minced in a beaker with a pair of scissors and used for Biochemical and molecular analysis.

2.7 Histopathological analysis:
Liver tissues were processed routinely, embedded in paraffin, sectioned, deparaffinized and rehydrated using the standard techniques (Bancroft, J.D. and M. Gamble, 2002). The extent of CCl_4 induced necrosis was evaluated by assessing the morphological changes in the liver sections stained with hematoxylin and eosin (H and E), using standard techniques.

2.8 Tissue Homogenate:

Some liver tissue was separated and homogenized in phosphate buffer to yield 25% homogenates. The homogenate was centrifuged for 15 minutes at 3000 rpm. The supernatant was stored at -80ºC then used for biochemical analysis.

2.8. Biochemical Assays:

The liver was removed, morphological changes were observed. Serum AST, ALT, ALP, TP and total and direct bilirubin concentrations were determined using colorimetric methods as described in the kits instruction (Diamond Co, Hannover, Germany). The absorption of the test samples were read at 505nm for AST and ALT, 510 nm for ALP, 546 nm for TP, 578 nm for total bilirubin and 546 for direct bilirubin.

Serum MDA level was determined as described by Draper and Hadley (Draper & Hadley, 1990). The principle of the methods is spectrophotometric measurements of the color produced by the reaction of thiobarbituric acid (TBA) with MDA. The concentration of MDA was then calculated as expressed as μmoles/L.

Serum reduced glutathione concentration (GSH) was measured by the method described by Beutler *et al.* (1963). This method depends on spectrophotometric estimation at 412 nm. The concentration of GSH was calculated as expressed as μmoles/L.

Serum activity of SOD, GPX and CAT enzymes were determined by Autoanalyzer (Roche-Hitachi, Japan) using commercial kits according to the methods described by Kakkor *et al.* (1984); Hissin and Hiff (1976) and Sinha (Sinha,(1972), respectively.

2.9. DNA Fragmentation Assay:

DNA fragmentation assay was conducted using the procedure of Wu *et al.* (2006). Tissue samples (50 mg) were homogenized in 10 volumes of a Tris-EDTA (TE) solution pH 8.0 (5 mM Tris-HCl, 20 mmol EDTA) and 0.2 percent triton X-100. 1.0 mL aliquot of each sample was centrifuged at 27,000 ×g for 20 min to separate the intact chromatin (pellet) from the fragmented DNA (supernatant). The pellet and supernatant fractions were assayed for DNA content using a freshly prepared DPA (Diphenylamine) solution for reaction. Optical density was read at 620 nm at (Optima D+31) spectrophotometer. The results were expressed as an amount of percent fragmented DNA (Sahreen *et al.*, 2013) .

2.10. DNA Ladder Assay:

DNA was isolated from tissue samples to estimate DNA damages (Wu *et al.* 2006). 5 μg of DNA of rats separately was loaded in 1.5 percent agarose gel containing 1.0 μg/mL ethidium bromide including DNA standards (0.5 μg per well). After electrophoresis gel was studied under gel doc system and was photographed through digital camera

2.11. Statistical analysis

All results were expressed as mean value ± S.D. The statistical analysis was performed using analysis of variance followed by student's-test with $p < 0.001$ considered statistically significant. All values are means ± SD (n = 6), * $p < 0.05$ compared with CCl_4 group, ** $p < 0.05$ compared with control (normal) group.

3. Results

3.1. Effects of zamzam water (ZW) on body weight and, relative liver weight:

Table 1 shows that body weights of the experimental animals were not affected by CCl_4, and ZW. However, a significant elevation of relative liver and spleen weight was seen in CCl_4-treated group, indicating that CCl_4 induced hypertrophy of these tissues. By contrast, ZW in combination with CCl_4 significantly reduced the elevated weight of liver, suggesting the possibility of ZW to give protection against liver injury upon CCl_4 induction.

Table 1. Effects of ZW on body weight (g) and Liver, and the relative weight of liver and spleen in normal and hepatotoxicity rats.

