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# Biochemical Changes During Starvation: Impact of Zamzam Water on Rat Models

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## Abstract

**Introduction and aim of the work:** We still need a complete understanding of the biochemical changes that occur during starvation and the impact of Zamzam water. The objective of this study is to investigate the biochemical impacts of Zamzam water on rats, as well as to compare the effects of Zamzam water and regular water on rats under conditions of severe starvation. **Materials and Methods:** We divided the rats into three groups: the normal control group, the starved group that drank tap water, and the starved group that drank Zamzam water. The biochemical analysis was carried out for Rats [total bilirubin, protein, urea, creatine, phosphorus, magnesium (Mg), iron (Fe), low-density lipoprotein (LDL), and high-density lipoprotein (HDL)]. **Results:** Rats-starved groups showed a significant reduction in body weight without a significant difference between the normal water and Zamzam groups. Biochemical analysis of the blood samples revealed lower levels of amylase, LDL, phosphorus, and total protein in the starved rats compared to the control group. On the other hand, the levels of creatinine and GOT were higher. On the other hand, there is no significant change in the other biochemical analyses between the control group and the starved groups. However, when comparing the two starved groups with each other, the experiments revealed that there are more increases in GPT, GOT, and HDL in the Zamzam group than in the tap water group, but in general, there are no significant changes in the other biochemical tests between the Zamzam water and tap water groups. Independent T-sample tests were conducted to compare means between groups, while a one-way ANOVA test was employed to assess differences among the three groups. The results revealed significant decreases in total protein levels in both starved groups compared to the control group, indicating the catabolic effects of prolonged fasting on protein metabolism. However, no significant differences were observed in most biochemical parameters between the starved groups with normal water and Zamzam water, except for amylase, creatinine, and magnesium levels. The one-way ANOVA test showed significant differences in total protein, amylase, creatinine, and magnesium levels among the three groups. These findings highlight the complex interactions between nutritional status, hydration, and metabolic pathways in response to starvation. Further research is warranted to elucidate the underlying mechanisms and clinical implications of these observations

**KEYWORDS:** Biochemical changes, Zamzam water, starvation.

## INTRODUCTION

Water is one of the most valuable natural resources on the earth's planet. Water is a necessary nutrient for cell survival, and it first serves as a construction ingredient. It controls and regulates our body temperature through sweating and respiration, which are required to perform many physiological functions within the human body properly. Water makes up 75% of an infant's body weight and 55% of an elderly person's body weight, and it is essential for cellular balance and survival. Topography, geological formations, and climatic conditions greatly influence water quality, which impacts human health.(Balasubramanian et al. 2015), (Bingqi Zhu et al. 2012), (Bingqi Zhu et al. 2011), (Vidyasagar 2007), (BingQi Zhu and Yang 2007), (Subramani, Elango, and Damodarasamy 2005), (Cazier and Gekas 2001), and (Helena et al. 2000). Human activities, such as industrialization and urbanization, produce effluents that affect natural water quality (Bingqi Zhu 2016).

One of the most important resources used by the pharmaceutical industry is water. It may be used as an excipient or to reconstitute products during synthesis, and manufacturing, or as a cleaning agent for vessels and equipment. Different levels of water quality are required for various medicinal applications. Water quality control, particularly microbiological quality control, is a significant problem, and the pharmaceutical sector invests significant resources in the development and operation of water purification systems (Shomar 2012). Zamzam is holy water considered a wonderful gift from God referred to as Allah in the Quran. Zamzam water is a natural treasure for Muslims in Saudi Arabia and across the globe. They believe in its importance either religiously or for its holistic medicinal value (Shomar 2012). The holy water is drawn from the well of Zamzam, which is located near Kaaba, Masjid Al-Haram, Mecca, Saudi Arabia. Based on the medicinal and cultural factors, we were curious to study the quality and medicinal benefits of Zamzam water. In this research, physicochemical factors and microbial load analysis were employed to assess the purity of Zamzam water, while the healing properties of the water were evaluated to showcase its medicinal benefits (Moni et al. 2022).

