

Potential Role of Zamzam Water in Some Chronic Diseases in Rats

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ABSTRACT

Water from holy zamzam is consumed by many Muslims in the world due to its beneficial role for the health of human beings. Therefore the present study was designed to evaluate the effect of zamzam water in diabetes mellitus, nephrotoxicity and hepatotoxicity in rats as well as to determine the chemical analysis of this water. Fifty six male albino rats weighting (150±5gm) were divided into four main groups and each group was divided into two subgroups a and b, one administered tap water (Tw) and the other subgroup administered zamzam water (zw). The 1st main group was normal rat (-ve control), the 2nd group was diabetic (induced by alloxan 150 mg/kg b.w.), the 3rd was nephrotoxic (induced by gentamicin 100 mg/kg b.w.) and the 4th group was hepatotoxic by carbon tetrachloride (2ml/ kg b.w.). The experiment lasted after four weeks. Zamzam water significantly decreased serum glucose, total lipids, total cholesterol, triglycerides, LDL-cholesterol and elevated HDL- cholesterol. Moreover the liver and kidney markers were also improved in the groups that treated with zamzam water relative to its control taking tap water. Zamzam water has a protective role against hazardous effects of diabetes mellitus, nephrotoxicity and hepatotoxicity in rats.

Keywords: Zamzam water – Diabetes mellitus – Nephrotoxicity - Hepatotoxicity – Glucose – Liver markers – Kidney markers.

INTRODUCTION

Water is one of the main dietary components. Its quality plays important role for the health of human being. Zamzam water is natural water consumed by millions of Muslims worldwide¹. Zamzam water is unique in its character as compared to other water. There are neither bacteria nor moulds that caused changes in odour and taste². Zamzam water has no signs of biological growth³. This water has been reported to be alkaline and also rich in minerals which render it a potential antioxidant source^{4,5}. The alkaline nature of zamzam is associated with the richness with certain elements like magnesium⁶.

Besides zamzam water has indicated that it is sodium chloride water and of meteoritic nature. The four toxic elements arsenic (AS), cadmium (Cd), lead (Pb) and selenium (Se) are less than the danger level of human consumption. Chemically zamzam water is extremely suitable for drinking purposes⁷.

Zamzam water contains fluorides that have an effective germicidal action⁸.

Diabetes mellitus (DM) is currently a major public health concern, because its incidence and prevalence are elevated increasing, reaching epidemic proportions⁹.

Recent advances in understanding the etiology and pathogenesis of diabetes have led to revised classifications in which diabetes is divided into general classes which are type I diabetes, type II diabetes and the gestational diabetes¹⁰. Diabetes can be diagnosed by the presence of

four classic signs that include polyuria, polyphagia, polydipsia and hyperglycemia¹¹.

Diabetes is reported to be a multifactorial disease which manifested by hyperglycemia¹², abnormalities of lipoprotein¹³, increased basal metabolic rate^{14,15}, defects in scavenging enzymes and changes in intermediary metabolism of most food substances¹⁶.

Nephrotoxicity is the most common kidney problem and happens when body is exposed to a toxin or drug. When kidney injury occurs, body is unable to rid of excess urine, wastes from the body and also blood electrolytes (such as magnesium and potassium). A number of therapies like aminoglycoside, antibiotics and chemotherapeutic agents can adversely affect the kidney resulting in acute renal failure, chronic interstitial nephritis and nephritic syndrome¹⁷.

The liver has a pivotal role to detoxify the toxic substances and synthesizes useful compounds. Liver toxicity is well known. Toxicity may occur when the liver has become overloaded with toxic materials that come from the diet, water, air, drugs, etc¹⁸.

Liver diseases are considered to be a worldwide problem and are associated with a significant morbidity and mortality. The contributory factors for the diseases of liver in developed countries are excessive consumption of alcohol and viral infections, while in the developing countries the causative factors are environmental toxin, parasitic disease, hepatitis viruses and some drugs

(chemotherapeutic agents, antibiotics, high doses of carbon tetrachloride (CCl₄), paracetamol¹⁹. Based on the aforementioned findings this research was designed to evaluate the beneficial effects of zamzam water on experimentally induced diabetes, renal toxicity and hepatotoxicity in rats.

MATERIALS AND METHODS

Materials

Zamzam water was obtained from Makkah, Saudi Arabia to perform chemical analysis as well as biological study. All chemicals used in this study were obtained from Sigma Chemical Company (St. Louis, USA).