Group	Body weight (g)	Relative weight (g/g body weight, %)	
		Liver	Spleen
G I: Normal (+ve control)	200.00 ± 16[*]	5.08 ± 0.25[*]	0.41 ± 0.09[*]
G II: CCl₄ (-ve control)	198.00 ± 13.9[**]	7.85 ± 0.14[**]	0.62 ± 0.19[**]
G III: (ZW)	209.00 ± 17.6[*]	5.11 ± 0.35[*]	0.43 ± 0.06[*]
G IV: CCl₄ +(ZW)	203.00 ± 16.4[**]	5.93 ± 0.31[**]	0.49 ± 0.18[*]

3.2. Biochemical analysis:

Tables 2 shows that serum levels of AST, ALT and ALP were significantly higher in CCl_4 treated rats, compared with the normal control rats, indicating liver damage. Administration of ZW as sole source of drinking water, kept the serum levels of AST, ALT and ALP near the control. Administration of ZW to rats treated with CCL_4 induced significantly lower in serum AST, ALT and ALP concentrations, compared with untreated rats.

Table 2. Effects of ZW on serum level concentrations of ALT, AST, and ALP in normal and hepatotoxicity rats.

Group	Serum levels		
	ALT (mg/L)	AST (mg/L)	ALP (mg/L)
G I: Normal (+ve control)	310.22 ± 2.85[*]	272.35 ± 3.38[*]	560.95 ± 4.42[*]
G II: CCl$_4$ (-ve control)	511.85 ± 3.99[**]	576.99 ± 11.96[**]	913.59 ± 5.98[**]
G III: (ZW)	306.89 ± 2.52[*]	272.93 ± 1.45[*]	569.91 ± 8.38[*]
G IV: CCl$_4$ +(ZW)	354.31 ± 4.58[**]	369.23 ± 6.37[**]	644.33 ± 5.33[**]

Table 3 shows the effect of drinking ZW on serum levels of total protein (TP) and both total and direct bilirubin. The present results indicated that CCl$_4$ treated rats had significant higher level (p<0.05) of serum TP and total and direct bilirubin, compared with those of the normal rats. CCl$_4$ rats treated with drinking ZW had significant lower levels of serum TP and total and direct bilirubin levels, compared with those of CCl$_4$ group.

Table 3. Effects of ZW on serum level concentration of TP, total bilirubin, and direct bilirubin in normal and hepatotoxicity rats.

Group	Serum levels		
	Total protein (g/L)	Total bilirubin (mg/L)	Direct bilirubin (mg/L)
G I: Normal (+ve control)	79.27 ± 2.97[*]	3.95 ± 0.09[*]	5.59 ± 0.11[*]
G II: CCl$_4$ (-ve control)	107.16 ± 7.72[**]	8.60 ± 0.16[**]	10.20 ± 0.29[**]
G III: (ZW)	80.62 ± 4.64[*]	4.48 ± 0.22[*]	6.01 ± 0.35[*]
G IV: CCl$_4$ +(ZW)	83.85 ± 4.94[**]	5.24 ± 0.13[**]	6.78 ± 0.19[**]

Table 4. Effects of ZW on serum concentration of malondialdehyde and reduced glutathionein in normal and hepatotoxicity rats.

Group	Serum levels	
	MDA (μmol/L)	GSH (μmol/L)
G I: Normal (+ve control)	14.03 ± 0.24[*]	466.31 ± 8.6[*]
G II: CCl$_4$ (-ve control)	25.76 ± 0.12[**]	310.08 ± 5.1[**]
G III: (ZW)	15.63 ± 0.19[*]	458.14 ± 3.6[*]
G IV: CCl$_4$ +(ZW)	19.43 ± 0.07[**]	420.84 ± 7.2[**]

The results of the effect of drinking ZW on serum MDA and GSH levels in experimental rats is recorded in Table 4. Results showed that CCl$_4$ rats group had significant increase in serum MDA level,

compared with those of the normal rats. Rats' treated with CCl$_4$ and drinking ZW (ZW+ CCl$_4$) had significant decrease in serum levels of MDA, compared with the untreated hepatotoxicity rats (CCl$_4$+tap water).

