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, HbA1c, 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 HbA1c but not in fasting blood sugar. Both HbA1c 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 HbA1c significantly in type 2 diabetic patients. Further research is needed in this area to confirm the results and explore the mechanism behind HbA1c lowering effect produced by Zamzam water.

Keywords: Zamzam water; Antioxidants; Diabetes mellitus; Glycated hemoglobin; HbA1c

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 functioning 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, 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
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 cartons (40 bottles each). Control water was bought from a 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 glycaated Hemoglobin (HbA1c)>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 (gliclazidamide, metformin, rosiglitazone) and were instructed to continue on their medications and to follow the same lifestyle 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 HbA1c 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 HbA1c), antioxidant parameters, lipid profile, kidney function (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 HbA1c.

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), HbA1c, lipid profile, Liver Function Tests (LFT) and Renal Function Tests (RFT): FBG, HbA1c, 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. HbA1c was assayed automatically by Hb Gold Analyzer, (using Gold Reagent Kit- HbA1c) 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 group using 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 A1c (HbA1c) 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 HbA1c. On the other hand, patients in the control group did not show a significant change in the level of HbA1c. 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 hematocrit (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 diabetes mellitus. The rise in creatinine and uric acid in Zamzam water group may be due to its high concentration of heavy metals and low pH. Zamzam water could be a potential alternative antioxidant source for patients with diabetes mellitus.

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

| Parameter                | Ordinary water | Zamzam water |
|--------------------------|----------------|--------------|
| Calcium Carbonate (ppm)  | 30-340         | 28-32        |
| Magnesium (ppm)          | 19-24          | 23-27        |
| Chromium (ppb)           | ND             | 0.7-0.75     |
| Manganese (ppb)          | 0.07-0.10      | ND           |
| Cobalt (ppb)             | 0.3-0.4        | ND           |
| Copper (ppb)             | 0.5-1.0        | ND           |
| Zinc (ppb)               | 1-2            | ND           |
| Arsenic (ppb)            | ND             | 19-26        |
| Strontium (ppb)          | ND             | 700-800      |
| Cadmium (ppb)            | ND             | 0.2-1.0      |
| Lead (ppb)               | ND             | 0.05-0.1     |
| Nitrate (ppb)            | 3-4            | ND           |
| pH                       | 7.0            | 7.75-8.0     |

ND=Not Detectable

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.

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

| Parameter                                      | Ordinary bottled Water | Zamzam Water |
|-----------------------------------------------|------------------------|--------------|
| Cholesterol                                   | 201 ± 5                | 206 ± 6      | 0.926   | 197 ± 8      | 196 ± 6      | 0.720   |
| Triglycerides                                 | 134 ± 13               | 145 ± 13     | 0.373   | 162 ± 27     | 155 ± 20     | 0.186   |
| HDL                                           | 45 ± 2                 | 46 ± 3       | 0.465   | 43 ± 2       | 42 ± 2       | 0.986   |
| LDL                                           | 134 ± 5                | 133 ± 7      | 0.534   | 123 ± 7      | 127 ± 6      | 0.260   |

Values represent mean ± SEM

No significant differences

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.

| Parameter                                      | Ordinary bottled Water | Zamzam Water |
|-----------------------------------------------|------------------------|--------------|
| 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
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 H₂O₂ 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 HbA₁c 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 [17]. This study has been funded by King Abdulaziz City for Science and Technology, Riyadh, Saudi Arabia.

**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.

| Parameter | Ordinary bottled Water | Zamzam Water |
|-----------|-------------------------|--------------|
| ESR (mm/h) | Baseline: 12 ± 2 | Final: 19 ± 4 | p-value: 0.04 |
|            | Baseline: 15 ± 0.3 | Final: 15.5 ± 0.3 | p-value: 0.41 |
| HCT (%)    | Baseline: 43 ± 0.6 | Final: 41 ± 0.5 | p-value: 0.00 |
| RBC (million/mm³) | Baseline: 6.4 ± 1 | Final: 5.2 ± 0.1 | p-value: 0.244 |
| MCV (fL)   | Baseline: 80 ± 1.1 | Final: 80 ± 1.1 | p-value: 0.561 |
| MCH (pg)   | Baseline: 27 ± 0.4 | Final: 28 ± 0.5 | p-value: 0.000 |