Different commercial diagnostic kits were purchased from BioMerieux Company (L'Etoile/France) and from Eagle Diagnostics (Dallas, TX, USA).

The animals used in the biological experiment were fifty six male albino rats (Sprague Dawley strain) of an average weight 150 ± 5 g). The rats were obtained from animal house of Agriculture Research Center (ARC).

Methods

Chemical Analysis

The procedures of zamzam water analysis were based on American Public Health Association (APHA)²⁰.

Animal Experiment

Diet Preparation

Basal diet and the vitamin mixture were prepared according to the method described by Campbell,²¹ on diet bases: Protein (12%), fat (8), salt mixture (4%), vitamin mixture (1%), cholin chloride (0.25%), and cellulose (5%) sucrose (10%), L-cystein (0.18%) and the remainder was starch (table 1). The salt mixture was prepared according to Reeves et al²².

Experimental Animal Design

The fifty six albino rats were divided into four groups as follow:

The first main group (14 rats) was considered as negative normal control and divided into two groups:

Subgroup 1a (7 rats) fed on basal diet + tap water

Subgroup 1b (7 rats) fed on basal diet + zamzam water

The second main group (14 rats) was injected with alloxan (150 mg/ kg b.wt.) intraperitoneally²³ to induce hyperglycemia, after 4 days, blood samples were obtained from rats eyes to estimate glucose levels. Rats with fasting serum glucose more than 200 mg /dl were considered as diabetics and then divided into two groups:

Subgroup 2a (7 rats) fed on basal diet + tap water.

Subgroup 2b: (7 rats) fed on basal diet + zamzam water

The third main group (14rats) was injected with gentamicin (amino glycosides antibiotics) about (100 mg/kg /day) for 5 days intraperitoneally to induce nephrotoxicity, according to the methods described by²⁴ and then divided into two groups:

Subgroup 3a: (7 rats) fed on basal diet + tap water.

Subgroup 3b: (7 rats) fed on basal diet + zamzam water.

The fourth main group (14rats) was subcutaneously injected with carbon tetrachloride (Ccl₄) in paraffin oil (50% V/V 2ml/kg b.wt.) twice a week for two weeks, to induce chronic damage in the liver²⁵. The rats injected with Ccl₄ were divided into two groups:

Subgroup 4a: (7 rats) fed on basal diet + tap water.

Subgroup 4b: (7 rats) fed on basal diet + zamzam water.

Animals were kept in stainless steel cages. Food and water were allowed ad libitum to the animals during the whole experiment which lasted for 4 weeks.

Biological evaluation

The biological effect of zamzam water were assessed by the determination of food intake, body weight gain and feed efficiency ratio throughout the experimental period.

The feeding experiment continued for four weeks after injection of rats and at the end of experiment, the rats were fasted for 12 hr., and then sacrificed under ether anesthetize. Blood samples were collected from the hepatic portal vein and were received into clean dried centrifuge tubes and left to clot at room temperature, then centrifuged for 10 minutes at 3000 r.p.m to separate serum. Serum was carefully separated into dry clean and kept frozen at (-20°C) till analysis.

Livers, Kidneys, pancreas, were carefully removed, cleaned, washed in saline solution and dried in filter paper and weighed for the determination of. Organs to body weight percent. The percent of the organ to body weight was calculated as follows: (organ weight / animal weight) x 100).

Biochemical Assessment

Determination of serum glucose was carried out according to methods by Trinder²⁶. Total lipids were determined according to method of Zollner and Kirsch²⁷. Total cholesterol was determined according to method by Allain et al²⁸.

The triglycerides in serum were colorimetrically determined according to Fossati and Principe²⁹. The high density lipoprotein cholesterol (HDL-Ch) was determined according to and Lopez- Virella³⁰. The concentration of very low density lipoprotein (VLDL-C) was estimated according to the Friedewald's equation³¹.

$VLDL-C \text{ (mg/dl)} = \text{Triglycerides} / 5$

The low density lipoprotein cholesterol (LDL-Ch) was determined according to Wieland, and Seidel³².

Kidney function was assessed by determination of serum urea according to the method described by Fawcett, and Scott³³, and creatinine was determined according to kinetic method of Henry³⁴.

