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Additive effects of Zamzam water in reducing fasting blood glucose and LDL-cholesterol in rats fed on a ketogenic diet

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Abstract

Purpose – The ketogenic diet (KD) has gained popularity due to its ability to improve type 2 diabetes, weight loss, antioxidant and anti-inflammatory activity. However, long-term use of the KD might not be safe due to its adverse effects. This study examined the effects of the KD alone or in combination with Zamzam water (holy water for Muslims) on glucose homeostasis, lipid parameters and oxidant-antioxidant variables in rats.

Design/methodology/approach – Based on the diet given for ten weeks, three groups of adult male Wistar rats were made (12 rats/group): (1) rats which fed on a chow diet and ordinary water, (2) rats which fed on KD and ordinary water and (3) rats which were given KD along with Zamzam. Fasting blood glucose (FBG), serum insulin, insulin resistance (HOMA-IR), LDL cholesterol, HDL cholesterol, superoxide dismutase and malondialdehyde were compared by one-way ANOVA followed by post-hoc Tukey's HSD test among groups.

Findings – Rats which fed on KD and Zamzam water had significantly reduced FBG and LDL cholesterol compared to the rats which fed on a chow diet and ordinary water (p-values 0.001), and KD and ordinary water (p-value 0.004 and 0.006, respectively). Serum insulin, insulin resistance, HDL cholesterol, superoxide dismutase and malondialdehyde did not differ significantly.

Originality/value – Consumption of KD along with Zamzam for ten weeks significantly reduces FBG and LDL cholesterol. KD alone does not decrease these parameters in ten weeks duration.

Keywords Ketone diet, Zamzam water, Fasting blood glucose, LDL cholesterol

Paper type Research paper

Introduction

From the Islamic perspective, Zamzam water is the holy water. This water comes from "the Zamzam well" in al-Masjid al-Haraam (the Sacred Mosque in Mecca, Saudi Arabia). As a custom, Muslims bring Zamzam water for their relatives while returning from Mecca after performing Hajj/Umrah.

According to Al Bukhari (n.d.), Prophet Ibrahim left his wife (Hajar) and son (Ismail) in the hot desert of Mecca by order of God. Hajar failed to find any water over there, but suddenly water sprang out of the land where Ismail scraped with his feet. Regarding Zamzam water, Prophet Mohammad (PBUH) said, "It is blessed; it (even) serves as food" (Al-Hajjaj, 2007).

Zamzam water is rich in minerals and has an alkaline pH (Shomar, 2012). Bacterial growth cannot occur in Zamzam water (Khalid, Ahmad, Khalid, Ahmed, & Irfan, 2014). It can prevent

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nephrolithiasis in rats in which renal stone formation was induced experimentally (Al-Ghamdi, 2012). It can even reduce oxidative stress and glycated hemoglobin levels in type 2 diabetic patients (Bamosa *et al.*, 2013). It can also decrease fasting blood glucose and LDL cholesterol (Abdel-Azeem, Mubarak, Abd-Elhady, & Badawi, 2017).

Despite a religious belief in the health benefits of Zamzam, usage of Zamzam water in medicine is rare. That is because of two primary reasons: (1) lack of research and scientific evidence on Zamzam's effects on health and (2) a ban by the Saudi Arabian government on the export of Zamzam water for commercial use.

The ketogenic diet (KD) (a diet high in fat content and low in carbohydrate and protein content) has gained popularity in recent years due to its ability to improve type 2 diabetes (Bolla, Caretto, Laurenzi, Scavini, & Piemonti, 2019), neurological disorders such as epilepsy (D'Andrea Meira *et al.*, 2019), weight loss (Abbasi, 2018), antioxidant and anti-inflammatory activity (Pinto, Bonucci, Maggi, Corsi, & Businaro, 2018), etc. However, there are concerns that KD may not be beneficial in the long run due to its adverse effects such as constipation (Wibisono *et al.*, 2015), kidney stones (Sampath, Kossoff, Furth, Pyzik, & Vining, 2007), dehydration, loss of electrolytes and nutrients deficiencies (Kang, Chung, Kim, & Kim, 2004).