Values of serum GSH level in untreated hepatotoxicity rats (CCl$_4$+tap water), were significantly lower compared with those of the normal rats. CCl$_4$ rats group supplemented with ZW caused significant elevation in serum GSH level in hepatotoxicity rats, compared with those of untreated hepatotoxicity rats drinking tap water only. Results indicate that the increases in serum MDA levels and the decreases in serum GSH levels were overcome with ZW application.

Table 5. Effects of ZW on serum concentration of SOD, GPX, and CAT in normal and hepatotoxicity rats.

Group	Serum levels		
	SOD (mmol/L)	GPX (μmol/L)	CAT (mmol/L)
G I: Normal (+ve control)	1054.36 ± 15.2[*]	205.08 ± 10.5[*]	738.92 ± 19.59[*]
G II: CCl$_4$ (-ve control)	745.32 ± 26.44[**]	80.65 ± 6[**]	504.33 ± 23.56[**]
G III: (ZW)	1046.59 ± 23.59[*]	204.28 ± 3.42[*]	717.46 ± 19.61[*]
G IV: CCl$_4$ +(ZW)	961.67 ± 34.7[**]	189.71 ± 3.8[**]	675.017 ± 8.99[**]

Concerning the effect of ZW on antioxidant system in affects rats (Table 5) results revealed that untreated rats had significantly lower values of SOD, GPX and CAT enzymes, compared with those of the normal healthy rats. Administration of ZW induced significantly higher in serum activity of SOD, GPX and CAT enzymes, compared with those of untreated hepatotoxicity rats.

3.3. Histopathological analysis:

Liver of Rats in the control group showed normal histological structure. The hepatic lobules composed of hepatocytes with well preserved cytoplasm, prominent nucleus and nucleolus. They are arranged in rows that surround the collaterals of hepatic vein, which is situated in the centre of the hepatocytes rows. The blood sinusoids, separated between the hepatocytes, transfer blood from the portal blood vessels into the central veins. Large phagocytic macrophages can be seen attached to the endothelial lining of these blood sinusoids (Fig. 1).
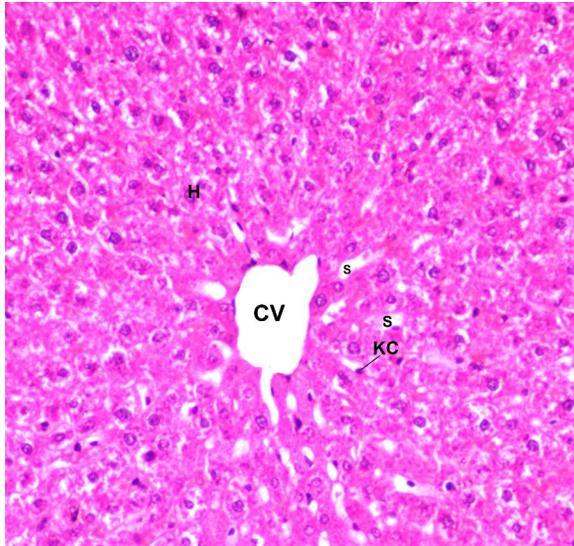
Figure 1. Liver section stained with hematoxylin and eosin. Hepatic tissue of control rats, showing normal appearance and central vein (CV), hepatocytes (H), Blood sinusoids and kupffer cells (KC). Original magnifications 200 X.