Liver Function was assessed by determination of serum aspartate aminotransferase (AST), and serum alanine aminotransferase (ALT) according to the procedure described by Reitman, and Frankel³⁵. Determination of serum alkaline phosphatase (ALP) was carried out according to Belfield and Goldberg³⁶, and serum total bilirubin according to the method described by Walter and Gerade³⁷.

Statistical Analysis

The results were expressed as mean \pm SD. Data was analyzed by one way analysis of variance (ANOVA) the differences between means were tested for significance using least significant difference (LSD) test at ($p < 0.05$). SPSS, (2008).

RESULTS

Chemical estimation of Zamzam and Tap water

The chemical analysis of zamzam water (Zw) and tap water (Tw) was shown in table (2).

Results are measured with part per million (ppm) or milligrams per liter (mg/l).except PH.

Zamzam water was proved to have significant amounts of some inorganic elements when compared to tap water. These elements include sodium (Na) 133:37.8mg/l, calcium (Ca) 130:35.2 mg/l, magnesium (Mg) 32:13.9 mg/l, chloride (Cl) 91:30 mg/l, potassium (K) 13:5.7 mg/l, fluoride (Fl) 0.68:0.0, Nitrate (NO₃) 26:19 mg/l, bicarbonate (HCO₃) 195.4:144 mg/l, sulfate (SO₄) 293.58:30.1 mg/l and total dissolved solids (TDS) 607:233 mg/l respectively .The pH of zamzam water is more than tap water (7.8, 6.8 respectively).

Nutritional evaluation

The results of body weight gain (BWG), Food intake (FI) and feed efficiency ratio (FER) of normal, diabetic, nephrotoxic and hepatotoxic groups were represented in table (3).

As shown in the table, the mean values of BWG, FI and FER of the diabetic group (2a) were significantly lower than those of normal group (1). The values reported for BWG, FI and FER of normal group were 45.91 ±1.10g, 504.28 ±3.14g and 0.090±0.01 respectively and the values for diabetic rats (2a) were 24.56±3.24g, 397.14 ± 3.71 g and 0.061 ±0.008 respectively.

These values were significantly improved in the diabetic group (2b) that treated with zamzam water (35.36 ± 1.98g, 497.0 ± 2.82g and 0.071 ±0.02 respectively).

Our data also revealed that BWG, FI and FER were significantly decreased in nephrotoxic rats (3a) (27.69± 2.45g, 392.42 ± 3.15g and 0.069 ±0.020 respectively) compared with normal group 1 (45.91 ± 1.10g, 504.28 ± 3.14g and 0.09 ± 0.01 respectively). These parameters were also improved in rats treated with zw (3b).

Concerning the hepatotoxic group (4a), a significant decrease was also observed in the mean values of BWG, FI and FER when compared with normal group 1 (table 3). The effect of zw on the organs weight percentage (pancreas, Liver and kidney) of normal diabetic, nephrotoxic and hepatotoxic groups was shown in table (4).

Results showed that no marked difference was noticed between weight percent of pancreas, liver or kidney of normal rats either given tap water or zamzam water (groups 1a and 1b)

On the other hand data illustrated that the percent weight of pancreas, liver and kidney of diabetic group (2a), nephrotoxic group (3a) and hepatotoxic group (4a) were increased significantly when compared with normal group (1). Zamzam water administration to the previous diseased groups resulted in significant reduction in the weight percent of these organs (groups 2b, 3b and 4b).

Serum glucose and lipid profile

Data in table (5) showed serum glucose level of normal group (1a, 1b), diabetic groups (2a, 2b) nephrotoxic groups (3a, 3b) and hepatotoxic groups (4a, 4b).

It was clear that serum glucose level was not significantly changed between normal groups 1a and 1b (86.7± 5.52 and

Table 1: Composition of basal Diet (g/Kg diet).