Values represent mean ± SEM

**References**

1. Shaw JE, Sicree RA, Zimmet PZ (2010) Global estimates of the prevalence of diabetes for 2010 and 2030. Diabetes Res Clin Pract 87: 4-14.
2. Wild S, Roglic G, Green A, Sicree R, King H (2004) Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care 27: 1047-1053.
3. Shi Y, Vanhoutte PM (2009) Reactive oxygen-derived free radicals are key to the endothelial dysfunction of diabetes. J Diabetes 1: 151-162.
4. Elmarakby AA, Sullivan JC (2012) Relationship between oxidative stress and inflammatory cytokines in diabetic nephropathy. Cardiovasc Ther 30: 49-59.
5. Tiganis T (2011) Reactive oxygen species and insulin resistance: the good, the bad and the ugly. Trends Pharmacol Sci 32: 82-89.
6. Pilocco D, Zaccardi F, Di Stasio E, Romiti F, Santini SA, et al. (2010) Oxidative stress, nitric oxide, and diabetes. Rev Diabet Stud 7: 15-25.
7. Wei W, Liu Q, Tan Y, Liu L, Li X, et al. (2009) Oxidative stress, diabetes, and diabetic complications. Hemoglobin 33: 370-377.
8. Reuter S, Gupta SC, Chaturvedi MM, Aggarwal BB (2010) Oxidative stress, inflammation, and cancer: how are they linked? Free Radic Biol Med 49: 1603-1616.
9. Narayana K (2008) An aminglycoside antibiotic gentamicin induces oxidative stress, reduces antioxidant reserve and impairs spermato genesis in rats. J Toxicol Sci 33: 85-96.
10. Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, et al. (1973) Selenium: biochemical role as a component of glutathione peroxidase. Science 179: 588-590.
11. Beem KM, Richardson DC, Rajagopalan KV (1977) Metal sites of copper-zinc superoxide dismutase. Biochemistry 16: 1930-1936.
12. Horn A Jr, Pariitha GL, Melo KV, Fernandes C, Horner M, et al. (2010) An iron-based cytosolic catalase and superoxide dismutase mimic complex. Inorg Chem 49: 1274-1276.
13. Cucireanu M, Caruntu ID, Paduraru O, Stoica B, Jerca L, et al. (2009) The protective effect of montelukast sodium on carbon tetrachloride induced hepatopathy in rat. Prostaglandins Other Lipid Mediat 88: 82-88.
14. Nassini R, André E, Gazzieri D, De Siena G, Zanasi A, et al. (2010) A bicarbonate-alkaline mineral water protects from ethanol-induced hemorrhagic gastric lesions in mice. Biol Pharm Bull 33: 1319-1323.
15. Huang KC, Yang CC, Lee KT, Chien CT (2003) Reduced hemodialysis-induced oxidative stress in end-stage renal disease patients by electrolyzed reduced water. Kidney Int 64: 704-714.
16. Hofer T, Marzetti E, Xu J, Seo AY, Gulec S, et al. (2008) Increased iron content and RNA oxidative damage in skeletal muscle with aging and disuse atrophy. Exp Gerontol 43: 563-570.
17. Jin D, Ryu SH, Kim HW, Yang EJ, Lim SJ, et al. (2006) Anti-diabetic effect of alkaline-reduced water on OLETF rats. Biosci Biotechnol Biochem 70: 31-37.
18. WHO (2005) Nutrients in drinking water. World Health Organization, Geneva.
19. Shomar B (2012) Zamzam water: concentration of trace elements and other characteristics. Chemosphere 86: 600-605.
20. Johansson LH, Borg LA (1988) A spectrophotometric method for determination of catalase activity in small tissue samples. Anal Biochem 174: 331-336.
21. Wheeler CR, Salzman JA, Elsayed NM, Omaye ST, Korte DW Jr (1990) Automated assays for superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase activity. Anal Biochem 184: 193-199.
22. Baker MA, Cermigil GJ, Zamanik (1990) Microtiter plate assay for the measurement of glutathione and glutathione disulfide in large numbers of biological samples. Anal Biochem 190: 360-365.
23. Armstrong D, Browne R (1994) The analysis of free radicals, lipid peroxides, antioxidant enzymes and compounds related to oxidative stress as applied to the clinical chemistry laboratory. Adv Exp Med Biol 366: 43-58.

24. Liu D (1996) The roles of free radicals in amyotrophic lateral sclerosis. J Mol Neurosci 7: 159-167.

25. Shirahata S, Kabayama S, Nakano M, Miura T, Kusumoto K, et al. (1997) Electrolyzed-reduced water scavenges active oxygen species and protects DNA from oxidative damage. Biochem Biophys Res Commun 234: 269-274.

26. Hanaoka K (2001) Antioxidant effects of reduced water produced by electrolysis of sodium chloride solutions. Journal of Applied Electrochemistry 12: 1307-1313.

27. Lee MY, Kim YK, Ryoo KK, Lee YB, Park EJ (2006) Electrolyzed-reduced water protects against oxidative damage to DNA, RNA, and protein. Appl Biochem Biotechnol 135: 133-144.

28. Kim MJ, Jung KH, Uhm YK, Leem KH, Kim HK (2007) Preservative effect of electrolyzed reduced water on pancreatic beta-cell mass in diabetic db/db mice. Biol Pharm Bull 30: 234-236.

29. Jain SK, McVie R (1994) Effect of glycemic control, race (white versus black), and duration of diabetes on reduced glutathione content in erythrocytes of diabetic patients. Metabolism 43: 306-309.

30. Testa R, Testa I, Manfrini S, Bonfigli AR, Plantanelli L, et al. (1996) Glycosylated hemoglobin and fructosamines: does their determination really reflect the glycemic control in diabetic patients? Life Sci 59: 43-49.

31. Santos-Oliveira R, Purdy C, da Silva MP, dos Anjos Carneiro-Leão AM, Machado M, et al. (2011) Haemoglobin A1c levels and subsequent cardiovascular disease in persons without diabetes: a meta-analysis of prospective cohorts. Diabetologia 54: 1327-1334.