In group II, CCl$_4$-induced histological alterations in the liver parenchyma. The hepatic lobules showed significant ballooning degeneration in the centrolobular region, necrosis, hepatocytic degeneration with pyknotic nuclei and cytoplasmic vaculations, in addition, congestion and dilatation in the portal veins; periductal and perivascular inflammatory cells infiltration and oedema in the portal area (Figs. 2A, B&C).
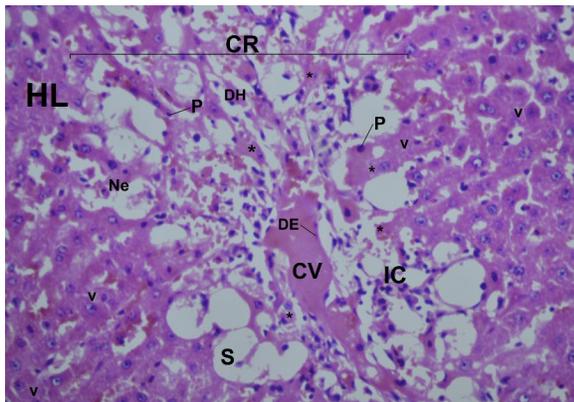

Figure. 2A. Liver section stained with hematoxylin and eosin. Hepatic tissue of CCl$_4$-treated rats in group II, showed a hepatic lobule (HL) with degenerated epithelium (DE) of the central vein (CV), ballooning degeneration (*) in the centrolobular region (CR), hepatocyte necrosis (Ne), degenerated hepatocytes (DH) with pyknotic nuclei (P) around the central vein region and mild inflammatory cell infiltration (IC). Original magnifications 400 X.
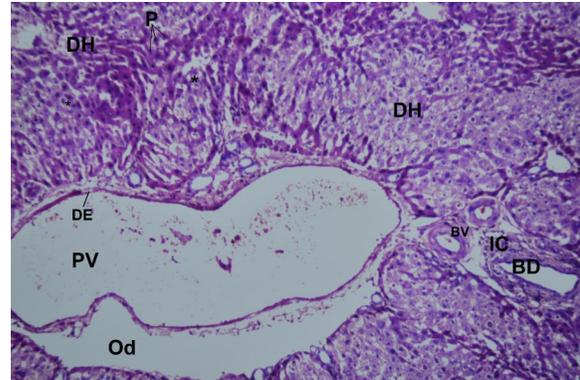

Figure 2B. Liver section stained with hematoxylin and eosin. Hepatic tissue of CCl$_4$-treated rats in group II, showed portal region with dilated portal vein (PV) with degenerated epithelium (DE), perivascular odema (Od), periductal (BD) and perivascular (BV) inflammatory cell infiltration (IC). (*): ballooning degeneration, (DH): degenerated hepatocytes, (P): pyknotic nuclei. Original magnifications 64 X.
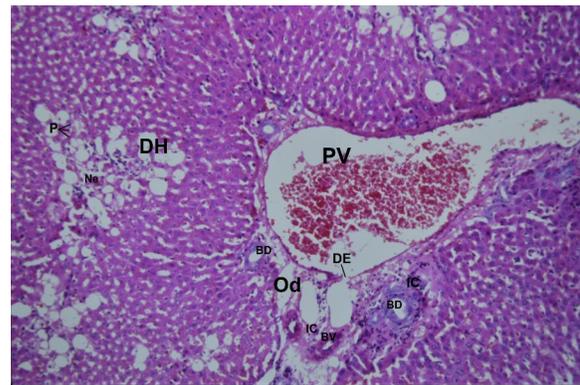

Figure 2C. Liver section stained with hematoxylin and eosin. Hepatic tissue of CCl$_4$-treated rats in group II, showed portal region with dilated and congested portal vein (PV) with degenerated epithelium (DE), perivascular odema (Od), periductal (BD) and perivascular (BV) inflammatory cell infiltration (IC). The hepatic lobules (HL) showed necrosis (Ne) and degenerated hepatocytes (DH) with pyknotic nuclei (P). Original magnifications 64 X.

