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Amelioration of Severe Carbon Tetrachloride Toxicity by Zamzam Water in Rats

Abdelsalam Elnour[2], Abdullah Bamosa[2]*, Abdullah Al Meheithif[1] and Khaled Aleissa[3]

[1]*Ministry of Water and Electricity, Riyadh, Saudi Arabia*
[2]*Department of Physiology, College of Medicine, University of Dammam, Dammam, Saudi Arabia*
[3]*Nuclear Research Institute, King Abdulaziz City for Science and Technology, Riyadh, Saudi Arabia*

Abstract

Zamzam water is alkaline mineral natural water consumed by millions of Muslims worldwide, who believe in its global healing effects. It is known that environmental toxins and oxidative stress are common mechanisms in the pathogenesis and complication of many diseases. Thus, the aim of the current study is to find out if Zamzam water can minimize liver toxicity induced by carbon tetrachloride. In three experiments, the effects of Zamzam water were compared to ordinary bottled water. This involved measurement of bilirubin, liver enzymes and different antioxidant parameters in serum of rats. In experiments 1 and 2, single injection of high and low doses was tested, respectively. In experiment 3, a repeated (4 days) very small dose was tested. In the three experiments, test groups were injected with carbon tetrachloride dissolved in olive oil, while control animals were injected with equivalent volume of olive oil only. In all experiments, carbon tetrachloride was associated with significant rise in serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT). In the single high dose experiment, serum AST and ALT were significantly lower in carbon tetrachloride treated rats given Zamzam water, than those given ordinary bottled water. This same trend persisted in the single small dose experiment and disappeared in very small repeated dose one. Plasma level of all tested antioxidant parameters in the three experiments did not follow a pattern which could explain the amelioration effect of Zamzam water on carbon tetrachloride liver toxicity. These results indicate that Zamzam water is a potential protective agent against carbon tetrachloride liver toxicity in rats. Further studies are needed to explore the mechanism of the beneficiary effect of Zamzam water, and to evaluate the effect of Zamzam water on other conditions of oxidative stress.

Keywords: Zamzam water; Antioxidants; Carbon tetrachloride; Liver enzymes

Introduction

The susceptibility of animals to oxidative stress depends on the critical balance between its antioxidant capacity and the level of oxidative stress [1]. Although some of the trace elements in drinking water are essential components of known antioxidant enzymes (selenium, copper, zinc), the contribution of drinking water to the daily requirements and intake of these elements, and thus, in boosting of antioxidant mechanisms, is not clear. However, special types of drinking water have been shown to foster the ability of animal to combat oxidative stress [2-7]. Specifically, Tsai et al. [6] demonstrated that electrolyzed reduced water protected the liver against carbon tetrachloride toxicity in mice. In addition, this water minimized UV light induced skin damage, a process mediated by oxidative processes [4]. Slowing of the aging process, another process aggravated by oxidative damage, has also been linked to use of electrolysed reduced water [5-8]. 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 richness 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. Despite the low levels of elements or trace elements in water, their contribution is still likely, at least for some of them [9]. Thus, if harmful contaminants of water are taken care of, water, in addition to its hydration property, may have other important effects.

Zamzam is natural water consumed by millions of Muslims worldwide because of their religious belief. The well is located in Makkah in the holy mosque (Haram). When they visit Makkah, pilgrims tend to take good quantities of Zamzam water to their countries. This natural water has been found to be alkaline and rich in many minerals [10], which make it a potential antioxidant agent. Thus, the aim of the current study is to investigate if Zamzam water can minimize liver toxicity induced by carbon tetrachloride in rats.

Materials and Methods

Animals

Winstar rats weighing 350-400 g were used. All experimental animals were housed individually and given free access to ordinary bottled water for one acclimatizing week. In each of three experiments, the rats were then divided in two groups; one group continued on ordinary bottled water, while the other was shifted to the test water, Zamzam water, for a further week. On the day immediately following this, each group was divided in two subgroups; one was injected intra peritoneally with the calculated dose of carbon tetrachloride (experimental), and the other with equivalent volume of olive oil and served as control group.