Water is a basic need and is essential in carrying out various physiological functions in the human body (Cazier and Gekas 2001). Humans can survive without food for a month, but they can survive without water for only seven days (Vidyasagar 2007). Water is one of the main dietary components. Its quality plays an important role in the health of the human being. Zamzam water is natural water consumed by millions of Muslims worldwide (Al-Shihri 2005). Many Muslims believe that the water of the Zamzam well is divinely blessed, able to satisfy both hunger and thirst, as well as cure illness. Pilgrims endeavor to consume this water during their pilgrimage, while those residing nearby may consume it more frequently (Careem 2005).

For medicinal or religious reasons, many Muslims drink Zamzam water. Millions of pilgrims drink it and frequently carry bottles back to their home countries. The prophet praised it, saying it was a blessing and tasted like food. Zamzam drinking water is unique in its natural characteristics; it has special optical parameters that are different from those of bottled and distilled water. Zamzam Water is located inside the Holy Mosque, about 20 meters east of the Ka'ba in Makkah Al-Mukarramah, Saudi Arabia. Zamzam's hand-excavated well is about 30.5 m deep, with an internal diameter ranging from 1.08 to 2.66 meters. In several ways, Zamzam water stands out from other water sources. Firstly, bacteria are unable to thrive at their source. Secondly, it remains free from mold and maintains its original color, taste, and smell over time. Unlike most wells, where biological growth and vegetation are common, the presence of algae in other water sources can render the water unpalatable due to changes in taste and odor. However, in the Zamzam water well, there is no indication of any biological growth, ensuring its continued purity and taste (Mashat 2010).

The chemical composition of Zamzam water includes various inorganic elements, namely sodium (Na), calcium (Ca), magnesium (Mg), potassium (K), bicarbonate (HCO3), chloride (Cl), fluoride (Fl), nitrate (NO3), sulfate (SO4), and the total dissolved salts (TDS) (Al-Zuhair, Al-Ghamdi, and Noorwali 2005). This water has been reported to be alkaline and also rich in minerals which render it a potential antioxidant source (Nassini et al. 2010), and (Shomar 2012). The alkaline nature of Zamzam is associated with the richness of certain elements like magnesium. Additionally, Zamzam water has been identified as sodium chloride water with meteoritic origins. Moreover, the levels of the four toxic elements, arsenic (As), cadmium (Cd), lead (Pb), and selenium (Se), are below the threshold considered dangerous for human consumption. Chemically Zamzam water is extremely suitable for drinking purposes(Naeem, Alsanussi, and Almohandis 1983). Zamzam water contains fluorides that have an effective germicidal action Zamzam water is colorless and without a smell, but has a special taste, with its pH at 7.9–8.0, indicating that it is alkaline to some extent. The mineral mass concentrations in Zamzam water are as follows: sodium 133 mg/L, calcium 96 mg/L, magnesium 38.88 mg/L, potassium 43.3 mg/L, bicarbonate 195.4 mg/L, chloride 163.3 mg/L, fluoride 0.72 mg/L, nitrate 124.8 mg/L, and sulfate 124.0 mg/L (Al Zuhair and Khounganian 2006).

The review article highlighted that Zamzam water may offer therapeutic benefits for certain diseases. It emphasized its distinctive analytical properties and noted its potent anti-inflammatory effects. In particular, Zamzam water strongly blocked tumor necrosis factor (TNFα) and interleukin 1 (IL1), showing that it might be useful for changing immune responses (Khalid et al. 2014a). Zamzam water exerts its analytical action through an indirect modulation of the endocrine, immune, and growth systems of the body (Ali et al. 2009). Clinical examination findings indicated that among the mixed dentition group, no statistically significant differences were observed. However, in the permanent dentition group, the mean decayed, missing, filled teeth (DMFT) score was notably lower among children who consumed Zamzam water(Al Zuhair and Khounganian 2006). As Zamzam water has strong anti-inflammatory and oncolytic action(Naeem, Alsanussi, and Almohandis 1983), (El-Zaiat 2005), (El- Kashef 1994), and (Ali et al. 2009). Zamzam water (ZW) is a well-known alkaline water that differs from the physical and chemical properties of other types of water. The physical analysis of Zamzam water revealed its alkaline nature, as well as the presence of trace elements such as selenium, zinc, and magnesium. These elements play a role in the formation of antioxidant enzymes, contributing to their overall antioxidant properties (Taha, Elazab, Qutub, et al. 2023), (Taha, Elazab, Baokbah, et al. 2023). An earlier investigation examined its ability to reduce the level of HbA1c, which represents glycated hemoglobin, in diabetic patients following two months of consumption (Bamosa et al. 2013). Another experimental research has recorded its beneficial antioxidant effects on the aging process (Hofer et al. 2008) and stress induced by gentamicin (Abdullah et al. 2012). Another study investigated the influence of Zamzam water on anxiety and depression induced by STZ (streptozotocin) and explored the potential neuroprotective mechanisms involved (Taha, Mahmoud, et al. 2023).