Ingredients	Amount (g/kg)
Casein	120
Corn oil	80
Salt mix.	40
Vit. mix.	10
Cellulose	50
Choline chloride	2.5
sucrose	100
Corn starch	597. 5
Total	1000

Table 2: Chemical Composition of ZamZam Water and Tap Water.

parameters	ZamZam Water	Tap Water
Color	Colorless	Colorless
Odor	Odorless	Odorless
Turbidity	0.6	0.1
PH	7.8	6.8
Total Hardness	210	146
Calcium hardness	125	88
Magnesium	85	58
Hardness		
Calcium	130	35.2
Magnesium	32	13.9
Sodium	133	37.8
potassium	13	5.7
Carbonate	0.0	0.0
Bicarbonate	195.4	144
Chloride	91	30
Sulfate	239.58	30.1
Ammonia	0.0	0.0
Nitrite	0.0	0.0
Nitrate	26	19
Iron	0.16	0.3
Manganese	0.04	0.05
Fluoride	0.68	0.0
Total Dissolved Solids	607	233

87.87 ±4.23mg/dl respectively). Results revealed that serum glucose level increased significantly in diabetic group (2a), nephrotoxic group (3a) and hepatotoxic group (4a) when compared with normal group 1a (294 ± 1.45, 120 ± 4.70, 122.2 ± 5.21 and 86.7 ± 5.52mg/dl respectively).

It was also noticed that serum glucose levels was significantly decreased in groups 2b, 3b and 4b (123± 7.07, 101.0± 5.60 and 89.45± 5.27 mg/dl respectively) when compared to groups 2a, 3a and 4a (294.21± 1.45, 120.0± 4.70 and 122.20± 5.21 respectively). This improvement in glucose level was attributed to zamzam water administration to these diseased groups.

The values reported for serum total cholesterol, total lipids, LDL cholesterol, HDL-cholesterol and the triglycerides of the different experimental groups are shown in table (5).

It can be noticed that all lipid parameters except HDL cholesterol were increased significantly in diabetic group (2a), nephrotoxic group (3a) and hepatotoxic group (4a) when compared with normal -ve control group (1a). Also

Table 3: Body weight gain (BWG), food intake (FI) and feed efficiency ratio (FER) of the different experimental groups.

Groups	Parameters		
	Body weight gain (g)	Food intake (g)	Feed efficiency ratio (FER)
G1: Normal (-ve control)			
1a (Tw)	45.91 ± 1.10 ^a	504.28 ± 3.14 ^a	0.090 ± 0.01 ^a
1b (Zw)	45.17 ± 2.50 ^a	505.28 ± 2.42 ^a	0.089 ± 0.02 ^a
G2: Diabetic			
2a (Tw)	24.56 ± 3.24 ^c	397.14 ± 3.71 ^c	0.061 ± 0.008 ^b
2b (Zw)	35.36 ± 1.98 ^b	497.0 ± 2.82 ^b	0.071 ± 0.02 ^b
G3: Nephrotoxic			
3a (Tw)	27.69 ± 2.45 ^c	392.42 ± 3.15 ^c	0.069 ± 0.020 ^b
3b (Zw)	44.78 ± 1.91 ^a	496.57 ± 2.50 ^b	0.089 ± 0.017 ^a
G4: Hepatotoxic			
4a (Tw)	26.57 ± 2.80 ^c	392.85 ± 3.57 ^c	0.067 ± 0.01 ^{ab}
4b (Zw)	39.43 ± 4.05 ^b	492.71 ± 2.05 ^b	0.079 ± 0.008 ^a

Data were expressed as mean ± SE

No significant difference between the values that share the same superscript letters in each column ($P \leq 0.05$).

Table 4: Effect of zamzam water on organ weight percentage of the different experimental groups.

Groups	Organ		
	Pancreas%	Liver %	Kidney %
G1: Normal (-ve control)			
1a (Tw)	0.16±0.05 ^b	3.50±0.04 ^b	0.52±0.04 ^b
1b (Zw)	0.16±0.04 ^b	3.51±0.16 ^b	0.55±0.03 ^b
G2: Diabetic			
2a (Tw)	0.31±0.02 ^a	4.14±0.39 ^a	0.62±0.08 ^a
2b (Zw)	0.16±0.01 ^b	3.58±0.16 ^b	0.53±0.05 ^b
G3: Nephrotoxic			
3a (Tw)	0.34±0.27 ^a	4.59±0.13 ^a	0.67±0.09 ^a
3b (Zw)	0.16±0.15 ^b	3.57±0.29 ^b	0.56±0.04 ^b
G4: Hepatotoxic			
4a (Tw)	0.30±0.04 ^a	4.59±0.12 ^a	0.70±0.05 ^a
4b (Zw)	0.16±0.01 ^b	3.52±0.27 ^b	0.54±0.01 ^b

Data were expressed as mean ± SE

No significant difference between the values that share the same superscript letters in each column ($P \leq 0.05$).

data demonstrated that administration of zamzam water to the above mentioned groups reduced, total lipids, total cholesterol, LDL-Ch, triglycerides and increased HDL-Ch (groups 2b, 3b, 4b respectively).

