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Zamzam Water Ameliorates Oxidative Stress and Reduces HemoglobinA1c in Type 2 Diabetic Patients

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Abstract

Zamzam water is alkaline natural water which makes it potentially capable of enhancing antioxidant power. We have carried out this study in type 2 diabetic patients to evaluate the effect of Zamzam water on their oxidant-antioxidant status, glycemic control and lipid profile. Forty nine type 2 diabetic patients were recruited from Dammam University primary health care unit in Alkhobar. The patients were randomly divided into two groups each drank one liter/day of water for two months; one group received ordinary bottled water while the other drank Zamzam water. Baseline and post treatment levels of antioxidant parameters, fasting blood sugar, HbA_{1c}, lipid profile, LFT, RFT, and CBC were measured in both groups. Zamzam group patients showed a significant increase in the serum levels of total antioxidant capacity, Catalase, Superoxide dismutase, and glutathione. However, total antioxidant capacity and superoxide dismutase were decreased significantly, while catalase and glutathione were not changed significantly in the control group. Serum TBARS was not changed significantly in both group. Patients receiving Zamzam water had a significant decrease in HbA_{1c} but not in fasting blood sugar. Both HbA_{1c} and fasting blood sugar did not change significantly in the control group. Other parameters either did not change or showed little significant change but within the normal limits, of these parameters, in both groups. In conclusion drinking Zamzam water enhanced antioxidant power and reduced HbA_{1c} significantly in type 2 diabetic patients. Further research is needed in this area to confirm the results and explore the mechanism behind HbA_{1c} lowering effect produced by Zamzam water.

Keywords: Zamzam water; Antioxidants; Diabetes mellitus; Glycated hemoglobin; HbA_{1c}

Introduction

Diabetes is a serious health hazard currently affecting more than 220 million people worldwide and expected to afflict 400 million by 2030 [1,2]. Diabetes being a metabolic disorder produces, in the long run, cell dysfunction in almost all organs in the body. The most serious complications of diabetes are: coronary artery disease, nephropathy, retinopathy and neuropathy. Oxidative stress is thought to play a major role in the development of most of these complications [3-5].

Oxidative stress, an imbalance between oxidant and antioxidant mechanisms in animal bodies, has been implicated in many diseases and their complications [6-8]. The imbalance may result either form excessive exposure to pro-oxidants or from compromised antioxidant mechanisms. The later may result from deficiency of essential elements or from incapacitation of the antioxidant machinery through the pathologic insult of disease, while the earlier might emanate from exposure to exogenous toxins or the pathologic stress of disease [6,9]. Oxidative stress thus may occur in normal animals when antioxidant mechanisms are not working properly as in dietary deficiencies of vitamin E, vitamin C or the essential elements like selenium, zinc, and manganese among others. The later elements are essential components of the antioxidant enzymes glutathione peroxidase, superoxide dismutase and catalase [10-12]. Another important cause of oxidative stress is the exaggerated endogenous production of free radicals by disease processes as in diabetes mellitus and cancer [6]. Exposure to exogenous toxins is still another mode for inducing oxidative stress as in the toxicity of some drugs like gentamicin [9] or industrial chemicals like carbon tetrachloride [13]. Apparently, then oxidative stress can be combated by strategies that promote and foster the antioxidant defense mechanisms.

Water has been shown to strengthen the antioxidant capacity of animal bodies [14]. 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 [15] and slow the aging process for which oxidative stress has been proposed as the main contributor [16]. This water has also been shown to improve the glycemic control in diabetic rats by unknown mechanisms [17]. 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 [18]. 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 beliefs. The well is located in Makkah in the holy mosque (Haram). When they visit Makkah, pilgrims

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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 [19], which make it a potential antioxidant agent. Therefore, this study was designed to investigate the effect of 2 months Zamzam water ingestion on oxidant-antioxidant status, glycemic control, and lipid profile in type 2 diabetic patients.

Materials and Method

Water samples

Zamzam water samples were obtained directly from the well. The samples were treated with UV for sterilization. Zamzam water was then prepared in small sterilized bottles (330 ml) by a specialized factory. The bottles were packed in cartoons (40 bottles each). Control water was bought from local market, in bottles of the same size (330 ml). All labels were removed from bottles and they finally had similar shape to the test (Zamzam) water bottles. Each patient, in both groups, was instructed to drink three bottles a day and was given the quantity for the whole period of the study (2 months). The amount of water consumed was monitored by asking the patient, on the final visit, how many bottles were left with him. The study was conducted in all aspects related to research ethics according to Declaration of Helsinki.

