Biochemical and histological effects of five weeks ingestion of Zamzam water on the liver and kidneys of Wistar rats

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Zamzam water is a natural alkaline water which has become alkaline as a result of the natural environment. It comes from what is considered as one of the oldest springs in the world. The water contains high concentrations of alkaline minerals as well as trace and heavy metals. The aim of the current study is to evaluate the effects of five weeks ingestion of Zamzam water on the liver and kidney functions of rats. Adult female Wistar rats weighing 150–200 g were divided into two groups, with 15 rats in each. The control group was supplied daily by bottled water and the Zamzam water group was supplied daily by 500 ml of Zamzam water for five weeks. The rats were weighed weekly and, at the end of the experiment, blood samples were collected from all rats for the biochemical determination of serum levels of aspartate transaminase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), urea, creatinine, albumin, and uric acid, using calorimetric methods. Liver and kidney tissues were fixed in 10% neutral buffered-formalin solution and further embedded in wax blocks for routine hematoxylin and eosin (H&E) staining and were examined for histopathological changes using a light microscope. The results of the current study showed that there was a significant increase (P < 0.05) in the weight of the Zamzam group when compared to the control group after five weeks of ingestion. Liver and kidney function tests did not show any significant difference when compared with the controls (P > 0.05). In addition, histological examination of the liver and kidney tissues did not show any toxicological changes. In conclusion, the results showed that the ingestion of Zamzam water did not alter serum levels of kidney function tests and liver enzymes; and did not result in a noticeable change in the liver and kidney histology. Thus, the high concentrations of elements in Zamzam water do not induce hepatotoxicity or nephrotoxicity and the water is considered safe for long-term consumption.

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1. Introduction

Zamzam water is a holy water consumed daily by millions of Muslims around the world. It is a natural alkaline water that has never been chemically preserved, treated, or chlorinated and the pH value ranges from 7.9 to 8 (Al Zahrani et al., 2019). Studies reported that the Zamzam well is free from any type of microbial contamination (Al-Barakah et al., 2016; Halim et al., 2016). Zamzam water is colorless and odorless with a high concentrations of alkalizing minerals such as potassium, sodium, magnesium, calcium, and bicarbonate (Shomar, 2012). Trace elements that are found naturally in Zamzam water include iron, copper, silver, zinc, chromium, cobalt, manganese, aluminum, and other elements (Shomar, 2012). Natural heavy metals such as arsenic, cadmium, selenium, and lead are also determined in Zamzam water (Abu-Taweel, 2017; Al Nouri et al., 2014). In 2010, King Abdullah Ibn Abdul-Aziz Project for Zamzam water was approved for dealing with the automated cleaning of Zamzam water. The water is now being supplied to public by King Abdullah project for strategic Zamzam water storage in Makkah. This project is the only source for Zamzam water which has been established more than ten years ago. Zamzam water samples from King Abdullah project have all constituents in the normal permissible range (Al-Hussain, n.d.). Nowadays, political debates are still appearing after a BBC report in 2011 (Lynn, 2011) about the toxicity of Zamzam water.
Moreover, few studies have been published that examine the effects of Zamzam water on health. In addition, studies on the trace and heavy metal contents of Zamzam water have contradictory results. Some reported that arsenic and nitrate levels were three times higher than World Health Organization (WHO) limits (Shomar, 2012) and others reported that arsenic levels were within acceptable limits (Al-Barakah et al., 2016). In vitro studies on cancer cells have reported that treating cancer cells with Zamzam water induced antiproliferative and apoptotic effects and it was suggested that the anticancer effects could be due to the high pH of the water and the presence of heavy metals such as arsenic (Al-Doghaithier et al., 2016; Omar et al., 2017; Al Zahrani et al., 2019). A study on rats has observed a reduction in tumor size after treating the rats with Zamzam water, and the authors suggested that the possible reason for the reduction in the size of the tumor was due to the biochemical nature of Zamzam water (Ali et al., 2009).