Liver of group III showed normal hepatic architecture (Fig. 3). Liver of rats in group IV, injected with CCl$_4$ and treated with ZW, showed normal histological structure of the hepatic lobules, central veins, intact hepatic cells and some portal veins are congested in the portal areas (Fig. 4).
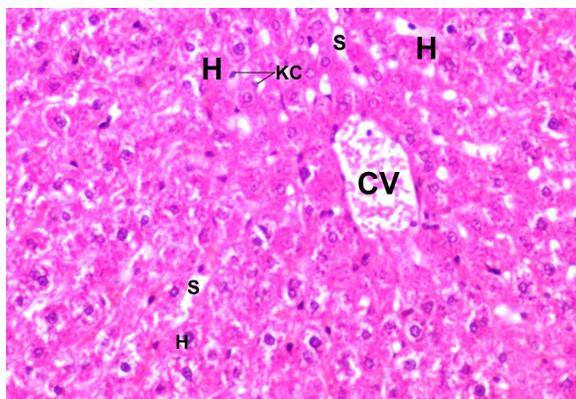
Figure 3. Liver section stained with hematoxylin and eosin. Hepatic tissue of rats in group III showed normal histological structure of a hepatic lobule with congested central vein (CV), normal hepatocytes (H), blood sinusoids (S) and kupffer cells (KC). Original magnifications 400 X".
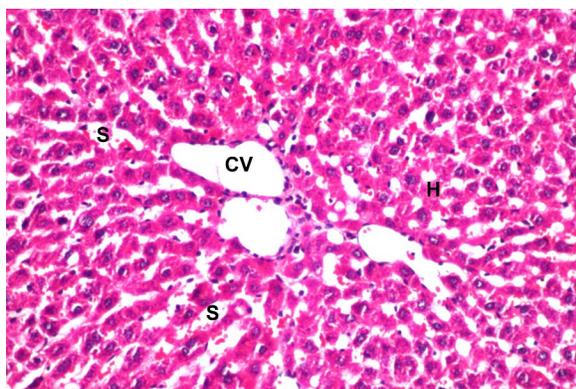

Figure 4. Liver section stained with hematoxylin and eosin. Hepatic tissue of rats in group IV restoring its normal structure. (CV): central vein, (H): hepatocytes, (S): sinusoids. Original magnifications 200 X.

3.4. Molecular analysis:

DNA ladder assay and DNA fragmentation tests show that the treatment of rats with CCl_4 increased the percentage of DNA fragmentation significantly as compared to control group. By contrast, the group of rats showed significant lower percentage of DNA fragmentation in the ZW -treated group.

A peculiar type of continuous DNA fragmentation pattern was observed in electrophoresis (Fig. 5).

DNA fragmentation percentage and DNA ladder assay were intimately correlated with each other. Treatment of ZW showed marked repairing potential against CCl_4 hepato-faliure effects.
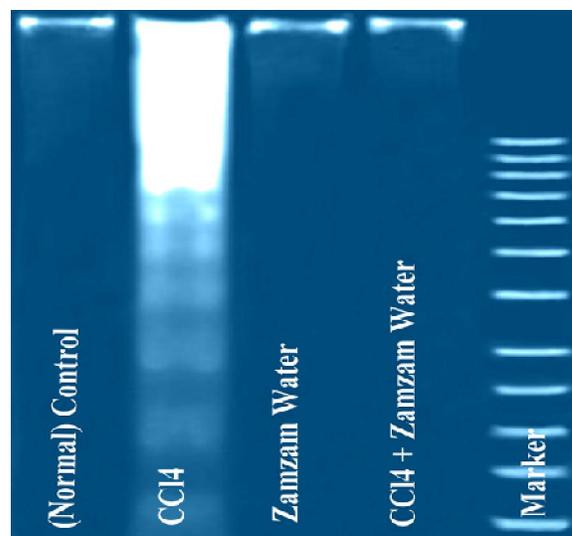

Figure 5. Agarose gel showing DNA damage by CCl_4 and protective effects of ZW in rats' liver tissue. Lanes from left showing groups 1-4 respectively and low molecular weight marker.

