



Experimental Article

## Effects of Zamzam water on glycemc status, lipid profile, redox homeostasis, and body composition in rats

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### المخلص

**أهداف البحث:** أظهرت الدراسات آثارا مفيدة لماء زمزم للعديد من الأمراض كالسكري، وتسمم الكلى، وتسمم الكبد، والضغط العصبي. تم تصميم الدراسة الحالية لتقييم تأثيرات ماء زمزم على حالة السكر في الدم، ومستوى الدهون، وتوازن مضادات الأكسدة، وتكوين الجسم في الفئران السليمة.

**طرق البحث:** تم تقسيم ٢٤ فأرا إلى مجموعتين متساويتين. بحيث تم تغذية الفئران على حمية تشاو جنبا إلى جنب مع ماء الصنبور، أو ماء زمزم كمصدر للسوائل فقط. بعد ١٠ أسابيع، تم قياس مستوى السكر في الدم أثناء الصيام، والأنسولين في الدم، ومقاومة الأنسولين، والبروتين الدهني منخفض الكثافة، والبروتين الدهني عالي الكثافة، وديسميوتاز سوبرأوكسيد، وبيروكسيد الدهون. كما تم وزن الكتلة الدهنية والجسدية (العضلات والعظام فقط) وحساب وزن الجسم المتبقي.

**النتائج:** انخفضت النتائج التالية بشكل ملحوظ في مجموعة ماء زمزم مقارنة بمجموعة ماء الصنبور السكري في الدم أثناء الصيام ٩٦.٥ مقابل ١٤٧.١ ملغم/دسل، والأنسولين في الدم ٠.٤٤ ضد ١.٣١، ومقاومة الأنسولين ١.٨٩ ضد ٨.٤٠. ولم يكن هناك أي تغير ملحوظ في البروتين الدهني منخفض الكثافة، والبروتين الدهني عالي الكثافة، وديسميوتاز سوبرأوكسيد، وبيروكسيد الدهون، ووزن الجسم، والكتلة الدهنية، والجمّة، ووزن الجسم المتبقي (المطلقة والنسبية على حد سواء).

**الاستنتاجات:** أظهرت دراستنا أن استهلاك ماء زمزم لمدة ١٠ أسابيع يؤدي إلى انخفاض مستوى السكر في الدم أثناء الصيام، والأنسولين في الدم ومقاومة

الأنسولين. ولكن لا يوجد أي تأثير لماء زمزم على مستوى الدهون، وتوازن مضادات الأكسدة وتكوين الجسم.

**الكلمات المفتاحية:** السكر في الدم؛ الأنسولين؛ مقاومة الأنسولين؛ البروتين الدهني منخفض الكثافة؛ ماء زمزم

### Abstract

**Objectives:** Previous studies have demonstrated that Zamzam water exerts beneficial effects on several ailments such as diabetes mellitus, nephrotoxicity, hepatotoxicity, and stress. The present study aimed to assess the effects of Zamzam water on glycemc status, lipid profile, redox homeostasis, and body composition in healthy rats.

**Methods:** Twenty-four rats were divided into two equal groups. Rats were fed a chow diet along with either tap or Zamzam water as the only fluid source. After ten weeks, fasting blood glucose, serum insulin, insulin resistance, low density lipoproteins (LDL) cholesterol, high density lipoprotein (HDL) cholesterol, superoxide dismutase, and lipid peroxidation were measured. Adipose pads and carcass (musculoskeletal only) were weighed and residual body weight was calculated. The groups were compared using independent sample *t* test (unpaired).

**Results:** The following parameters were significantly reduced in the Zamzam water group compared to the tap water group: fasting blood sugar, 96.5 vs. 147.1 mg/dl ( $p = 0.00$ ); serum insulin, 0.44 vs. 1.31  $\mu$ U/l ( $p = 0.00$ ); and insulin resistance, 1.89 vs. 8.40 ( $p = 0.00$ ). LDL cholesterol, HDL cholesterol, superoxide dismutase, lipid peroxidation, weight of the body, fat pads, and carcass, as well as residual body weight (both absolute and relative) showed no significant changes.

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**Conclusion:** Zamzam water intake for ten weeks decreases fasting blood sugar, serum insulin, and insulin resistance. However, Zamzam water has no effect on lipid profile, redox homeostasis, and body composition.