Liver and kidney functions

The effect of zamzam water (ZW) on liver and kidney functions in normal, diabetic, nephrotoxic and hepatotoxic groups was represented in table (6).

Data showed that there was no significant change in liver enzymes (ALT, AST and ALP) and bilirubin levels between the two normal groups 1a (given tap water) and 1b (given zw water).

As shown the parameters of liver function (ALT, AST ALP and bilirubin) were all deranged due to injection of alloxan and the resulting hyperglycemia. Marked increase in the activities of these enzymes was noted in the diabetic group (2a) compared with normal groups (1a). The values

reported for ALT, AST, ALP and bilirubin for normal rats were 20.12 ± 1.20, 20.33 ± 3.30, 84.18 ± 1.90 U/L and 0.968 ± 0.55 respectively and the values for diabetic rats were 46.32 ± 1.50, 46.24 ± 2.24, 91.48 ± 0.22 and 2.73 ± 0.15 U/L respectively. Also the parameters of kidney function namely urea and creatinine were significantly higher than normal levels in the diabetic group (2a). The reported values for urea and creatinine in normal group (1a) were 17.92 ± 0.78 and 0.597 ± 0.11 mg/dl respectively. In case of diabetic group (2a) the values were 53.97 ± 1.78 and 1.45 ± 0.14 mg/dl respectively.

Moreover the serum levels of ALT, AST, ALP, bilirubin, creatinine and urea decreased significantly in the diabetic group treated with ZW (2b) compared with the diabetic group which given tap water (2a).

The previous table (6) showed the effect of zw on liver function markers (ALT, AST, ALP and bilirubin) in nephrotoxic rats. Data showed that the levels of these enzymes increased significantly in nephrotoxic groups (3a) that given tap water when compared with normal group (1a).

On the other hand, the levels of these enzymes were decreased significantly in nephrotoxic group (3b) treated with ZW.

Also nephrotoxic group (3a) showed significant increase in urea and creatinine levels when compared with normal groups.

Concerning the effect of zamzam water (zw) on liver function markers in hepatotoxic rats (table 6), results illustrated a significant elevations in serum levels of ALT, AST, ALP and bilirubin levels were observed in CC14 treated rats (group 4a) compared with the normal control group (1a).

Administration of zamzam water (as sole source of drinking water) to rats treated with CC14, induced significant decrease in serum ALT, AST, ALP and bilirubin levels (group 4b) compared with untreated rats (group 4a) and kept their levels near the normal control.

Furthermore, the urea and creatinine levels were increased significantly in the hepatotoxic group 4a (given Tw) compared with the normal group (-ve control). Meanwhile

Table 5: Effect of zamzam water on glucose level and lipid profile in normal, diabetic, nephrotoxic and hepatotoxic groups.

Groups	Parameter						
	Glucose mg/dl	Total lipids mg/dl	Total cholesterol mg/dl	Triglycerides mg/dl	LDL-Ch mg/dl	HDL-ch mg/dl	VLDL-ch mg/dl
G1: Normal (-ve control)							
1a (Tw)	86.77± 5.52 ^C	304.0± 5.77 ^C	109.98± 2.12 ^C	84.53± 4.37 ^b	24.28± 7.25 ^C	68.78± 3.19 ^a	16.90± 2.28 ^b
1b (Zw)	87.90± 4.23 ^C	307.06± 4.53 ^C	110.04± 1.68 ^C	86.97± 4.36 ^b	23.13± 3.25 ^C	69.51± 1.87 ^a	17.39± 2.30 ^b
G2: Diabetic							
2a (Tw)	294.21± 1.45 ^a	532.50± 1.76 ^a	180.03± 5.52 ^a	172.49± 3.95 ^a	102.90± 0.77 ^a	42.69± 2.17 ^b	34.43± 1.81 ^a
2b (Zw)	123.0± 7.07 ^b	350.01± 4.72 ^b	130.96± 5.45 ^b	88.11± 5.95 ^b	43.71± 2.51 ^b	69.59± 3.19 ^a	17.62± 1.19 ^b
G3: Nephrotoxic							
3a (Tw)	120.0± 4.70 ^a	510.50± 6.19 ^a	180.13± 4.94 ^a	150.3± 5.76 ^a	108.34± 2.35 ^a	41.73± 2.41 ^b	30.05± 2.45 ^a
3b (Zw)	101.0± 5.60 ^b	343.07± 5.89 ^b	130.44± 2.00 ^b	82.18± 5.68 ^b	43.44± 8.13 ^b	70.56± 3.12 ^a	16.43± 2.6 ^b
G4: Hepatotoxic							
4a (Tw)	122.20± 5.21 ^a	491.09± 4.99 ^a	173.13± 2.65 ^a	170.82± 5.78 ^a	95.32± 3.92 ^a	43.65± 3.63 ^b	34.16± 1.15 ^a
4b (Zw)	89.45± 5.27 ^b	327.68± 4.16 ^b	121.51± 3.98 ^b	84.65± 8.30 ^b	35.40± 3.87 ^b	69.18± 4.04 ^a	16.93± 1.66 ^b