4. Discussions

Carbon tetrachloride (CCl_4) is one of the most commonly used hepatotoxins in the experimental study of liver diseases. The hepatotoxic effects of (CCl_4) are largely due to its active metabolite, trichloromethyl radical (Johnson DE & Kroening 1998). These activated radicals bind covalently to the macromolecules inducing peroxidative degradation of biomembranes of endoplasmic reticulum, riched in polyunsaturated fatty acids, that leads to the formation of lipid peroxides, which is one of the principle causes of hepatotoxicity of CCl_4 (Kaplowitz et al., 1986). This is evidenced by an elevation in the serum marker enzymes namely (AST), (ALT), (ALP), total bilirubin, (GGTP) and decrease in protein.

These activated radicals bind covalently to the macromolecules and induce peroxidative degradation of membrane lipids of endoplasmic reticulum rich in polyunsaturated fatty acids. This leads to the formation of lipid peroxides. This lipid peroxidative degradation of biomembranes is one of the principle causes of hepatotoxicity of CCl_4 (Kaplowitz et al., 1986).

In the assessment of liver damage by CCl_4, the determination of enzyme levels such as AST and ALT is largely used. Necrosis or membrane damage releases the enzyme into circulation and hence it can be measured in the serum. A high level of AST indicates liver damage, such as that caused by viral hepatitis as well as cardiac infarction and muscle injury. AST catalyses, means the conversion of alanine to pyruvate and glutamate, is released in a similar manner.

AST (do u mean ALT) catalyses, the conversion of alanine to pyruvate and glutamate and is released in a similar manner. Therefore ALT is more specific to the liver, and is thus a better parameter for detecting liver injury. Elevated levels of serum enzymes are indicative of cellular leakage and loss of functional integrity of cell membrane in liver (Drotman & Lawhan, 1978). Serum (ALP), bilirubin and TP levels, on other hand, are related to the function of hepatic cell. Increase in serum level of ALP is due to increased synthesis, in presence of increasing biliary pressure (Muriel & Garcipiana, 1992).

Administration of CCl_4 caused a significant ($P<0.001$) elevation of enzyme levels such as AST, ALT, ALP, GGTP and total Bilirubin, and decrease in TP when compared to control. There was a significant ($P<0.001$) restoration of these enzyme levels on administration of ZW. The reversal of increased serum enzymes in CCl_4-induced liver damage by the extract may be due to the prevention of the leakage of intracellular enzymes by its membrane stabilizing activity. This is in agreement with the commonly accepted view that serum levels of transaminases return to normal with the healing of hepatic parenchyma and the regeneration of hepatocytes (Thabrew & Joice, 1987). Effective control of ALP, bilirubin and TP levels points towards an early improvement in the secretary mechanism of the hepatic cells.

The efficacy of any hepatoprotective drug is dependent on its capacity of either reducing the harmful effect or restoring the normal hepatic physiology that has been distributed by a hepatotoxin. ZW decreased CCl_4 induced elevated enzyme levels in tested groups, indicating the protection of structural integrity of hepatocytic cell membrane or regeneration of damaged liver cells.

The increase in lipid peroxidation (LPO) level in liver induced by CCl_4 suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanism to prevent formation of excessive free radicals. Treatment with ZW significantly reverses these changes. Hence it is likely that the mechanism of hepatoprotection of ZW is due to its antioxidant effect. Decrease in enzyme activity of SOD is a sensitive index in hepatocellular damage and is the most sensitive enzymatic index in liver injury. Curtis and Mortiz (1972) have been reported that SOD is the most important enzymes in the enzymatic antioxidant defense system. It scavenges the superoxide anion to form hydrogen peroxide and thus diminishing the toxic effect caused by this radical. ZW causes a significant increase in hepatic SOD activity and thus reduces reactive free radical induced oxidative damage to liver.

CAT is an enzymatic antioxidant widely distributed in all animal tissues, and the highest activity is found in liver. CAT decomposes hydrogen peroxide and protects the tissues from highly reactive hydroxyl radicals (Chance & Greenstein, 1992). Therefore reduction in the activity of CAT may result in a number of deleterious effects due to the assimilation of superoxide radical and hydrogen peroxide. Elevation in the level of CAT produced by ZW support its hepatoprotective effect.