Two protocols were employed; single dose protocol (experiment 1 and 2) and repeated very small dose protocol (experiment 3). Fifteen

***Corresponding author:** Bamosa Abdullah, Department of Physiology, College of Medicine, University of Dammam, Dammam, Saudi Arabia, E-mail: bamosa@ud.edu.sa

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rats were included in experiment 1 (single high dose), five served as controls and injected with olive oil intraperitoneally, and 10 as test group and injected with 2 ml/kg CCl_4 intra peritoneally. Fifteen rats were included in experiment 2 (single low dose), 5 were injected with olive oil and served as controls, while 10 were injected with 1 ml/kg body weight of CCl_4 intra peritoneally and served as test group. In both experiment 1 and 2, rats were sacrificed 24 hours after the intra-peritoneal injections. Twenty rats were included in the repeated very small dose protocol (experiment 3). Test group included fifteen rats injected intraperitoneally with a small dose of CCl_4 (0.3 ml/kg body weight), daily for four days, before sacrificing the animals. The other five rats were injected with equivalent volume of olive oil in the same way, and served as controls.

Chemicals

Carbon tetrachloride solution was prepared by dissolving carbon tetrachloride in olive oil, in a ratio of 1:1.

Collection of blood samples and laboratory analysis

On the last day of each experiment and at 8 o'clock in the morning, rats were anaesthetized with 0.5 ml of a 2:3 ketamine zylocain mixture. The abdomen was exposed, and samples of blood were extracted from the abdominal aorta. The blood was centrifuged at 2500 rpm, and then serum was used for evaluation of total and direct bilirubin, liver enzymes γ-glutamyltranspeptidase (GGT), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP), and the antioxidant parameters; total antioxidant capacity, catalase, superoxide dismutase, glutathione and thiobarbituric acid reactive substances (TBARS).

Laboratory analysis

Catalase, superoxide dismutase, glutathione and Thiobarbituric acid reactive species (TBARS) were analyzed by Cayman kits (Cayman Chemical Co Inc, Ellsworth Rd, Ann Arbor, USA). All the analyses were based on methods previously described.

Liver function tests (LFT) were automatically assayed using Dimension Clinical Chemistry System (Dimension Max. Germany). The sampling, delivery, mixing, processing and printing of the results were automated. The assays performed using Flex® reagent cartridges, supplied by Dade Behring, Germany.

Statistical analysis

The differences between groups were analyzed by one way analysis of variance using SPSS. Differences were considered significant at p values less than 0.05.

Results

Zazmam water samples had higher levels of Arsenic, Nitrate, Selenium and other trace elements, and pH than ordinary bottled water samples (Table 1). Water consumption did not differ significantly between the two groups, before and after carbon tetrachloride injections.

Tables 2-4 summarize the results of serum bilirubin and liver enzymes in the three experiments. In all experiments, serum ALP was not significantly different between the groups. In experiment 1 (single high dose), direct and total bilirubin, GGT, AST, ALT increased significantly in rats injected with carbon tetrachloride compared to their respective control injected with olive oil, for both bottled water and Zamzam water. Furthermore, comparing the two subgroups injected with carbon tetrachloride, the one on bottled water had significantly higher AST and ALT than the one on Zamzam water.

In experiment 2 (single low dose), as shown in table 3, carbon tetrachloride injection was not associated with significant increase in serum bilirubin or GGT. Injection of carbon tetrachloride was, however, associated with significant increase in serum AST, for both ordinary water and Zamzam water, and a significant increase in serum ALT for ordinary water only.

In experiment 3 (repeated very small dose), as shown in table 4, injection of carbon tetrachloride was not associated with changes in serum bilirubin and GGT. A significant increase in serum AST and

Parameter	Ordinary water	Zamzam water	Parameter	Ordinary water	Zamzam water
Calcium Carbonate (ppm)	28-32	300-340	Arsenic (ppb)	ND	19-26
Magnesium (ppm)	23-27	19-24	Selenium (ppb)	ND	3-4
Chromium (ppb)	ND	0.7-0.75	Strontium (ppb)	ND	700-800
Manganese (ppb)	ND	0.07-0.10	Cadmium (ppb)	ND	0.2-1.0
Cobalt (ppb)	ND	0.3-0.4	Lead (ppb)	ND	0.05-0.1
Copper (ppb)	ND	0.5-1.0	Nitrate (ppb)	3-4	70-90
Zinc (ppb)	ND	1-2	pH	7.0	7.75-8.0

ND=not detectable

Table 1: Ranges of some elements, salts and pH of ordinary and Zamzam water samples.