Data were expressed as mean ± SE

No significant difference between the values that share the same superscript letters in each column ($P \leq 0.05$).

urea and creatinine levels of hepatotoxic group 4b (treated with zw) decreased significantly when compared with hepatotoxic group 4a (administered with tw).

DISCUSSION

The above results of the chemical analysis of zamzam water and tap water are in agreements with many studies. Koshak,² found that zw is clearly different from normal water in various ways: first there is no bacterial contamination. Second there is no moulds that may change colour, taste or smell, additionally zw contains high level of Na estimated as 133 mg/l. The increase of nitrate level in Zw plays an important role due to the antibacterial action³⁸. Thus, high nitrate levels coupled with microbiological safety might be a favorable attribute of zamzam water and could be responsible for some of its benefits³⁹. Zamzam water contains multimineral like Mg, Ca, Fe, Na, Fl, K, HCO₃, NO₃, SO₄ and totally dissolved salts (TDS),^{40,41}. Moreover zamzam water is considered as the richest of all waters in the world in calcium⁴². The pH of zamzam water is more than tap water⁴³. In contrast it was reported that zw had likely more harmful elements as compared to tap water such as Cl, nitrites, Na and Fl. Although their levels were nearly low and within the safety range⁴⁴, their harmful effects were controlled by other elements that either retard absorption or bad effect of these elements.

The results of body weight gain, food intake and feed efficiency ratio showed significant decrease in the diabetic, nephrotoxic and hepatotoxic groups as compared with normal control group. This finding is consistent with previous reports of weight loss in diabetic rats after injection of alloxan as a result of increased catabolism^{45,46}. Our data are also in consistent with Ademiliuyi et al.,⁴⁷ who indicated that the body weight was significantly reduced in rats administered gentamicin as compared to normal control. Khan, et al.⁴⁸ showed that the toxicity of CCl₄ caused body weight loss and abnormal tissue weight increase in rats. These results are in accordance with those of Wahba⁴⁹ who reported that liver weights of alloxan diabetic rats were higher than in normal rats. This increase appears to be due to the diabetogenic effect of alloxan which resulted in insulin deficiency. Moreover our data are in agreement with Adewole et al.,⁵⁰ and Khan et al.,⁴⁸ who reported that the toxicity of carbon tetrachloride (CCl₄) indicated significant increase in tissue weight as a share of total weight of liver and kidney, when compared with normal control.

The improvement in the organs weights percent may be attributed to the antioxidant character of zamzam water which protects the body against degenerative diseases. This may explain the protective action of zm particularly regarding the pancreas, against the free radicals generation known to occur in diabetes^{51,39}.

Table 6: Effect of Zamzam water on liver and kidney functions in normal, diabetic, nephrotoxic and hepatotoxic groups.