In view of the beneficial effects of Zamzam water, KD and the limitations of long-term use of KD, we hypothesize that simultaneous intake of KD and Zamzam water may produce beneficial effects on glycemic status, lipid profile and oxidant-antioxidant status more efficiently and in a shorter duration than KD alone. If our hypothesis is proven, Zamzam water and KD together may be promising therapeutic agents for hyperglycemia and hyperlipidemia. To the best of our knowledge, the present study is the first one exploring the effects of Zamzam water together with KD.

Materials and methods

Animals

Adult male Wistar rats (210 ± 16.4 g body weight) were obtained from the animal house of our university, Saudi Arabia. Before commencing the study, the rats were acclimatized for seven days at 25 ± 2°C temperature, 50 ± 15% humidity and 12h of a light-dark cycle. The ethical approval was obtained from the Institutional Review Board of our university (IRB-PGS-2017-01-128), and all experiments followed the "Guide for the Care and Use of Laboratory Animals" of our university (Policy and guidelines for the care and use of animals in scientific research).

Experimental design:

A total of 36 rats were divided into three groups (12 rats/group) as follows:

(1) Rats which fed on a chow diet and ordinary water

(2) Rats which fed on a KD and ordinary water

(3) Rats which fed on a KD and Zamzam water

Diet composition:

Chow diet. Chow diet contained crude protein (20%), crude fat (4%), crude fiber (3.50%), carbohydrate (4%), minerals and vitamins, as supplied by Saudi Grains Organization.

Ketogenic diet. The KD (a diet high in fat content and low in carbohydrate and protein content) was prepared locally by increasing the fat content in the rat chow diet. Briefly, 50 kg powder chow diet was mixed with 28 kg melted animal fat, hence providing the typical ratio of fats to carbohydrates and protein (in terms of grams) as 3:1 (Hartman & Vining, 2007), which approximately resulted in 90% of total calories intake from fat, 6% from protein and 4% from carbohydrates.

Zamzam water. Pure Zamzam water was acquired from the "General Presidency of the Grand Mosque and the Prophet's Mosque, KSA" through a registered supplier at King Fahad International Airport, Dammam. The composition of pure Zamzam water as measured by ionic chromatography in our lab has been published previously (Bamosa *et al.*, 2013) and given in Table 1.

Each rat consumed about 500 ml of Zamzam or ordinary water per week.

Determination of glycemic status. Glycemic status was determined by measuring (1) blood glucose by glucometer, (2) serum insulin by ALPCO Insulin ELISA kit (Catalog Number: 80-INSHU-E01.1) after an overnight fast of 12 hours and c) insulin resistance by homeostasis model assessment method (HOMA-IR = [fasting insulin (μ U/ml) × fasting glucose (mmol/l)]/22.5 (Antunes, Elkfury, Jornada, Foletto, & Bertoluci, 2016).

(HOMA-IR: homeostasis model assessment for insulin resistance).

Insulin levels were measured as per the steps mentioned in the ALPCO Insulin ELISA kit manual. Briefly, all reagents and microplates were brought to room temperature. 25 µl of each standard, control and sample were pipetted into their respective wells and the microplate was incubated for an hour. After that, the microplate was washed six times, 100 ml of the substrate was added to each well and incubated for 15 minutes. Then, a stop solution was added to each well, and the absorbance was read at 450 nm through a microplate reader. A standard curve was constructed to measure the insulin levels.

Determination of lipid profile. LDL cholesterol and HDL cholesterol were estimated by a Sigma-Aldrich HDL and LDL/VLDL Quantitation Kit (catalog number MAK045). In this kit, serum HDL and LDL/VLDL are first separated by centrifugation of serum samples at 2000g for 10 min. The supernatant fraction (HDL) is transferred to a new tube. The precipitant containing the LDL/VLDL fraction is centrifuged again to remove any remaining trace HDL supernatant. Then the cholesterol concentration of each is determined by a coupled enzyme assay as described in the kit manual, which results in a colorimetric (570 nm) product proportional to the cholesterol present.