Parameters	Ordinary water		Zamzam water	
	Control*	Carbon tetrachloride	Control*	Carbon tetrachloride
Direct bilirubin (mg/dl)	0.00 [a]	0.33 ± 0.07 [b]	0.00 [a]	0.21 ± 0.07 [b]
Total bilirubin (mg/dl)	0.12 ± 0.2 [a]	0.62 ± 0.11 [b]	0.12 ± 0.02 [a]	0.43 ± 0.10 [b]
γglutamyltranspeptidase (U/l)	0.00 [a]	2.11 0.51 [b]	0.00 [a]	2.40 ± 0.80 [b]
Aspartate aminotransferase (U/l)	149 ± 19 [a]	3355 ± 565 [b]	135 ± 10 [a]	1947 ± 578 [c]
Alanine Aminotransferase (U/l)	76 ± 7 [a]	4751 ± 716 [b]	53 ± 4 [a]	2940 ± 758 [c]
Alkaline phosphatase (U/l)	200 ± 19 [a]	190 ± 16 [a]	165 ± 14 [a]	190 ± 19 [a]

* Rats Injected with olive oil injection

Means in the same row with the same letter are not significantly different.

Table 2: Total bilirubin, direct bilirubin and liver enzymes in rats given ordinary and Zamzam water, with and without high dose (2 ml/kg, experiment 1) carbon tetrachloride treatment.

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	Ordinary water		Zamzam water	
	Control*	Carbon tetrachloride	Control*	Carbon tetrachloride
Direct bilirubin (mg/dl)	0.0	0.0	0.0	0.0
Total bilirubin mg/dl)	0.1 ± 0.0 ^{a}	0.15 ± 0.01 ^{ac}	0.12 ± 0.02 ^{ac}	0.17 ± 0.02 ^{bc}
γGlutamyltranspeptidase(U/l)	0.80 ± 0.37 ^{a}	1.00 ± 0.24 ^{a}	0.40 ± 0.24 ^{a}	0.93 ± 0.25 ^{a}
Aspartate aminotransferase (U/l)	189 ± 37 ^{bc}	585 ± 108 ^{a}	155 ± 19 ^{b}	497 ± 70 ^{ac}
Alanine aminotransferase (U/l)	60 ± 4 ^{a}	414 ± 97 ^{b}	91 ± 12 ^{ac}	344 ± 70 ^{bc}
Alkaline phosphatase (U/l))	240 ± 43 ^{a}	214 ± 16 ^{a}	205 ± 19 ^{a}	210 ± 17 ^{a}

*Rats Injected with olive oil injection

Means in the same row with the same letter are not significantly different.

Table 3: Total bilirubin, direct bilirubin and liver enzymes in rats given ordinary and Zamzam water, with and without low dose (1 ml/kg, experiment 2) carbon tetrachloride treatment.

Parameters	Ordinary water		Zamzam water	
	CONTROL*	Carbon tetrachloride	CONTROL*	Carbon tetrachloride
Direct bilirubin (mg/dl)	0.02 ± 0.02 ^{a}	0.01 ± 0.01 ^{a}	0.0 ^{a}	.04 0.02 ^{a}
Total bilirubin (mg/dl)	0.16 ± 0.02 ^{a}	0.21 ± 0.01 ^{a}	0.18 ± 0.02 ^{a}	0.24 ± 03 ^{a}
γglutamyltranspeptidase(U/l)	1.4 ± 1.4 ^{a}	1.4 ± 0.6 ^{a}	0.0 ^{a}	.80 ± 0.4 ^{a}
Aspartate aminotransferase (U/l)	293 ± 24 ^{a}	937 ± 198 ^{b}	353 ± 30 ^{a}	1328 ± 263 ^{b}
Alnine aminotransferase (U/l)	68 ± 6 ^{a}	987 ± 284 ^{b}	81 ± 4 ^{a}	1417 ± 216 ^{b}
Alkaline phosphatase (U/l)	281 ± 17 ^{a}	271 ± 23 ^{a}	278 ± 16 ^{a}	476 ± 128 ^{a}

*Rats Injected with olive oil injection

Means in the same row with the same letter are not significantly different.