Groups	Parameter					
	ALT (U/L)	AST (U/L)	ALP (U/L)	Bilirubin (U/L)	Creatinine mg/dl	Urea mg/dl
G1: Normal (-ve control)						
1a (Tw)	20.12 ±1.20 ^C	30.33±3.30 ^d	84.18±1.90 ^b	0.968±0.55 ^c	0.597±0.11 ^C	17.92±0.78 ^C
1b (Zw)	22.13±1.80 ^C	24.71±2.56 ^c	86.01±2.70 ^b	0.978±0.08 ^C	0.555±0.15 ^C	17.41±1.22 ^C
G2: Diabetic						
2a (Tw)	46.32 ±1.50 ^a	46.24 ±2.24 ^a	91.48 ±0.22 ^a	2.73±0.15 ^a	1.45 ±0.14 ^a	53.97 ±1.78 ^a
2b (Zw)	25.97±1.60 ^b	33.6 ±5.70 ^b	84.46 ± 2.70 ^b	1.35±0.21 ^b	0.66 ±0.10 ^b	20.95 ±0.44 ^b
G3: Nephrotoxic						
3a (Tw)	57.74 ±3.07 ^a	56.58±7.53 ^a	95.34±1.07 ^a	2.48±0.21 ^a	2.41±0.18 ^a	65.78±2.12 ^a
3b (Zw)	23.46±2.51 ^b	35.06±5.01 ^b	87.75±0.32 ^b	1.38±0.39 ^b	0.957±0.45 ^b	38.68±5.75 ^b
G4: Hepatotoxic						
4a (Tw)	84.75±1.75 ^a	87.14±3.70 ^a	122.68±0.70 ^a	2.37±0.11 ^a	2.36±0.14 ^a	71.02±1.60 ^a
4b (Zw)	28.96±3.11 ^b	31.48±3.21 ^b	87.84±0.20 ^b	1.42±0.23 ^b	0.64±0.23 ^b	37.92±1.80 ^b

Data were expressed as mean ± SE

No significant difference between the values that share the same superscript letters in each column ($P \leq 0.05$).

Serum glucose level was significantly increased in the diabetic group compared to normal control. These results are in agreement with Bukhar et al.,⁵² who revealed that blood glucose was significantly increased in alloxan induced diabetic rats compared with negative control.

Our emerged data demonstrated that zamzam water treatment significantly decreased serum glucose level which in agreement with Jin et al.⁵³ who reported that zamzam water has been shown to improve the glycemic parameters in diabetic rats by various mechanisms. Zamzam water is alkaline natural water and rich in many minerals⁵⁴ which makes it potentially capable of enhancing antioxidant power⁵¹. This was in agreement with the previous finding that reported a significant reducing effect of glucose resulted from alkaline water in animals⁵⁵.

The previous results showed that glucose level increased in nephrotoxic rats (gentamicin injected rats). Meanwhile rats treated with zamzam water showed significant decrease in serum glucose level.

These data are coincided with those of Manikandan et al.⁵⁶ and Gowrisri et al.⁵⁷. Moreover, Boschero and Delattre⁵⁸ illustrated that gentamicin (GM) reduced insulin release from pancreas islets; it is conceivable that the decrease of insulin simultaneously increased glucose level. Besides the reduced glomerular filtration rate induced by GM. In addition, GM administration has been proven to cause marked alternation of some enzymes involved in carbohydrate metabolism⁵⁹. Khan et al.⁶⁰ also documented that there was glucosuria in gentamicin treated rats and this could be due to proximal tubular necrosis.

The present data also demonstrated that serum glucose level was slightly increased in carbon tetra chloride treated rats as compared to control groups (normal). These results were supported by Alhazza et al.,⁶¹ and Ozturk et al.⁶² and who reported that glucose level increased by CCl₄ intoxication.

The results reported from this study show that most of the lipid parameters of the diabetic rats were disturbed. It was noticed a significant increase in the level of total lipids,

LDL-Ch, total cholesterol and triglycerides and a reduction in HDL-Ch concentration. Dyslipidemia has been reported in many studies to associate diabetes and perhaps remain for a time after treatment of hyperglycemia⁶³.

Our results in this regard are in agreement with Chigozie and Chidinima⁶⁴ and Kannan et al.⁶⁵ who reported that induction of diabetes caused hyperlipidemia due to higher level of cholesterol.

Tama and Sagiran⁶⁶ also found that administration of zamzam water can increase HDL level and decrease LDL level of diabetic rats.

The increase of lipid parameters in the nephrotoxic group were supported by Rashid et al.,⁶⁷ who found that gentamicin causes hypercholesterolemia as is evident by the rise in level of cholesterol in gentamicin treated group. Ademiluyi et al.⁴⁷ also confirmed an increase in the levels of triglycerides, total cholesterol and LDL-cholesterol coupled with a concomitant decrease in HDL-cholesterol in gentamicin administrated rats. Also the increase in lipid parameters in hepatotoxic rats was in agreement with Khan, et al.,⁴⁸ who stated that administration of CCl₄ increased triglycerides, total cholesterol, LDL-Ch while decreased HDL-Ch levels.