## OBJECTIVE & HYPOTHESIS

This work aims to study the biochemical effects of Zamzam water on rats, as well as the difference between Zamzam and normal water effects on rats during severe starvation.

## MATERIALS & METHODS

### Animals

Male Wistar rats were purchased from the Applied Medical Science College Animal House, Jazan University, KSA. The animals were maintained under standard laboratory conditions: temperature of 21±2 °C, relative humidity of 50±5 %, and a normal photoperiod (12-h dark, 12-h light). The experimental and animal care procedures were approved by the Animal Care and Use Committee of Applied Medical Science, Jazan University, KSA (from 6/3/2019 to 17/6/2019) and conformed to the Guide for the Care and Use of Laboratory Animals by the National Institutes of Health (NIH Publication No. 85–23).

### Starvation protocol

Male rats with a body weight of 306.3±16.5 g were randomly divided on the first day into three groups, the first was the control which was given normal water, and normal feeding while the second group was given normal water without feeding, and the third group were given Zamzam water (purchased from Makkah) without feeding. Weight is a useful indicator of development. Thus, the rats were weighed on days 1, 4, 6, 8 and 11. Rats in the starvation group were individually housed in cages to prevent aggression, and their access to normal chow was restricted for 11 days. Throughout the experiment, they had unlimited access to drinking water. Special care was taken to minimize environmental stress for the rats during the starvation period. Their behavior, movement, and appearance were regularly monitored following established guidelines. Daily observations showed no significant decrease in activity levels. However, at the end of the 11-day starvation period, three rats from the starved group died, and their data were excluded from the baseline analysis.

**Biochemical Analysis**

We measured body weight and tissue mass and conducted a blood chemistry assay. We anesthetized the rats with petroleum ether after 11 days of starvation. We collected blood samples into tubes for serum separation. All the samples contained the following substances: total bilirubin, protein, urea, creatine, phosphorus, magnesium (Mg), iron (Fe), low-density lipoprotein (LDL), and high-density lipoprotein (HDL). We measured these using a commercial reference laboratory kit from the Human Gesellschaft für Biochemica und Diagnostica mbH at Max-Planck-Ring 21, 65205 Wiesbaden, Germany. We conducted the tests in the clinical laboratory of the Applied Medical Science College at Jazan University, KSA.

**Statistical Analysis:**

Statistical analysis was performed using SPSS software (version 27). Independent T-sample tests were conducted to compare means between groups, while a one-way ANOVA test followed by post-hoc analysis (Tukey's test) was employed to assess differences among the three groups. Results were considered statistically significant at $p < 0.05$

## RESULTS

This study aimed to investigate the biochemical responses to starvation and different types of water consumption in rats. Male Wistar rats were divided into three groups: a control group with ad libitum access to food and water, a starved group with normal water, and a starved group with Zamzam water. Thus, the rats were weighed on days 1, 4, 6, 8 and 11. The rate of weight changes in the control group increases daily, but decreases daily in the Zamzam water and normal water groups (Figure 1). After an 11-day starvation period, blood samples were collected and analyzed for various biochemical parameters, including albumin, total protein, GPT, GOT, bilirubin, amylase, creatinine, urea, glucose, HDL, LDL, iron, magnesium, and phosphorus. Independent T-sample tests were used to compare means between the control and starved groups, while a one-way ANOVA test was conducted to assess differences among the three groups. The following sections present the findings from these analyses, highlighting significant changes and differences in the biochemical markers studied.