**Keywords:** Blood glucose; Insulin; Insulin resistance; LDL cholesterol; Zamzam

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## Introduction

Muslims globally believe in the blessings of Zamzam water, as Prophet Mohammad (PBUH) said in his hadith, “Zamzam is blessed, it serves as food”.<sup>1</sup> Compared to ordinary water, Zamzam water is unique in its characteristics. A study conducted at King Saud University in 2017 proved that Zamzam water has no microbial growth and is excellent for drinking (class I water quality index). In addition, the concentration of anions, cations, and trace metals has been found to be within acceptable limits, as set by the Saudi Arabian Standard Organization for drinking water.<sup>2</sup> Another study indicated that Zamzam water differed from distilled and bottled water. Zamzam had higher concentration of calcium (Ca), magnesium (Mg), sodium (Na), and chloride (Cl). Toxic elements such as arsenic (As), cadmium (Cd), lead (Pb), and selenium (Se) have been found to be below maximum toxic limits set by different regulatory agencies.<sup>3</sup>

Al Doghithie et al. (2016) suggested the anticancer potential of Zamzam water by showing that it induces apoptosis of human colon cancer HCT-116 cells.<sup>4</sup> Ali et al. have shown that Zamzam water induces the expression of new aquaporin (AQPs) channels across the cell membrane and increases their number.<sup>5</sup> Bamosa et al. have found a significant increase in antioxidant parameters and a significant decrease in glycated hemoglobin (HbA1c) levels in patients with type 2 diabetes after two months of Zamzam supplementation.<sup>6</sup> Another study has reported that Zamzam increased total antioxidant capacity in gentamicin-induced stressed rats.<sup>7</sup>

Previous studies have demonstrated the beneficial effects of Zamzam water in pathological conditions such as diabetes, nephrotoxicity, hepatotoxicity, and stress. Studies exploring the effects of Zamzam water on healthy animals or humans are scarce.<sup>8,9</sup> Therefore, the present study aimed to examine the effects of Zamzam in healthy rats, which were fed a normal chow diet (CD).

## Materials and Methods

This was a quasi-experimental research. The experimental protocol was approved by the Institutional Review Board of Imam Abdulrahman Bin Faisal University (IRB-PGS-2017-01-128). All experiments were performed in accordance with “Guide for the Care and Use of Laboratory Animals” prepared by our university, and every effort was made to minimize animal suffering.<sup>10</sup>

Chow diet (CD) was supplied by Saudi Grains Organization and consisted of the following (%/100 g): crude protein 20%; crude fat 4%; crude fiber 3.50%; carbohydrate 51.4%; minerals: ash 6%; salt 0.50%; calcium 1%; phosphorus 0.60%; moisture 13%; vitamins, including A 20 IU/g, D 2.20 IU/g, and E 70 IU/kg; and metabolized energy (ME) 2850 kcal/kg.

Pure Zamzam water was obtained from the General Presidency for the affairs of the Grand Mosque and the Prophet’s Mosque, KSA. Major constituents of Zamzam water as have been previously measured by ionic chromatography in our lab<sup>6</sup> are shown in Table 1.

Twenty-four male Wistar albino rats (body weight: 150–200 g; age: 10–12 weeks old) were obtained from the animal house of our university and housed individually in labeled cages under standard laboratory conditions (adequate ventilation; temperature:  $25 \pm 2$  °C; relative humidity:  $50 \pm 15\%$ ; and 12 h light/dark cycle) with free access to chow diet and drinking water during the acclimatization week.

Following the acclimatization week, rats were assigned into the following two groups with simple randomization technique (12 rats/group):

1. Tap water group: Rats fed on CD and tap water as the only fluid source.
2. Zamzam water group: Rats fed on CD and Zamzam water as the only fluid source.

The experiment lasted 10 weeks. After 10 weeks, rats were weighed and then anesthetized with ketamine (40 mg/kg) and xylazine (5 mg/kg). A central abdominal incision was made, and the internal organs were removed. Fasting blood sugar was measured with an automatic blood glucometer (Contour TS, Bayer Healthcare AG, Leverkusen, Germany). Five mL blood was withdrawn from an exposed aorta in EDTA-coated tubes. Serum was separated by centrifugation and frozen at  $-80$  °C until assayed.

### Serum parameters

Serum insulin, HDL, LDL, superoxide dismutase (SOD), and thiobarbituric acid reactive substances (TBARS) were measured with an ALPCO Insulin ELISA kit (Catalog Number: 80-INSHU-E01.1), a Sigma–Aldrich HDL and LDL/VLDL Quantitation kit (catalog number MAK045), an EnzyChrom Superoxide Dismutase Assay Kit (ESOD-100), and a Quanti-Chrom TBARS Assay Kit (DTBA-100), respectively.

Insulin resistance was calculated with the homeostasis model assessment method according to the following formula:<sup>11</sup>

$$\text{HOMA-IR} = [\text{fasting insulin } (\mu\text{U/ml}) \times \text{fasting glucose } (\text{mmol/l})] / 22.5$$

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

### 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. Residual body weight was calculated as total body weight minus the weights of the carcass and total fat.<sup>12</sup>

Statistical analysis was processed using Statistical Package for Social Sciences (SPSS) version 20 (IBM, Armonk, NY, USA). Normality of data was checked using the Shapiro Wilk test. The values of the measured parameters were expressed as mean  $\pm$  SD. The groups were compared using an independent sample t test. A p-value of less than 0.05 was considered significant.