Glutathione is one of the most abundant tripeptide, non-enzymatic biological antioxidant present in the liver. It removes free radical species such as hydrogen peroxide, superoxide radicals and maintains membrane protein thiols. Also it is substrate for GPx (Prakash et al., 2001). Decreased level of GSH is associated with an enhanced lipid peroxidation in CCl_4 treated rats. Administration of ZW significantly ($P<0.001$) increased the level of GPx and GST in a dose dependent manner.

Extensive vascular degenerative changes and centrilobular necrosis in hepatocytes was produced by CCl_4. Treatment with ZW produced only mild degenerative changes and absence of centrilobular necrosis, indicating its hepatoprotective efficiency. Free radical mediated process has been implicated in pathogenesis of most of the diseases. The protective effect of ZW on CCl_4 induced hepatotoxicity in rats appears to be related to inhibition of lipid peroxidation and enhancement of antioxidant enzyme levels in addition to free radicals scavenging action.

It was reported that CCl_4 resulted in the oxidative damage to liver proteins in rats. Oxidative damage to proteins is very important as it can contribute secondary damage resulting in hampering the DNA repair enzymes and loss of reliability of damage polymerases during DNA replication. The DNA damage in various tissues like brain, testis and liver was reported by Manierea et al. (2005).

From the present study, it can be assumed that exposure to CCl_4 may cause accumulation of many toxic species in cells thus damaging both DNA and lipids. In fact, treatment with ZW ameliorated the toxic effects on DNA as revealed by DNA fragmentation percentage and ladder assay. The present study clearly augments the defensive mechanism of ZW against oxidative stress induced by CCl_4 and provides confirmation about its therapeutic use in hepatic abnormalities.

The observed antioxidant and hepatoprotective activity of ZW may be due to the antioxidant characters. Further studies to characterize the active principles and to elucidate the mechanism are in progress.

5. Conclusion

In conclusion, ZW caused a protective effect against CCl_4-induced liver damage and improved the biochemical parameters. Also, ZW has a hepatoprotective effect against injury in the liver of CCl_4-treated rats. Therefore, ZW may be used to protect against toxic effects of CCl_4 and other chemical agents in liver. In the future, examination of the liver protective effect of ZW against CCl_4 in dose dependent manner could be investigated.

Corresponding Author:
Dr. Ashraf Saif
Al-Leith University College
Umm Al-Qura University
Makkah, Saudi Arabia.
E-mail: aaa_saif@hotmail.com