Heavy metal toxicity has proven to be a major threat and there are several health risks associated with it (Jan et al., 2015). It is well known that trace and heavy metals have been linked to hepatotoxicity and nephrotoxicity. Previous reports have shown that the exposure to high levels of heavy metals such as arsenic demonstrates acute and chronic liver injury and other autoimmune diseases (Das et al., 2012; Dhanraj et al., 2020; Samanta et al., 2020). In addition, many studies reported that acute and chronic exposure to one type of heavy metal or a combination of heavy metals such as lead, mercury, arsenic, and cadmium can be detrimental to the kidneys (Orr and Bridges, 2017). Although millions of people consume Zamzam water every day, as far as we are aware, no research has been published about the possible toxic effects of long-term ingestion of Zamzam water on humans or rats. Based on this, the aim of the current study is to evaluate the biochemical and histological effects of the long-term consumption of Zamzam water on the liver and kidneys of Wistar rats.

2. Materials and methods

2.1. Animals

Thirty locally bred adult female Wistar rats initially weighing 150–200 g were used in this study. The rats were housed in groups in the animal house at King Fahd Medical Research Center (KFMRC), King Abdulaziz University, Jeddah, Kingdom of Saudi Arabia (KSA), at a temperature of 22 ± 2 °C under a 12 h dark–light cycle (light on at 0700 h) and given a standard pelleted diet (Grain Soil and Flourmills Organization, Jeddah, KSA) and water ad libitum with free access to water. The experimental protocol was approved by the animal ethics committee and proceeded according to the guidelines established by the Research Ethics Committee at King Abdulaziz University, Jeddah, KSA.

2.2. Zamzam water samples

Water samples were provided by the General Presidency of the Affairs of the Two Holy Mosques. The samples were stored in small sterilized glass bottles until used. The water samples were taken directly from the Zamzam well which is located in the holy city of Makkah, KSA. All the water samples were neither filtered nor treated. The pH of the water was measured using pH meters (GC 817) (APHA, 1998). Major elements and nitrate were determined using ICS-5000 ion chromatography system ( Dionex, Thermo Fisher Scientific Inc., USA). Lead, arsenic, and chromium were determined using Perkin Elmer Optima, 4300 DV ICP-OES, Shelton, USA.

2.3. Study design

The animals were divided into two groups and were housed for four days prior to the start of the experiment in order to adapt to the conditions of the experiment. Each group comprised 15 rats, as follows: the control group (n = 15), which was supplied with 500 ml of bottled drinking water, and the Zamzam group (n = 15), which was supplied daily with 500 ml of Zamzam water for five weeks. Food and water consumption were measured for each group and all rats were weighed weekly throughout the duration of the study.

2.4. Blood sample collection and biochemical analysis

At the end of the experimental period, blood samples were drawn by capillary tube from the retro orbital plexus of each rat under anesthesia with diethyl ether. Five ml of blood was collected in a plain tube. Blood was allowed to stand to clot for 30 min at room temperature and was then centrifuged for 10 min at 3000 rpm. After centrifugation, the serum was carefully separated and then stored at −80 °C until the analysis. The samples were stored until they were analyzed for determinations of serum aspartate transaminase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), uric acid, urea, creatinine, and albumin using enzymatic methods on an automated chemical analyzer (Dimension R clinical chemistry system, Siemens Healthcare Diagnostics Inc, Glasgow, UK.).

2.5. Histological analyses

Liver and kidney tissues were fixed in 10% neutral buffered formalin solution and further processed and embedded in paraffin blocks. The tissues were cut into 5 μm sections for routine hematoxylin and eosin (H&E) staining (Bancroft and Gamble, 2018) and were examined for histopathological changes using a light microscope.

2.6. Statistical analysis

All statistical analyses were performed using SPSS statistics software (version 22.0, IBM Corp., Armonk, NY, USA). Results were expressed as a mean ± standard error of mean (SEM). Data were analyzed statistically by independent t-test to compare the values of means between the two groups. P values < 0.05 were considered statistically significant.

3. Results

3.1. Analysis of Zamzam water samples

The analysis of Zamzam water samples showed that the pH was alkaline, and the total dissolved solids were within the acceptable limits of WHO. In addition, the levels of nitrate and heavy metals tested, lead and arsenic, were also within the permissible limits of WHO (see Table 1).

3.2. Weight and biochemical analysis

The effects of Zamzam water ingestion on the weight of all groups are shown in Table 2. The results revealed that the Zamzam group showed a significant increase in weight after five weeks compared to the control group. The mean weight for the Zamzam water group and the control group was 218.71 ± 5.68 gm and 203.71 ± 2.99 gm, respectively (P < 0.05). The biochemical effects of Zamzam water on liver enzymes are summarized in Table 3. The
results showed that there were no significant differences in serum concentrations of liver enzymes between both groups ($P > 0.05$). The effect of Zamzam water ingestion on kidney function did not show any significant difference between the control group and the Zamzam group (Table 4).