Table 4: Total bilirubin, direct bilirubin and liver enzymes in rats given ordinary and Zamzam water, with and without repeated very small dose (0.3 ml/kg, experiment 3) carbon tetrachloride treatment.

Parameters	Ordinary water		Zamzam water	
	Control*	Carbon tetrachloride	Control*	Carbon tetrachloride
Catalase (nM/min/ml)	85.22 ± 21.09 ^{a}	177.74 ± 16.88 ^{b}	53.55 ± 4.07 ^{a}	164.97 ± 18.17 ^{b}
Superoxide Dismutase (U/ml)	2.30 ± 0.34 ^{a}	12.99 1.13 ^{b}	2.35 ± 0.12 ^{a}	12.12 ± 0.50 ^{b}
Serum glutathione (μM)	4.18 ± 1.45 ^{ab}	20.26 ± 3.89 ^{c}	2.39 ± 0.83 ^{a}	10.30 ± 2.51 ^{b}
Total antioxidant capacity (mM)	2.52 ± 0.45 ^{a}	4.81 ± 1.19 ^{a}	2.63 ± 0.51 ^{a}	3.56 ± 0.41 ^{a}
TBARS (μM)	82.42 ± 21.83 ^{a}	28.80 ± 6.47 ^{a}	64.64 ± 8.77 ^{a}	49.77 ± 11.03 ^{a}

Means in the same row with the same letter are not significantly different.

Table 5: Serum catalase, superoxide dismutase, total antioxidant capacity, glutathione and tbars in rats given ordinary and Zamzam water, with or without high dose (2 ml/kg, experiment 1) carbon tetrachloride treatment.

	Ordinary water		Zamzam water	
	Control*	Carbon tetrachloride	Control*	Carbon tetrachloride
Catalase (nM/min/ml)	190.1 ± 36.8 $^{\alpha}$	179.0 ± 31.4 $^{\alpha}$	137.7 ± 37.0 $^{\alpha}$	145.1 ± 17.2 $^{\alpha}$
Superoxide Dismutase (U/ml)	3.10 ± 0.19 $^{\beta}$	3.98 ± 0.15 $^{\alpha}$	3.58 ± 0.11 $^{\alpha\beta\chi}$	3.79 ± 0.09 $^{\alpha\chi}$
Serum glutathione (μM)	13.00 ± 1.97 $^{\alpha}$	11.60 ± 1.35 $^{\alpha}$	8.93 ± 1.40 $^{\alpha}$	8.94 ± 1.03 $^{\alpha}$
Total antioxidant capacity (mM)	3.89 ± 0.19 $^{\alpha}$	3.69 ± 0.29 $^{\alpha}$	6.26 ± 0.50 $^{\beta}$	4.13 ± 0.28 $^{\alpha}$
TBARS (μM)	80.6 ± 14.0 $^{\alpha\beta}$	68.0 ± 9.4 $^{\alpha}$	115.0 ± 13.7 $^{\alpha\beta}$	100.94 ± 9.4 $^{\alpha\beta}$

Means in the same row with the same letter are not significantly different.

Table 6: Serum catalase, superoxide dismutase, total antioxidant capacity, glutathione and TBARS in rats given ordinary and Zamzam water, with or without low dose (1 ml/kg, experiment 2) carbon tetrachloride treatment.

ALT was, however, associated with the group injected with carbon tetrachloride. But, there was no significant difference in these enzymes between the two subgroups injected with carbon tetrachloride.

Tables 5-7 summarize the results of the antioxidant parameters; serum catalase, superoxide dismutase, glutathione, total antioxidant capacity and TBARS. In all experiments, serum total antioxidant capacity and TBARS did not differ significantly between any of the groups. In experiment 1 (table 5), serum catalase, superoxide dismutase and glutathione increased significantly with injection of carbon tetrachloride. However, the two subgroups injected with carbon tetrachloride did not differ significantly from each other. In experiment 2, carbon tetrachloride was not associated with significant change in serum catalase, or total antioxidant capacity. However, superoxide dismutase increased significantly with injection of carbon tetrachloride for the bottled water groups. In experiment 3, no significant difference was noted for any of the antioxidant parameters.