As shown in table (6) the parameters of liver function (ALT, AST ALP and bilirubin) and kidney function (urea and creatinine) were all deranged due to injection of alloxan and the resulting hyperglycemia.

These results are in agreement with various studies which indicated that alloxan has deleterious effects on liver and kidney^{68,69}. Disruption in liver function that is demonstrated with increasing in ALT and AST has been occurred one week after alloxan injection⁷⁰.

Many researchers have investigated an association between diabetic complications and disturbance in many tissues like diabetic nephrotoxicity and peripheral neuropathy. Oxidative stress is thought to have a major role in the progress of such complications^{71,72}.

The improvement of these parameters may be attributed to the antioxidant activity of zw^{51,39}.

The previous table (6) showed the effect of zw on liver function markers (ALT, AST, ALP and bilirubin) and kidney function markers (urea and creatinine) in nephrotoxic rats. Data showed that the levels of these markers increased significantly in nephrotoxic group (3a) that given tap water when compared with normal group (1a).

These results coincided with those of Faramobi and Ekor²⁴, Khan et al.⁷³ and Ademiluyi et al.⁴⁷ who reported that serum AST and ALT levels increase in patients receiving antibiotic injection. This was in accordance with our observations, where rats received gentamicin (GM) injection.

Moreover Randjelovic et al.,⁷⁴ and Gowrisri et al.,⁵⁷ who stated that GM administration to rats produces nephrotoxicity which was manifested by marked increase in serum creatinine and urea levels.

The improvement in kidney and liver functions in nephrotoxic rats after administration of zamzam water was related to its antioxidant capacity where it is similar to alkaline water and can boost the antioxidant mechanisms in rats stressed by gentamicin^{75,4}

A significant increase of serum transaminases activities (AST, ALT and ALP) of the hepatotoxic group (table 6) was in agreement with Alhazza et al.⁶¹ and Khan et al.⁴⁸ who reported that carbon tetrachloride (CCl₄) has harmful effects on the liver function.

Hepatotoxicity of carbon tetra chloride (CCl₄) is largely due to its active metabolite, trichloromethyl radical⁷⁶. Elevation of serum transaminases are indicators of cellular leakage and loss of functional integrity of hepatocellular membrane⁷⁷. On the other hand serum alkaline phosphatase (ALP) and bilirubin are closely related to the liver function. Elevation of ALP level may be due to increased its synthesis as a result of increasing biliary pressure⁷⁸. There was a marked restoration of these enzymes levels on administration of zw. This improvement may be attributed to the prevention of leakage of intracellular enzymes by the membrane stabilizing power (88). Zamzam water is a potential protective agent against CCl₄ hepatotoxicity in rats⁷⁹. Besides the protective role of zw on hepatotoxicity of CCl₄ in rats may be clearly appear to be due to inhibition of lipid peroxidation and activation of antioxidant enzymes, and to free radicals scavenging action⁸⁰.

Zamzam water minimizes the injurious effect of the famous toxin CCl₄ on the liver. It is interesting to note the similarity between zw and electrolyzed- reduced water (ERW) in this respect. ERW or alkaline water extremely treated the CCl₄- induced liver lesions and lowered the serum levels of hepatic enzyme markers ALT and AST⁸¹. The disturbance in the levels of serum urea and creatinine of the hepatotoxic group are supported by many studies which proved that CCl₄ produced significant toxic changes in kidneys, lungs, and testis as well as in blood by generating free radicals⁸².

Findings by Ogeturk et al.⁸³, and Adewole et al.⁵⁰ suggested that exposure to CCl₄ causes renal injuries as well as oxidative stress and marked depletion of endogenous antioxidant enzymes in kidneys. In addition severe

impairment in renal function was seen as evaluated by increased serum creatinine and blood urea nitrogen and decreased urea and creatinine clearance. Most of studies proved that CCl₄ enhance peroxidation of lipids, decreases renal microsomal NADPH cytochrome P450, and renal reduced/oxidized glutathione ratio (GSH/GSSG) in kidney cortex as well as renal microsomes and mitochondria⁸⁴.

CONCLUSION

Zamzam water is a potential protective agent against diabetes mellitus, liver toxicity and kidney toxicity due to its antioxidant capacity. Further studies are needed to explore the beneficiary effect of zamzam water and to evaluate its effect on other conditions of oxidative stress.

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