Determination of oxidant-antioxidant status. Superoxide dismutase and malondialdehyde (TBARS) were measured by an EnzyChrom Superoxide Dismutase (SOD) Assay Kit (ESOD-100) and a QuantiChrom TBARS Assay Kit (DTBA-100), respectively.

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

Note(s): ppm = part per million
ppb = part per billion
Source(s): Bamosa *et al.* (2013)

Table 1.
Chemical composition of Zamzam water

SOD assay provides a colorimetric means for quantitative determination of SOD enzyme activity in biological samples through xanthine oxidase (XO) catalyzed reaction. Prior to assay, all reagents are brought to room temperature. Then, 20 µl of each standard, control and sample are added to their respective wells. After adding assay buffer, xanthine, working reagent and XO enzyme in the quantity/order mentioned in the assay protocol, the color intensity at 440nm is used to determine the SOD activity in the sample.

The calorimetric TBARS Assay is based on the reaction of TBARS with thiobarbituric acid (TBA) to form a pink-colored product. The color intensity at 535 nm is directly proportional to TBARS concentration in the sample. First, all reagents and samples are equilibrated to room temperature. Then, 200 µl of each standard and sample are added to separate tubes. 200 µl of TBA reagent is added to standards and samples to produce the color reaction. Then, 100 µl from each tube is added to the wells, and color intensity is read at 535 nm.

Determination of body composition. Adipose pads (mesenteric, retroperitoneal, epididymal and abdominal fat) were dissected, blotted dry and weighed immediately with the Denver instrument Scale SI-603A. After dissection, the empty body (i.e. musculoskeletal, excluding internal organs, fat pads and tail) was weighed and classified as a carcass (Rolland *et al.*, 2002).

Statistical analysis

Data were statistically analyzed with Statistical Package for Social Sciences (SPSS) version 20 (IBM, Armonk, New York, United States). Shapiro–Wilk test was used to check the normality of data. Groups were compared by a one-way ANOVA test. A p-value of less than 0.05 was considered significant.

Results

Statistically significant differences were observed in fasting blood sugar and LDL cholesterol levels of all three groups (p-values 0.001 as seen in Table 2). A post-hoc Tukey's HSD test revealed that the rats which fed on the KD and Zamzam water had significantly reduced fasting blood glucose compared to the rats which fed on a chow diet and ordinary water (p-value 0.001), and KD and ordinary water (p-value 0.004). Likewise, rats which fed on the KD and Zamzam water had significantly reduced LDL cholesterol compared to the rats which fed on a chow diet and ordinary water (p-value 0.001), and KD and ordinary water (p-value 0.006) (Table 3).

Discussion

In our study, the rats which fed on the KD and ordinary water did not show a significant reduction in fasting blood glucose and LDL cholesterol. This is contrary to Yancy *et al.* (2005) who reported that a KD induced a significant reduction in fasting blood glucose in type 2 diabetes mellitus, and Dashti *et al.* (2004) who reported a KD-induced significant reduction in LDL cholesterol in obese subjects. The reason for this discrepancy could be the KD intake for a longer duration (16 weeks) in both studies compared to a shorter duration (10 weeks) in our study.

Our results showed that the rats which fed on a KD along with Zamzam water had significantly reduced fasting blood glucose and LDL cholesterol after ten weeks. Previous studies on Zamzam water have reported its hypoglycemic and hypolipidemic effects (Abdel-Azeem *et al.*, 2017). The Zamzam water, being alkaline in nature, may upregulate hexokinase (the main enzyme for glycolysis) (Quach *et al.*, 2016), thus leading to a decrease in fasting blood glucose. Likewise, Zamzam water has been shown to reduce LDL cholesterol (Tama & Sagiran, 2019), most probably due to its magnesium content that may activate the enzyme lecithin cholesterol acyl transferase (LCAT) (Ansari *et al.*, 2012). Our results suggest an additive effect of Zamzam water when given along with a KD in reducing blood glucose and LDL cholesterol.