Discussion

The results of the current study provide clear evidence that Zamzam water minimizes the injurious effects of the famous toxin, carbon tetrachloride on the liver. It is interesting to note the similarity between Zamzam water and electrolyzed-reduced water in this respect.

Parameters	Ordinary water		Zamzam water	
	Control*	Carbon tetrachloride	Control*	Carbon tetrachloride
Catalase (nM/min/ml)	242.7 ± 27.0 [a]	158.6 ± 35.3 [a]	135.3 ± 25.0 [a]	133.6 ± 25.5 [a]
Superoxide Dismutase (U/l)	4.67 ± 0.49 [a]	4.44 ± 0.0.20 [a]	4.66 ± 0.41 [a]	4.76 ± 0.0.29 [a]
Serum glutathione (µM)	7.11 ± 2.58 [a]	9.37 ± 0.69 [a]	8.93 ± 1.40 [a]	10.65 ± 0.61 [a]
Total antioxidant capacity (mM)	2.63 ± 0.19[a]	2.08 ± 0.36 [a]	2.47 ± 0.50 [a]	4.00 ± 0.26 [b]
TBARS (µM)	77.4 ± 17.0 [a]	64.6 ± 10.7 [a]	79.0 ± .7.2 [a]	94.5 ± 13.6 [a]

Means in the same row with the same letter are not significantly different.

Table 7: Serum catalase, superoxide dismutase, total antioxidant capacity, glutathione and tbars in rats given ordinary and Zamzam Water, with or without repeated very small dose (2 ml/kg, experiment 2) carbon tetrachloride treatment.

Tsai et al. [6], using mice, demonstrated that electrolysed-reduced water decreased the toxicity of carbon tetrachloride on the liver. Indeed, our results, especially with high carbon tetrachloride dose (experiment 1), are similar to those of Tsai et al. [6], who showed that electrolyzed reduced water causes significant reduction in serum levels of AST and ALT in mice injected with carbon tetrachloride. The lack of a significant effect of carbon tetrachloride on serum alkaline phosphatase is not unexpected. Serum alkaline phosphatase rises in cholestasis, rather than direct hepatocyte damage. Carbon tetrachloride is known to cause direct hepatocyte injury, but not cholestasis [11]. The lack of effects of Zamzam water in the repeated very small dose experiment requires further investigation.

The high serum catalase, superoxide dismutase and glutathione in the groups given carbon tetrachloride in the high dose experiment in the current study are not unexpected. Hepatocytes are rich in these substances. Indeed, serum levels of these have been shown to rise in different conditions associated with acute liver damage like acetaminophen [12], alcoholic hepatitis [13], and organophosphate insecticides [14]. In fact, it has been suggested that measurement of serum catalase can be used to diagnose severe liver disease [15]. The unexpected lack of effect of carbon tetrachloride on serum level of TBARS requires further evaluation. The results of antioxidant parameters do not show any indication of oxidative stress induced by carbon tetrachloride in the plasma. This has been attributed to rupture of liver cells, together with a decrease in plasma lipids, which impede detection of any potential changes in the antioxidant status of the plasma [16]. However, this does not exclude oxidative damage as the mechanism of carbon tetrachloride toxicity, because the picture might be different in liver tissue [17].

It is noteworthy that carbon tetrachloride induced oxidative stress is commonly used to test the antioxidant promoting effects of medicinal plants and drugs, and we followed similar protocols of acute (experiments 1 and 2) and sub-acute exposures (experiment 3). The exposure time of 24 hours (time from injection of carbon tetrachloride to sacrificing of animals in experiment 1 and 2), is the recommended time to show the toxic effects of carbon tetrachloride unequivocally [18]. In our study, the effects of carbon tetrachloride on serum AST and ALT are similar to previous studies [19], and given the sensitivity of serum levels of these two enzymes to liver disease [20]. Whether the protective mechanism of Zamzam water is similar to that of electrolysed-reduced water requires further investigation [21]. In conclusion, our results indicate that Zamzam water reduces liver toxicity induced by carbon tetrachloride in rats. Further studies are required to confirm the results of this study with liver histopathology and measurement of antioxidant parameters in liver tissue, and to evaluate the effects of Zamzam water in other causes, and situations of oxidative stress.

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