Patients

Forty nine uncontrolled diabetic patients were randomly selected from the primary health care clinic of the University of Dammam at Al-Khobar Government Hospital. Patients' randomization was through a simple 1:1 allocation to the test or control group. Only those patients with uncontrolled type 2 diabetes mellitus (defined by glycated Hemoglobin (HbA_{1c})>7%) were selected to be enrolled in the study. The nature of the study was explained to all patients and an informed consent was obtained. Patients were on oral hypoglycemic drugs (glipenclamide, metformin, rosiglitazone) and were instructed to continue on their medications and to follow the same life style before initiation of the study.

Exclusion criteria: patients of the following criteria were excluded from the study

1. Age less than 18 or more than 60.
2. Patient with HbA_{1c} less than 7%.
3. Patients with less than 90% compliance.
4. Patients not ready for regular follow-up.
5. Patients with nephropathy or major cardiac problems.

Protocol

At the first visit, an array of baseline investigation was carried on the patients. These fell into one of five categories including: indicators of diabetic control (fasting blood glucose and HbA_{1c}), antioxidant parameters, lipid profile, kidney (RFT) and liver function tests (LFT), as well as complete blood count. Following this, the patients were divided into one of two groups through simple randomization walk in method. The first group of patients (24 total, 8 females) with mean age of 41.9 years was supplied with ordinary bottled water (control). The second group (25 total, 7 females) with mean age 45.9 years was supplied with Zamzam water (test). They were asked to consume three bottles a day of the water they were provided (approximately one liter daily). The above investigations were repeated again at the end of the study.

Blood collection

After a 10-12 hours period of fasting, blood was drawn using venipuncture, between 08.00 and 09.00 am. Patients were asked not to smoke or engage in physical activity for 30 minutes prior to blood extraction. Blood was divided into three portions:

a. The first portion of blood was collected into plain tubes, allowed to clot, to separate the serum, which was used to determine glucose, lipid profile, LFT and RFT.

b. The second portion of blood was collected into plain tubes, to be used in antioxidant assay and other parameters measured in serum.

c. The third portion of blood was collected into EDTA–coated tube and used for complete blood count. The hemolysate was then used in estimation of $HbA_{1c.}$

Laboratory analysis

Catalase, superoxide dismutase, glutathione and thiobarbituric acid reactive species (TBARS): TBARS were analyzed by Cayman kits (Cayman Chemical Co Inc, Ellsworth Rd, Ann Arbor, USA). All the analyses were based on methods previously described (20-24).

Major constituents of the samples of Zamzam water used in the study were measured by ionic chromatography (ionic chromatograph, Metrohm, USA).

Serum levels of Fasting Blood Glucose (FBG), HbA_{1c}, lipid profile, Liver Function Tests (LFT) and Renal Function Tests (RFT): FBG, HbA_{1c}, lipid profile, LFT and RFT 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. HbA_{1c} was assayed automatically by Hb Gold Analyzer, (using Gold Reagent Kit- HbA_{1c}) provided by Drew Scientific Ltd. Germany [20-24].

Statistical analysis

The Statistical Package for Social Sciences (SPSS 14) was used for statistical analysis. Results after two months of water consumption were compared with their corresponding baseline values in the same groupusing paired *t*-test. Statistical significance was set at $p<0.05$.

Results

The average age of the test group (41.9 ± 1.9 years) did not differ significantly from that of the control group (45.7 ± 1.8 years). There was no significant difference between the two groups of patients in sex distribution and duration of diabetes. Chemical composition of Zamzam water and ordinary water samples, used in this study, is shown in table 1. The results indicate that Zamzam water is alkaline and has higher levels of nitrate, arsenic, selenium, chromium, and cadmium than ordinary water. Table 2 summarizes the baseline and final levels of serum antioxidant parameters and TBARS for both groups of diabetic patients included in the study. Interestingly, at the end of the two months water consumption, serum total antioxidant capacity, catalase, superoxide dismutase and glutathione were significantly higher than their baseline levels in the group of patients who received Zamzam water. On the contrary, final serum total antioxidant capacity and superoxide dismutase were significantly lower in patients who received the ordinary bottled water. However, glutathione and catalase were not significantly affected in the control group. TBARS did not differ significantly compared to its baseline level in both groups of patients.