References

1. Al Zuhair N and Khounganian RA (2006). Comparative Study between the Chemical Composition of Potable Water and Zamzam Water and its Effect on Tooth Structure in Saudi Arabia. In the Afaq (Arabic) Dental Journal, 11(1):36 – 37.
2. Alfadul, SM and Khan MA (2011). Water quality of bottled water in the kingdom of Saudi 362 Arabia: A comparative study with Riyadh municipal and Zamzam water, J. Environ. Sci. Health, 363 A. 2011, 46, 13, 1519-1528.
3. Analytical report of Zamzam water cited from the annual report of the ministry of agriculture and water resources 1971.
4. Bancroft and Gamble (2002). Theory and Practice of Histological Techniques. 5th ed., Churchill Livingstone, London, New York and Philadelphia.
5. Beutler E; Dubon B and Kelly M (1963). Improved method for determination of blood glutathione. J. Lab. Clin. Med., 61:882-888.
6. Challis DJ; Zeinstra JS and Anderson MJ (1987). Some effects of water quality on the performance of high yielding cows in an arid climate. Vet Rec., 120(1):12–5.
7. Chance B and Greenstein DS (1992). The mechanism of catalase actions-steady state analysis. Arch Biochem Biophys., 37:301–339.
8. Curtis JJ and Mortiz M. (1972). Serum enzymes derived from liver cell fraction and response to carbon tetrachloride intoxication in rats. Gastroenterol., 62:84–92.
9. Draper H and Hadley M (1990). Malondi-aldehyde determination as index of lipid peroxidation. Methods Enzymol., 186:421-431.
10. Drotman R and Lawhan G (1978). Serum enzymes are indications of chemical induced liver damage. Drug Chem Toxicol., 1:163–171.
11. Hissin PL and Hiff R (1976). A fluorometric method for determination of oxidized and reduced glutathione in tissues. Anal. Biochem., 74(1):214-226.
12. Hofer T; Marzetti E; Xu J; Seo AY; Gulec S; Knutson MD; Leeuwenburgh C and Dupont-Versteegden EE (2008). Increased iron content and RNA oxidative damage in skeletal muscle with aging and disuse atrophy. Exp. Gerontol. 43:563-570.
13. Huang KC; Yang CC; Lee KT and Chien CT (2003). Reduced hemodialysis-induced oxidative stress in end-stage renal disease patients by electrolyzed reduced water. Kidney Int., 64:704-714.
14. Jin D; Ryu SH; Kim HW; Yang EJ; Lim SJ; Ryang YS; Chung CH; Park SK and Lee KJ (2006). Anti-diabetic effect of alkaline-reduced water on OLETF rats. Biosci. Biotechnol. Biochem., 70:31-37.
15. Johnson DE and Kroening C (1998). Mechanism of early carbon tetra chloride toxicity in cultured rat hepatocytes. Pharmacol Toxicol., 83:231–239.
16. Kakkor P; Das B and Viswanathan PN (1984). A modified spectrophotometric assay of superoxide dismutase. Ind J Biochem Biophyso., 21:131-133.
17. Kaplowitz N; Aw TY; Simon FR; Stolz A (1986). Drug induced hepatotoxicity. Ann Intern Med., 104:826–839.
18. Khan AA and Alzohairy M (2011). Hepato-protective effects of camel milk against CCl_4-induced hepatotoxicity in Rats. Asian J Biochem., 6(2):171–180.
19. Manierea I; Godardb T; Doergec DR; Churchwellc MI; Guffroyd M; Laurentiea M and Poula JM (2005). DNA damage and DNA adduct formation in rat tissues following oral administration of acrylamide. Mutation Res., 580:119–129.
20. Muriel P and Garcipiana T. (1992). Silymarin protects against paracetamol-induced lipid peroxidation and liver damage. J Appl Toxicol., 12:439-442.
21. Nassini R; Andrè E; Gazzieri D; De Siena G, Zanasi A; Geppetti P and Materazzi S (2010). A bicarbonate-alkaline mineral water protects from

ethanol-induced hemorrhagic gastric lesions in mice. Biol. Pharm. Bull., 33:1319-1323.

22. Prakash J; Gupta SK and Singh N (2001). Chemopreventive activity of *Withania somnifera* in experimentally induced fibro sarcoma tumors in Swiss albino rats. Phytother. Res., 15:200-204.

23. Sahreen S; Khan MR; Khan RA (2013). Ameliorating effect of various fractions of *Rumex hastatus* roots against hepato- and testicular toxicity caused by CCl4. Oxid. Med Cell Longev., 2013:325406.

24. Sinha KA (1972). Colorimetric assay of catalase enzyme. Anal, Biochem., 47:389-394.

25. Thabrew M and Joice PA (1987). Comparative study of the efficacy of *Pavetta indica* and *Osbeckia octanda* in the treatment of liver dysfunction. Planta. Med., 53:239–241.

26. Tsai CF; Hsu YW; Chen WK; Chang WH; Yen CC; Ho YC and Lu FJ (2009). Hepatoprotective effect of electrolyzed reduced water against carbon tetrachloride-induced liver damage in mice. Food Chem Toxicol., 47(8):2031-6.

27. Wu B.; Ootani A. and Iwakiri R. *et al*., (2006). T cell deficiency leads to liver carcinogenesis in azoxymethane-treated rats. Experimental Biology and Medicine, 231 (1):91–98.

28. Zamzam Studies and Research Centre. Cited from The Saudi Geological Survey. 5th June, 2005.