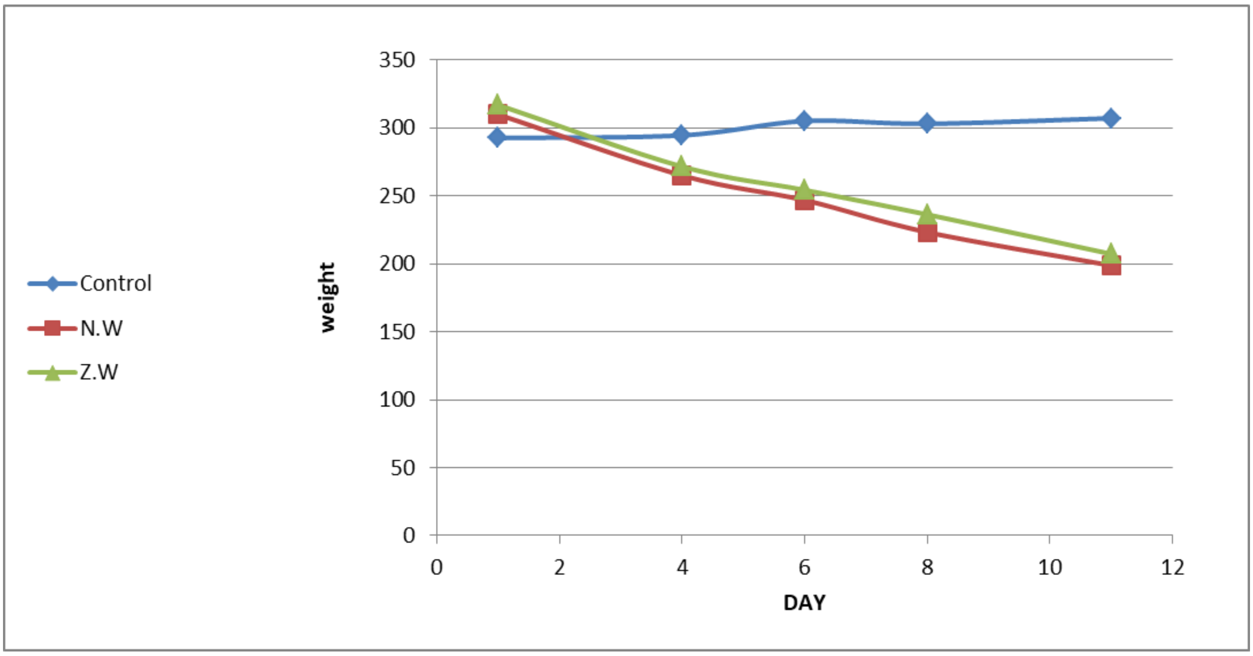


Figure -1: Changes in the rats' weight (gram) under conditions of severe starvation and daring in Zamzam water and normal water were compared with the control group.

NW: Normal Water

Z.W: Zamzam Water

We used an automated clinical chemistry analyzer to perform biochemical tests and quality controls. Table 1 displays the mean of all tests for the control group, Zamzam water group, and normal water group. The rate of weight changes in the control group increases daily and decreases daily in the Zamzam water and normal water groups (Figure 1). Biochemical analysis of rat blood samples after starvation and drinking normal water or Zamzam water revealed that amylase, LDL, phosphate, and total protein decreased in the case of starved rats compared with the control group, but there was an increase in creatinine and GOT blood levels. On the other hand, there is no difference in the other biochemical analyses between the control group and starved groups (Table 1). We looked at rat blood samples that had been starved and then given either normal water or Zamzam water. We found that the levels of amylase, LDL, phosphate, and total protein were lower in the rats that had been starved than in the rats that had been fed normally. On the other hand, the levels of creatinine and GOT were higher. On the other hand, there is no significant difference in the other biochemical analyses between the control group and starved groups (Table 1).

The comparison of means using independent T-sample tests revealed several significant findings. Firstly, when comparing the control group to the starved group with normal water consumption, a significant decrease in total protein levels was observed in the starved group ($p = 0.037$), indicating the impact of starvation on protein metabolism. However, no significant differences were detected in other biochemical parameters between these two groups (Table 1). Similarly, when comparing the control group to the starved group with Zamzam water consumption, a significant decrease in total protein levels was evident in the starved group ($p = 0.013$), reinforcing the metabolic effects of starvation. Again, no significant differences were noted in other biochemical parameters between these two groups (Table 2). Interestingly, when comparing the starved group with normal water to the starved group with Zamzam water, significant differences were observed in amylase ($p = 0.008$), creatinine ($p = 0.047$), and magnesium ($p = 0.024$) levels. These differences suggest potential variations in metabolic responses influenced by different water sources (Table 3).