Table 3 summarizes the baseline and final levels of fasting blood glucose and hemoglobin A_{1c} (HbA_{1c}) of all patients. Two months water consumption did not affect fasting blood glucose in both groups of patients. Interestingly, the group of patients who drank Zamzam water showed a significant decline in HbA_{1c}. On the other hand, patients in the control group did not show a significant change in the level of HbA_{1c}. All parameters in the lipid profile (Table 4) were not changed significantly in both groups.

Table 5 summarizes the results on blood urea, serum creatinine, uric acid and calcium. A significant rise in serum creatinine and uric acid was encountered in the group given Zamzam water. The same group of patients showed a significant decrease in serum calcium. Liver function tests were not changed significantly in both groups. The control group showed a significant elevation in ESR and mean corpuscular hemoglobin and a significant drop in heamatocrit (Table 6). However, Zamzam group showed no significance change in all parameters in the complete blood count except mean corpuscular volume which was increased a little (Table 6).

Discussion

The results reported in this study indicate, for the first time, a significant antioxidant enhancing power of Zamzam water in type 2

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.

	Ordinary bottled Water			Zamzam Water		
Parameter	**Baseline**	**Final**	**p-value**	**Baseline**	**Final**	**p-value**
Total antioxidant capacity (mM)	4.01 ± 0.14	3.2 ± 0.22	0.001	2.74 ± 0.20	3.81 ± 0.19	0.000
Catalase (nmol/min/ml)	95.0 ± 11.6	87.1 ± 8.4	0.224	119.4 ± 11.6	140.9 ± 11.6	0.001
superoxide dismutase (U/ml)	7.84 ± 0.56	6.12 ± 0.56	0.001	8.45 ± 0.76	9.86 ± 0.70	0.034
TBARS (μM)	42.6 ± 4.6	43.7 ± 4.8	0.714	41.3 ± 4.0	40.8 ± 5.0	0.884
Glutathione (μM)	3.90 ± 0.55	3.42 ± 0.37	0.269	4.65 ± 0.74	6.37 ± 0.74	0.009

Values represent mean ± SEM

Table 2: Baseline and final blood levels of antioxidant parameters in diabetic patients receiving ordinary bottled water (control) or Zamzam water (test) for two months.

	Ordinary bottled Water			Zamzam Water		
Parameter	**Baseline**	**Final**	**p-value**	**Baseline**	**Final**	**p-value**
Fasting blood glucose (mg/dl)	225 ± 15.9	214 ± 15.3	0.445	189.2 ± 13.1	176.1 ± 10.9	0.247
Hemoglobin A1c (%)	10.01 ± 0.27	9.83 ± 0.36	0.465	9.7- ± 0.37	8.96 ± 0.27	0.009

Values represent mean ± SEM

Table 3: Baseline and final levels of fasting blood glucose and hemoglobin A1c in diabetic patients receiving ordinary bottled water (control) or Zamzam water (test) for two months.

Parameter (mg/dl)	Ordinary bottled Water		Zamzam Water	
	Baseline	**Final**	**Baseline**	**Final**
Cholesterol	201 ± 5	206 ± 6	197 ± 8	196 ± 6
Triglycerides	134 ± 13	145 ± 13	162 ± 27	155 ± 20
HDL	45 ± 2	46 ± 3	43 ± 2	42 ± 2
LDL	134 ± 5	133 ±	123 ± 7	127 ± 6

Values represent mean ± SEM
No significant differences

Table 4: Baseline and final lipid profile in diabetic patients receiving ordinary bottled water (control) or Zamzam water (test) for two months.