### 3.3. Histopathological evaluation

A histopathological evaluation was carried out to confirm the biochemical findings and to identify any structural changes in the tissues. Microscopic examination of the liver tissue of the rats is illustrated in Fig. 1. The rats in the control group and Zamzam group showed normal histological structure of hepatic parenchyma. In the Zamzam group, there were no signs of injury, congestion, fatty acid accumulation, necrosis, or hemorrhagic regions around the central vein or sinusoids of the liver were observed.

### Table 1
The chemical analysis of Zamzam water.

| Parameter     | Unit | Concentration | WHO limits |
|---------------|------|---------------|------------|
| pH            |      | 8.2           | 6.5–8.5    |
| Total dissolved solids | mg/l | 489           | <1000      |
| Sodium        | mg/l | 75.3          | 200        |
| Calcium       | mg/l | 46.5          | 50         |
| Magnesium     | mg/l | 10.08         | 30         |
| Potassium     | mg/l | 28.15         | No limit listed |
| Phosphate     | mg/l | <0.01         | <0.15      |
| Chloride      | mg/l | 65            | 250        |
| Nitrate       | mg/l | 34            | 50         |
| Lead          | ppb  | <0.01         | 10         |
| Arsenic       | ppb  | 6.82          | 10         |
| Chromium      | ppb  | 0.55          | 50         |

### Table 2
The Effects of Zamzam water on weight (g) after five weeks of ingestion.

| Weeks | Control group | Zamzam group | $P$-value |
|-------|---------------|--------------|-----------|
| 1     | $183 \pm 1.29$ | $180.38 \pm 2.07$ | 0.302     |
| 2     | $196.5 \pm 1.82$ | $203.8 \pm 3.59$ | 0.094     |
| 3     | $199.5 \pm 2.478$ | $207.88 \pm 3.86$ | 0.089     |
| 4     | $202.08 \pm 9.7$ | $211.13 \pm 4.65$ | 0.094     |
| 5     | $203.71 \pm 2.99$ | $218.71 \pm 5.68$ | 0.038*    |

Values were represented as the mean ± SEM.

### Table 3
The Effects of Zamzam water ingestion on liver function in both groups after five weeks of ingestion.

| Liver enzyme | Control group | Zamzam group | $P$-value |
|--------------|---------------|--------------|-----------|
| ALT U/L      | 76.80 ± 4.13  | 66.20 ± 5.56 | 0.144     |
| AST U/L      | 78.80 ± 2.30  | 84.60 ± 2.32 | 0.094     |
| ALP U/L      | 151.10 ± 16.06 | 150.50 ± 12.30 | 0.977   |
| Albumin (g/L)| 13.70 ± 0.47  | 14.0 ± 0.42  | 0.641     |

Values were represented as the mean ± SEM.

### Table 4
The Effects of Zamzam water ingestion on the kidney function tests in both groups after five weeks of treatment.

| variable      | Control group | Zamzam group | $P$-value |
|---------------|---------------|--------------|-----------|
| Creatinine (umol/L) | 40.10 ± 1.17 | 41.50 ± 2.16 | 0.577     |
| Urea (mmol/L)  | 7.52 ± 0.27   | 7.81 ± 0.35  | 0.525     |
| Uric acid (umol/L) | 40.40 ± 2.33 | 44.70 ± 3.42 | 0.313     |

Values were represented as the mean ± SEM.

Regarding the effects of Zamzam water on the kidneys, histological studies showed that there were no effects on the kidneys (Fig. 2). The appearance of the glomerular architecture was similar to the control group. Moreover, all nephron cells were normal and showed clearly visible nucleoli with no degeneration, necrosis, or bleeding.

### 4. Discussion

Millions of Muslims across the world drink Zamzam water for use either religiously or medicinally and they visit the well of Zamzam every year during the Hajj pilgrimage. Few studies have been conducted on Zamzam water in relation to its use for the treatment of diseases. The analysis of Zamzam water sample showed that all the analyzed elements were within the acceptable limits of WHO. The samples of this study were taken directly from the well and were stored in glass bottles in order to reduce contamination. However, the variation of the concentrations of the elements of Zamzam water in previous studies could be due to the different sources of the water and storage conditions. In addition, Zamzam water is a natural ground water, so it is obviously that the concentrations of trace elements will keep changing according to many factors such as climate, geology and human action.