Furthermore, the one-way ANOVA test provided additional insights into the overall differences among the three groups. Significant differences were detected in total protein ($p = 0.017$), amylase ($p = 0.008$), creatinine ($p = 0.047$), and magnesium ($p = 0.024$) levels. However, no significant differences were found in albumin, GPT, GOT, bilirubin, urea, glucose, HDL, LDL, iron, or phosphorus levels among the three groups. These results underscore the complex interplay between starvation, water type, and specific biochemical parameters in rats. While starvation consistently led to decreased total protein levels compared to the control group, differences in amylase, creatinine, and magnesium levels highlight potential nuances in metabolic responses influenced by water type (Table 4).

**Table 1: Comparison of Biochemical Parameters between Control and Starved Groups**

| | Group Type | Mean | Std. Deviation | P- Value |
|---|---|---|---|---|
| Albumin | control | 3.415 | 0.091924 | 0.854 |
| | N. W. | 3.46 | 0.268701 | |
| Total Protein | control | 7.59 | 0.127279 | 0.037* |
| | N. W. | 6.9 | 0.141421 | |
| GPT | control | 103 | 2.828427 | 0.994 |
| | N. W. | 103.5 | 71.417785 | |
| GOT | control | 133.5 | 60.104076 | 0.945 |
| | N. W. | 141 | 115.96551 | |
| Bilirubin | control | 0.29 | 0.028284 | 0.405 |
| | N. W. | 0.245 | 0.049497 | |
| Amylase | control | 1730.5 | 53.033009 | 0.063 |
| | N. W. | 657 | 190.91883 | |
| Creatinine | control | 0.18 | 0 | 0.079 |
| | N. W. | 0.26 | 0.014142 | |
| Urea | control | 50.15 | 1.06066 | 0.472 |
| | N. W. | 49.15 | 1.202082 | |
| Glucose | control | 155.4 | 13.152186 | 0.528 |
| | N. W. | 168.8 | 20.364675 | |
| High Density Lipoprotein | control | 16.6 | 0.424264 | 0.243 |
| | N. W. | 12.65 | 2.333452 | |
| Low Density Lipoprotein | control | 9.55 | 1.343503 | 0.167 |
| | N. W. | 6.5 | 0.424264 | |
| Iron | control | 232 | 32.526912 | 0.126 |
| | N. W. | 340.5 | 45.961941 | |
| Magnesium | control | 2.955 | 0.035355 | 0.179 |
| | N. W. | 2.835 | 0.06364 | |
| Phosphorus | control | 7.935 | 0.487904 | 0.041* |
| | N. W. | 5.58 | 0.494975 | |

This table presents the comparison of mean values for various biochemical parameters between the control group and the starved group with access to normal drinking water. Independent T-sample tests were conducted to assess differences between the two groups.

**Group Type:** Indicates the group being compared (control group vs. starved group with normal water access).

**Mean:** Represents the mean value of the biochemical parameter for each group.

**Std. Deviation:** Shows the standard deviation of the mean values within each group.

**P-Value:** Indicates the significance level of the difference between the two groups. A p-value less than 0.05 is considered statistically significant*, and less than 0.01 consider as highly statistically significant**

**Table 2: Comparison of Biochemical Parameters between Control and Starved Groups with Zamzam Water Consumption**

| | Group Type | Mean | Std. Deviation | P-Value |
|---|---|---|---|---|
| Albumin | control | 3.415 | 0.091924 | 0.523 |
| | Z. W. | 3.25333 | 0.358515 | |
| Total Protein | control | 7.59 | 0.127279 | 0.013* |
| | Z. W. | 6.56667 | 0.277909 | |
| GPT | control | 103 | 2.828427 | 0.696 |
| | Z. W. | 153.66667 | 194.29445 | |
| GOT | control | 133.5 | 60.104076 | 0.431 |
| | Z. W. | 226.33333 | 157.823741 | |
| Bilirubin | control | 0.29 | 0.028284 | 0.805 |
| | Z. W. | 0.28333 | 0.020817 | |
| Amylase | control | 1730.5 | 53.033009 | 0.013* |
| | Z. W. | 530.66667 | 275.118762 | |
| Creatinine | control | 0.18 | 0 | 0.05 |
| | Z. W. | 0.28333 | 0.041633 | |
| Urea | control | 50.15 | 1.06066 | 0.205 |
| | Z. W. | 48.36667 | 0.665833 | |
| Glucose | control | 155.4 | 13.152186 | 0.289 |
| | Z. W. | 117 | 46.555236 | |
| HDL | control | 16.6 | 0.424264 | 0.199 |
| | Z. W. | 18.1 | 0.848528 | |
| LDL | control | 9.55 | 1.343503 | 0.151 |
| | Z. W. | 6.35 | 0.494975 | |
| Iron | control | 232 | 32.526912 | 0.149 |
| | Z. W. | 324.66667 | 71.765823 | |
| Magnesium | control | 2.955 | 0.035355 | 0.386 |
| | Z. W. | 2.99 | 0.01 | |
| Phosphorus | control | 7.935 | 0.487904 | 0.144 |
| | Z. W. | 6.71333 | 0.892711 | |