## Results

Rats in both groups were active and showed no abnormal signs throughout the study period. Zamzam water caused a significant reduction in fasting blood sugar, serum insulin

levels, and insulin resistance compared to tap water (Table 2). None of the parameters related to body composition (weight of the body, fat pads, carcass, and residual body weight) and redox homeostasis were affected by Zamzam in any of the experiments.

## Discussion

In this study, we aimed to determine the effect of Zamzam water on the metabolic parameters, oxidant-antioxidant status, and body composition of healthy rats.

Our study revealed a statistically significant decrease in fasting blood glucose levels in rats fed with Zamzam water. This result is in accordance with the study conducted by Abdel-Azeem et al., who reported significant hypoglycemic effects of Zamzam water in three different groups of rats, namely, alloxan-induced diabetic rats, gentamicin-induced nephrotoxic rats, and carbon tetra chloride-induced hepatoxic rats, compared with their respective controls.<sup>13</sup> Our results contradict those of Bamosa et al., who found that Zamzam water supplementation in uncontrolled diabetic patients for two months did not produce any significant change in fasting blood glucose.<sup>6</sup> The reason for this discrepancy could be that Bamosa et al. used Zamzam water as supplementation, in a limited quantity (1 L/day); contrastingly, in the present study, Zamzam water was the sole source of water. Furthermore, subjects in the study of Bamosa et al. were uncontrolled diabetics with abnormally increased blood sugar levels (more than 200 mg/dl); however, in our study, we used healthy rats with sugar levels within normal limits.

The mechanism through which Zamzam water decreases fasting blood glucose is not fully understood. Nonetheless, since

**Table 1: Chemical composition of Zamzam water.<sup>6</sup>**

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

ppm = part per million.

ppb = part per billion.

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

Parameters	Chow diet + Tap water	Chow diet + Zamzam water	P value
1. Fasting blood glucose (mg/dl)	147.1 $\pm$ 26.90	96.5 $\pm$ 12.27	*0.00
2. Serum insulin ( $\mu$ U/l)	1.31 $\pm$ 0.88	0.44 $\pm$ 0.10	*0.00
3. HOMA – IR	8.40 $\pm$ 5.50	1.89 $\pm$ 0.48	*0.00
4. LDL cholesterol (mg/dl)	0.30 $\pm$ 0.04	0.26 $\pm$ 0.06	0.12
5. HDL cholesterol (mg/dl)	0.14 $\pm$ 0.03	0.14 $\pm$ 0.03	0.99
6. Superoxide dismutase (U/ml)	0.09 $\pm$ 0.01	0.09 $\pm$ 0.01	0.50
7. Thiobarbituric acid reactive substance ( $\mu$ mol/l)	0.39 $\pm$ 0.09	0.38 $\pm$ 0.08	0.80
8. Pre-test body weight (g)	203.33 $\pm$ 34.66	206.67 $\pm$ 48.44	0.90
9. Post-test body weight (g)	363.09 $\pm$ 48.04	374.09 $\pm$ 31.06	0.53
10. Weight gain (g)	161.73 $\pm$ 44.80	170.45 $\pm$ 36.77	0.62
11. Weight of the fat pads (g)	9.04 $\pm$ 4.01	9.56 $\pm$ 2.34	0.71
12. Weight of the carcass (g)	174.0 $\pm$ 26.66	178.18 $\pm$ 16.27	0.66
13. Residual body weight (g)	180.04 $\pm$ 23.18	186.35 $\pm$ 25.77	0.55
14. Relative weight of the fat pads (g)	2.46 $\pm$ 0.94	2.55 $\pm$ 0.54	0.79
15. Relative weight of the carcass (g)	47.87 $\pm$ 2.63	47.75 $\pm$ 3.97	0.93
16. Relative residual body weight (g)	49.67 $\pm$ 2.41	49.71 $\pm$ 4.31	0.98

Values are mean  $\pm$  standard deviation, \*P value < 0.05.

HOMA-IR: Homeostasis model assessment for insulin resistance; LDL: Low density lipoprotein; HDL: High density lipoprotein.

Weight gain = Post-test body weight - Pre-test body weight.

Fat pads: mesenteric, retroperitoneal, epididymal, and abdominal fat.

Carcass: musculoskeletal excluding internal organs, fat pads, and tail.

RBM: residual body mass = Total body weight - (weight of carcass + total fat).

Relative weight of the fat pads = Fat pads/body weight \*100.