Parameters	Chow diet and ordinary water	Ketogenic diet and ordinary water	Ketogenic diet and Zamzam water	*p*-value*
Fasting blood glucose (mg/dl)	147.1 ± 26.90	141.5 ± 30.4	105.0 ± 10.27	0.001
Serum Insulin (μIU/ml)	37.61 ± 25.43	47.09 ± 43.64	45.42 ± 56.13	0.14
HOMA–IR	8.40 ± 5.50	11.7 ± 13.7	7.6 ± 9.6	0.60
LDL cholesterol (mg/ml)	30.22 ± 3.62	28.06 ± 4.28	19.79 ± 7.42	0.001
HDL cholesterol (mg/ml)	14.06 ± 2.93	12.75 ± 3.05	14.68 ± 2.35	0.46
Superoxide dismutase (U/ml)	0.09 ± 0.01	0.10 ± 0.01	0.10 ± 0.01	0.15
Thiobarbituric acid reactive substance (μmol/l)	0.39 ± 0.09	0.42 ± 0.17	0.42 ± 0.12	0.84
Pre-test body mass (g)	201.36 ± 35.6	220.0 ± 30.7	209.09 ± 37.0	0.46
Post-test body mass (g)	363.09 ± 48.04	350.18 ± 34.7	358.18 ± 45.9	0.78
Mass gain (g)	161.73 ± 44.8	130.18 ± 33.15	149.09 ± 28.88	0.14
Fat pads mass (g/100 g body weight)	2.46 ± 0.94	3.54 ± 1.08	4.00 ± 2.10	0.06
Carcass mass (g/100 g body weight)	47.87 ± 2.63	48.36 ± 2.05	49.52 ± 9.53	0.79

Table 2.
Comparison of study variables in rats which fed on a normal chow diet or ketogenic diet with either Zamzam water or tap water for a period of 10 weeks

Note(s): Values are mean ± standard deviation, *p-value obtained by one-way ANOVA
HOMA-IR: Homeostasis model assessment for insulin resistance; LDL: low-density lipoprotein; HDL: High-density lipoprotein
Mass gain = Post-test body mass – Pre-test body weight
Fat pads: sum of mesenteric, retroperitoneal, epididymal and abdominal fat
Carcass: musculoskeletal excluding internal organs, fat pads and tail

Variables	Groups compared	*p*-value
Fasting blood glucose	Ketogenic diet and Zamzam water vs chow diet and ordinary water	0.001
	Ketogenic diet and Zamzam water vs ketogenic diet and ordinary water	0.004
LDL cholesterol	Ketogenic diet and Zamzam water vs chow diet and ordinary water	0.001
	Ketogenic diet and Zamzam water vs ketogenic diet and ordinary water	0.006

Table 3.
Post hoc test results (tukey HSD)

Note(s): LDL: low-density lipoprotein

Our study failed to reveal any significant effect of the KD alone or combined with Zamzam water on oxidant–antioxidant balance. Our results are like Rhyu, Cho, and Roh (2014), who reported that the KD did not produce any significant change in levels of reactive oxygen species and superoxide dismutase enzyme. Zamzam water has also been reported to produce no effect on lipid peroxidation, as determined by serum concentrations of TBARS by Al Meheithif, Elnour, Bamosa, and Aleissa (2012) and Bamosa *et al.* (2013). Our results are contrary to Greco, Glenn, Hovda, and Prins (2016), who reported that the KD reduces oxidative stress and increases cytosolic and mitochondrial antioxidants. This result variation could be due to the differences in the composition of their ketone diet to ours.

Conclusion

Our results clearly show that KD intake alone for ten weeks does not produce any significant decrease in fasting blood glucose and LDL cholesterol. However, when KD is given along with Zamzam water, fasting blood glucose and LDL cholesterol are significantly reduced.

Therefore, Zamzam water and KD together may be promising therapeutic agents for hyperglycemia and hyperlipidemia. People who wish to use KD for a shorter duration may add Zamzam water to enhance the therapeutic effects of KD.

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