This table presents the comparison of mean values for various biochemical parameters between the control group and the starved group with access to Zamzam water. Independent T-sample tests were conducted to assess differences between the two groups

**Group Type:** Indicates the group being compared (control group vs. starved group starved group with Zamzam water access).

**Mean:** Represents the mean value of the biochemical parameter for each group.**Std. Deviation:** Shows the standard deviation of the mean values within each group.

**P-Value:** Indicates the significance level of the difference between the two groups. A p-value less than 0.05 is considered statistically significant*, and less than 0.01 consider as highly statistically significant**

**Table 3: Comparison of Biochemical Parameters between Starved Groups with Normal Water and Zamzam Water**

| | Group Type | Mean | Std. Deviation | P- Value |
|---|---|---|---|---|
| Albumin | N. W. | 3.46 | 0.268701 | 0.519 |
| | Z. W. | 3.25333 | 0.358515 | |
| Total Protein | N. W. | 6.9 | 0.141421 | 0.177 |
| | Z. W. | 6.56667 | 0.277909 | |
| GPT | N. W. | 103.5 | 71.417785 | 0.714 |
| | Z. W. | 153.66667 | 194.29445 | |
| GOT | N. W. | 141 | 115.96551 | 0.539 |
| | Z. W. | 226.33333 | 157.82374 | |
| Bilirubin | N. W. | 0.245 | 0.049497 | 0.461 |
| | Z. W. | 0.28333 | 0.020817 | |
| Amylase | N. W. | 657 | 190.91883 | 0.589 |
| | Z. W. | 530.66667 | 275.11876 | |
| Creatinine | N. W. | 0.26 | 0.014142 | 0.445 |
| | Z. W. | 0.28333 | 0.041633 | |
| Urea | N. W. | 49.15 | 1.202082 | 0.519 |
| | Z. W. | 48.36667 | 0.665833 | |
| Glucose | N. W. | 168.8 | 20.364675 | 0.193 |
| | Z. W. | 117 | 46.555236 | |
| High Density Lipoprotein | N. W. | 12.65 | 2.333452 | 0.155 |
| | Z. W. | 18.1 | 0.848528 | |
| Low Density Lipoprotein | N. W. | 6.5 | 0.424264 | 0.776 |
| | Z. W. | 6.35 | 0.494975 | |
| Iron | N. W. | 340.5 | 45.961941 | 0.783 |
| | Z. W. | 324.66667 | 71.765823 | |
| Magnesium | N. W. | 2.835 | 0.06364 | 0.175 |
| | Z. W. | 2.99 | 0.01 | |
| Phosphorus | N. W. | 5.58 | 0.494975 | 0.167 |
| | Z. W. | 6.71333 | 0.892711 | |

This table presents the comparison of mean values for various biochemical parameters between the starved group with access to normal water and the starved group with access to Zamzam water. Independent T-sample tests were conducted to assess differences between the two groups.