Relative weight of the carcass = Carcass/body weight \*100.

Relative residual body weight = residual body weight/body weight \*100.

alkaline water has been shown to produce hypoglycemia in a type 2 diabetic rat strain,<sup>14</sup> possibly by upregulating hexokinase (the main enzyme of glycolysis that facilitates cellular glucose uptake),<sup>15</sup> we propose that the alkaline pH of Zamzam water might be responsible for Zamzam-induced hypoglycemia. Previous experiments from our lab have shown that, compared to ordinary water, Zamzam is highly alkaline (pH 7 vs. 7.75–8.0).<sup>6</sup>

Zamzam water also significantly reduces serum insulin levels. Since glucose is the major stimulus for insulin secretion,<sup>16</sup> Zamzam-induced decrease in insulin levels seems to be secondary to hypoglycemia. A significant reduction in insulin resistance was observed in the Zamzam group, which can be explained by the Zamzam-induced decrease in blood glucose and insulin levels. To the best of our knowledge, to date, no studies have been conducted to determine the effects of Zamzam water on insulin resistance. Previous studies on Zamzam have measured Zamzam-induced changes in blood sugar alone, without measuring insulin resistance.<sup>6,13</sup>

Zamzam water produced no significant change in the levels of LDL and HDL cholesterol. As Zamzam water reduced insulin resistance, it should also reduce triglyceride levels because glucose and lipid metabolism is correlated, i.e., increased glucose levels ultimately increase the production of triglyceride rich lipoproteins. However, in the current study, there was no effect on triglyceride levels. A possible explanation for this could be the short duration of treatment in our study. We observed a decrease in the levels of LDL cholesterol, from  $0.30 \pm 0.04$  mg/dl in the tap water group to  $0.26 \pm 0.06$  mg/dl in the Zamzam water group, although this decrease was not statistically significant. Increased treatment duration may result in a significant reduction in LDL cholesterol. Our finding concur with those of Abdel-Azeem et al.,<sup>13</sup> who provided CD + zamzam water to 50% of the rats in their control group and CD + tap-water to the remaining rats. They did not find any significant change in any of the lipid profile parameters (total lipids, total cholesterol, triglycerides, LDL cholesterol, HDL cholesterol, and VLDL cholesterol) between the Zamzam and tap water-fed control group rats.

In our study, in contrast to previous studies,<sup>6,7</sup> consumption of Zamzam water had no significant effect on SOD activity. This is not consistent with the findings of Bamosa et al.,<sup>6</sup> who reported a significant increase in SOD following treatment of patients with type 2 diabetes with Zamzam water. Similarly, AlMehethif et al.<sup>7</sup> investigated the antioxidant potential in gentamicin-induced stressed rats fed with Zamzam water and reported a significant increase in SOD. These discrepancies may be the result of differences in the study subjects. Bamosa et al.<sup>6</sup> and AlMehethif et al.<sup>7</sup> examined the effects of Zamzam water in patients with type 2 diabetes and gentamicin-induced stressed rats, respectively. Gentamicin-induced stress and type 2 diabetes are the conditions well known for disturbed redox homeostasis.<sup>17,18</sup> It is possible that Zamzam water causes a significant increase in antioxidant levels in oxidative stress conditions, but has no effect on antioxidant levels in the absence of oxidative stress.

In our study, Zamzam water had no effect on lipid peroxidation, as determined by serum concentrations of TBARS. This result corresponded with a previous study by Al Meheithif et al.<sup>7</sup> and Bamosa et al.,<sup>6</sup> who both failed to demonstrate any significant effect of Zamzam on TBARS

in gentamicin-induced stressed rats and patients with type 2 diabetes, respectively.

No significant changes were observed in the Zamzam group in terms of absolute and relative body weights, residual body weight, as well as the fat pad and carcass weights.

## Conclusion

Drinking Zamzam water for ten weeks resulted in a significant decrease in the levels of fasting blood glucose, serum insulin, and insulin resistance in healthy rats. Zamzam water had no effect on body composition, SOD, and lipid peroxidation.

## Recommendations

We recommend further studies using Zamzam as the only source of water in humans with hyperglycemia or insulin resistance. The mechanism underpinning Zamzam-induced metabolic effects also needs to be studied further.

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## Conflict of interest

There is no conflict of interest.

## Ethical approval

The ethical approval of this research was obtained from the Institutional Review Board of Imam Abdulrahman Bin Faisal University through letter number IRB-PGS-2017-01-128.

## Authors contributions

GFA conducted research, collected and organized data, and wrote initial draft of the article. RL conceived and designed the study and wrote final draft of the article. MHA analyzed and interpreted data. AAS provided logistic support, analyzed and interpreted data. SC performed biochemical tests. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

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