	Ordinary bottled Water			Zamzam Water		
Parameter	**Baseline**	**Final**	**p-value**	**Baseline**	**Final**	**p-value**
Urea (mg/dl)	28.7 ± 1.6	28.2 ± 1.8	0.700	23.5 ± 1.3	25.2 ± 1.3	0.064
Creatinine (mg/dl)	0.65 ± 0.03	0.65 ± 0.03	0.694	0.65 ± 0.03	0.67 ± 0.03	0.021
Uric acid (mg/dl)	4.4 ± 0.2	4.6 ± 0.2	0.166	4.7 ± 0.3	5.1 ± 0.3	0.004
Calcium (mg/dl)	9.0 ± 0.07	9.2 ± 0.07	0.051	9.2 ± 0.06	9.0 ± 0.09	0.046

Values represent mean ± SEM

Table 5: Baseline and final serum urea, creatinine and uric acid in diabetic patients receiving ordinary bottled water (control) or Zamzam water (test) for two months.

	Ordinary bottled Water			Zamzam Water		
Parameter	**Baseline**	**Final**	**p-value**	**Baseline**	**Final**	**p-value**
ESR (mm/h)	12 ± 2	19 ± 4	0.04	18 ± 3	16 ± 3	0.532
Hemoglobin concentration (g/dl)	14.7 ± 0.3	15.5 ± 0.3	0.41	15.8 ± 0.3	14.4 ± 0.3	0.180
HCT (%)	43 ± 0.6	41 ± 0.5	0.000	42 ± 0.6	42 ± 0.8	0.118
RBC (mil/cmm)	6.4 ± 1	5.2 ± 0.1	0.244	5.3 ± 0.08	5.2 ± 0.08	0.078
MCV (fl)	80 ± 1.1	80 ± 1.1	0.561	80 ± 1.3	83 ± 1.3	0.005
MCH (pg)	27 ± 0.4	28 ± 0.5	0.000	27 ± 0.5	28 ± 0.5	0.463

Values represent mean ± SEM

Table 6: Baseline and final erythrocyte sedimentation rate (ESR), hemoglobin concentration, hematocrit (HCT), red cell count (RBCs), mean corpuscular volume (MCV) and mean corpuscular hemoglobin(MCH) in diabetic patients receiving ordinary bottled water (control) or Zamzam water (test) for two months.

diabetic patients. This is in general agreement with the previous results reporting antioxidant power of electrolyzed reduced water in chemical solutions [25,26]. Furthermore, electrolyzed-reduced water has been reported to enhance human lymphocyte resistance to the DNA strand breaks induced by H_2O_2 *in vitro* [27]. The antioxidant power of Zamzam water could be due to its alkaline pH and/or to its richness in many minerals needed for antioxidant enzymes activity. The other interesting finding in this study is the significant decrease in HbA_{1c} following 2 months ingestion of Zamzam water in type 2 diabetic patients. Unexpectedly, there was not a corresponding decrease in fasting blood glucose in these patients. This seems to be conflicting with the previous finding of a significant lowering effect of glucose produced by alkaline water in animals [17,28]. However, both of these studies used water samples of pH=10 which is higher than the pH of our samples of Zamzam water used in this study (pH=8). Since fasting blood glucose of our patients did not decrease significantly, an explanation for the decrease in HbA_{1c} is needed. It has been found that HbA_{1c} is negatively and significantly correlating with reduced glutathione in diabetic patients [29]. Furthermore, Testa et al. [30] found that serum antioxidant power influence glycated hemoglobin in type 2 diabetic patients. However, carrying out correlations between HbA_{1c} and antioxidant parameters revealed negative but insignificant correlations in our study. Therefore, the decrease in HbA_{1c} in our study could be due to combination of the insignificant decrease in blood glucose and the enhancement of antioxidant power. Since HbA_{1c} is a predictor of cardiovascular risk in people without diabetes [31], it is worthwhile to confirm and further investigate the cause of HbA_{1c} reduction found in this study. Our findings of no significant effect of Zamzam water on lipid profile seems to be conflicting with previous results reporting a significant decrease in total cholesterol and triglycerides produced by alkaline reduced water in OLETF rats [17]. However, differences in water samples and species could account for this discrepancy. All other significant changes produced by Zamzam water were small and lied within the normal limits of the concerned parameters. In conclusion, the present study shows, for the first time, an antioxidant enhancing power and a reduction in HbA_{1c} produced by Zamzam water in type 2 diabetic patients. Further research is needed with a larger sample size and longer Zamzam water ingestion period to confirm the results of this study.

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