Regarding the effects of Zamzam water ingestion on weight, the results of the current study showed that the weight of the rats significantly increased after five weeks of Zamzam water ingestion. Our result is contradictory to Al Meheithif et al.’s (2012) findings which did not report any significant difference in weight after
giving Zamzam water to rats for two weeks. This opposite result could be due to the short duration of their experiment. In agreement with our results, Abu-Taweel (2017) demonstrated that the exposure of perinatal dams to Zamzam water enhanced weight gain in their offspring. Interestingly, our findings are in agreement with the saying about Zamzam water by the Prophet Muhammad, peace be upon him, who said, “It is a blessing, and it is food that gives strength and gain in their offspring. Interestingly, our findings are in agreement with the saying about Zamzam water by the Prophet Muhammad, peace be upon him, who said, “It is a blessing, and it is food that gives strength and enhances weight gain.”

Almost all heavy metals are serious toxicants as carcinogens (Kim et al., 2015). Arsenic and nitrate have been recognized as pollutants in drinking water due to their toxicity (Ratnaike, 2003; Ward et al., 2018). Furthermore, it is well known that many physicians do not recommend drinking Zamzam water to their patients due to its high concentration of minerals, especially calcium. In the current study, although the concentrations of calcium and phosphorus are very high in Zamzam water compared to other natural or bottled water, the results showed that five weeks of ingestion of Zamzam water did not affect kidney function and did not enhance the formation of kidney stones such as calcium oxalate or calcium phosphate stones. Histopathological examination of the kidneys did not show any noticeable change in the tissues. Interestingly, Al-Ghamdi (2012) reported earlier that Zamzam water prevented calcium oxalate nephrotoxicity in rats. This suggests that Zamzam water is unique in its properties and in its components. Moreover, it is well known that heavy metals such as copper, mercury, lead, zinc, and arsenic are directly toxic to cells and demonstrate hepatotoxicity (Hasanein and Emamjomeh, 2019). The results of the current study provide clear evidence that Zamzam water has no toxic effects on liver enzymes. Histopathological examination did not show noticeable change in the histology of the liver. Studies on the trace and heavy metal contents of Zamzam water have conflict results. Some reported that bromate levels were 20 times higher than allowable limits in some Zamzam water samples (Al-Ansi et al., 2011), and this might be a reaction with plastic in the bottles. Others reported that arsenic levels were within acceptable limits (Al-Barakah et al., 2016). Al Meheithif et al. (2012) reported that the levels of nitrate in Zamzam water samples used in their study were three times higher than WHO limits and suggested that the high pH value and the presence of arsenic and lithium may cause a healing power. On the other hand, others have reported that the levels of the four toxic elements arsenic, lithium, lead, and selenium in Zamzam water were below the danger level for human consumption (Al Nouri et al., 2014; Al-Rawi and Fetters, 2012). Interestingly, in 2014, a study reported the hepatoprotective effects of Zamzam water against CCl₄-induced liver damage, and the authors observed significant improvements in biochemical parameters. Thus, they suggested that Zamzam water may be used to protect from the toxic effects of CCl₄ and other toxic chemical agents in the liver (Saif et al., 2014). However, another study on rats reported no differences in arsenic levels between Zamzam water and ordinary bottled water regarding safety for the duration of a three-week experiment (Al Meheithif et al., 2012). Furthermore, it was reported that alkaline water reduced oxidative stress in patients with chronic renal disease (Huang et al., 2003) and improved glycemic control in diabetic rats (Jin et al., 2006) without known mechanism. Al Meheithif et al., 2012 also showed that Zamzam water tends to potentiate antioxidant power in rats stressed with gentamicin. It is assumed that the beneficial effects of the Zamzam water might be due to the high pH and the unique perfectly balanced mineral composition of the water.

5. Conclusion

The results show that Zamzam water induced weight gain without altering serum levels of liver enzymes and kidney function. Moreover, it did not result in a noticeable change in the histology of the liver and kidneys. Thus, long-term ingestion of Zamzam water does not induce hepatotoxicity or nephrotoxicity. Further studies are needed to examine the effects of Zamzam’s heavy metals on human organs. Furthermore, a complete analysis of the contents of Zamzam water is needed to confirm that the trace and heavy metals that are in Zamzam water are safe for human consumption.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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