**Group Type:** Indicates the type of starved group being compared (normal water vs. Zamzam water).

**Mean:** Represents the mean value of the biochemical parameter for each group.

**Std. Deviation:** Shows the standard deviation of the mean values within each group.

**P-Value:** Indicates the significance level of the difference between the two groups. A p-value less than 0.05 is considered statistically significant*, and less than 0.01 consider as highly statistically significant**

**Table 4: Results of One-Way ANOVA Test Comparing Biochemical Parameters among Three Groups**

| | | Sum of Squares | Mean Square | P- Value |
|---|---|---|---|---|
| Albumin | Between Groups | 0.06 | 0.03 | 0.72 |
| Total Protein | Between Groups | 1.265 | 0.632 | 0.017* |
| GPT | Between Groups | 4357.69 | 2178.845 | 0.9 |
| GOT | Between Groups | 13660.548 | 6830.274 | 0.69 |
| Bilirubin | Between Groups | 0.002 | 0.001 | 0.392 |
| Amylase | Between Groups | 1906138.5 | 953069.27 | 0.008** |
| Creatinine | Between Groups | 0.013 | 0.007 | 0.047* |
| Urea | Between Groups | 3.823 | 1.912 | 0.225 |
| Glucose | Between Groups | 3666.434 | 1833.217 | 0.328 |
| High Density Lipoprotein | Between Groups | 31.703 | 15.852 | 0.068 |
| Low Density Lipoprotein | Between Groups | 13.043 | 6.522 | 0.056 |
| Iron | Between Groups | 14302.262 | 7151.131 | 0.235 |
| Magnesium | Between Groups | 0.03 | 0.015 | 0.024* |
| Phosphorus | Between Groups | 5.549 | 2.775 | 0.074 |

This table presents the results of a one-way ANOVA test comparing the mean values of various biochemical parameters among three groups.

**Sum of Squares:** Represents the sum of squares for each biochemical parameter between groups.

**Mean Square:** Indicates the mean square value for each biochemical parameter between groups.

**P-Value:** Indicates the significance level of the difference among the three groups for each biochemical parameter. A p-value less than 0.05 is considered statistically significant*, and less than 0.01 consider as highly statistically significant**

# DISCUSSION

Water is one of the main dietary components. Its quality plays an important role in the health of human beings [12]. Water is indispensable for life, serving as a fundamental necessity and playing a crucial role in numerous physiological functions within the human body (Cazier and Gekas 2001). Despite the limited sources of water and the rapid depletion of existing ones, Zamzam stands out as a unique water source, catering to the needs of billions of people around the world (Samy et al. 2017), (Khalid et al. 2014b), and (Naeem, Alsanussi, and Almohandis 1983). Zamzam Water is situated in the Mecca area, which is a region within Saudi Arabia. The review article highlighted that Zamzam water exhibits a potent anti-inflammatory effect and can potentially aid in the recovery from certain diseases (Khalid et al. 2014b). Additionally, the review article noted that Zamzam water exerts an analytic action by indirectly influencing the endocrine, immunological, and growth systems of the body (Abd-Allah 2022). Zamzam water is natural water consumed by millions of Muslims worldwide (Al-Shihri 2005). Zamzam water is unique as compared to other water. There are neither bacteria nor molds that cause changes in odor and taste. Zamzam water has no signs of biological growth (Bassam H. H. Mashat 2010). Zamzam water, with its alkaline properties, exhibits antioxidant, anti-inflammatory, and antiapoptotic properties against gentamicin-induced testicular toxicity in vivo (Taha, Elazab, Saati, et al. 2023).

The combination of globe artichoke extract with Zamzam water exhibited the highest toxicity against P. solenopsis in the control group. This finding suggests potential applications of Zamzam water in pest management and highlights the need for further research on the combination of globe artichoke leaf extract and Zamzam water (Abd-Allah 2022).

Starvation is a serious deficiency of energy intake for a long period (Cahill Jr 2006) and leads to a lot of changes in metabolic reactions. During the food restriction, the glucose level declines between 24 and 72 hours, resulting in release of glucagon (Allison 1980). In these conditions, the body switches from carbohydrate to fat and protein usage to produce glucose and energy (McCray, Walker, and Parrish 2005), and (Fuentebella and Kerner 2009). Therefore, glucose is replaced with fatty acids and ketone bodies as the main energy source (Crook, Hally, and Panteli 2001).

To the best of our knowledge, this study is the first study to investigate the biochemical effects of zamzam water on rats during starvation. Our results revealed that there is a decrease in the rats' weight during severe starvation; this is a normal situation due to the usage of stored lipids, and proteins in the production of energy during severe starvation. This is in agreement with the study of (Lee et al. 2015) who observed that the weight was significantly decreased during starvation (192±6.7 g reduction), Whereas control group rats gained body weight (47.2±6.2 g).

In (Abdullah et al. 2012) study, the effects of carbon tetrachloride on serum GOT and GPT are similar to previous studies, and given the sensitivity of serum levels of these two enzymes to liver disease (Dufour et al. 2000). Further investigation is needed to determine whether the protective mechanism of Zamzam water is similar to that of electrolyzed-reduced water (Hanaoka et al. 2004). The findings suggest that Zamzam water diminishes liver toxicity triggered by carbon tetrachloride in rats. However, additional studies are necessary to validate these results, utilizing liver histopathology and assessing antioxidant parameters in liver tissue. Moreover, further research is needed to explore the effects of Zamzam water in various other contexts and situations of oxidative stress. In our results liver function tests revealed that there is an increase in GPT in Zamzam water group more than control and normal water groups. In albumin, there are no significant differences in Zamzam, control, and normal water groups, but in the case of GOT there is an increase in Zamzam water group more than in control and normal water groups, and in the case of Total bilirubin there is a decrease in normal water group less than control and Zamzam water groups. So further investigations were needed to know the rule of Zamzam water in severe starvation on the liver.

Other study indicated the safety of using Zamzam water in normal rats for medium period of time (Abdullah et al. 2012). However, calcium and chromium concentrations, manganese, copper, and nitrate were reported (Shomar 2012). Our results revealed that Phosphorus concentration in the control group was more than in  normal water and Zamzam water groups, and Zamzam water group more than the normal water group. Magnesium (Mg) determination shows that the results are most probably the same in all groups. Iron (Fe) determination shows that there is a decrease in control less than normal water and Zamzam water groups.

Zamzam water is microbiologically safe and is subjected to routine monitoring in this respect. Thus, the nitrate levels of Zamzam water may not pose any danger even for bottle-fed infants since it is microbiologically safe (Abdullah et al. 2012). Also concluded that this study found no differences between Zamzam water and ordinary bottled water regarding safety for the duration of the two experiments conducted. No signs of arsenic toxicity were also detected. Moreover, Zamzam water tends to potentiate antioxidant power in rats stressed with gentamicin (Abdullah et al. 2012).

Other studies showed Cadmium-induced cytotoxicity in a concentration- and time-dependent manner in both colon cancer Caco-2 cells and normal human liver cell line HL-7702 (Aziz et al. 2014). Calcium and magnesium are the major ions present in ZW. Epidemiologic studies have pointed out the importance of calcium to magnesium ratio in reducing the risk of cancer (Dai et al. 2007). Thus, ZW may be a beneficial source of the proper ratio of calcium to magnesium ions. Furthermore, the concentrations of toxic elements such as arsenate, lead, and selenium are higher than any other water but still lower than the danger level of human consumption (Alfadul and Khan 2011). However, the difference between the therapeutic dose and toxicity is very small. it is presumed that the presence of small amounts of elements such as lithium and arsenic and the combined balanced mixture between other ions, as well as the alkaline nature of ZW could explain its healing properties (Abdullah et al. 2012). Another study demonstrated comparable effects of Zamzam water, bottled water, and mineral water on methemoglobin (MetHb) concentration in young rats. Interestingly, even with high levels of nitrate in older Zamzam samples, no significant difference in MetHb concentration was observed. This suggests that the prolonged consumption of Zamzam water does not have adverse effects on the hemoglobin levels of young rats.

In a particular research study, results from various sample collections conducted at different times consistently revealed that the total concentration of arsenic in Zamzam water exceeds the WHO standards for drinking water by threefold. This underscores the necessity for comprehensive and long-term research on the toxicology and risk assessment associated with arsenic exposure in drinking water (Shomar 2012).

The combined therapy involving dapagliflozin and Zamzam water accelerates the resolution of inflammation by boosting the activity of antioxidant enzymes and inhibiting the NF-κB transcription factor. Consequently, this leads to reduced levels of pro-inflammatory cytokines such as TNFα, IL-1β, and IL6, as well as a decrease in the number of CD45 cells. Additionally, it enhances cell proliferation by elevating Ki-67 expression and promotes angiogenesis by upregulating CD34, eNOS, VEGF-A, EGF-β1, FGF, AngII, and HIF-1α. Moreover, it facilitates the remodeling process by enhancing TGF-β1 and Col1A1. In summary, this combined therapeutic approach presents a promising strategy for managing diabetic patients with wound injuries, undergoing surgical procedures, or experiencing diabetic foot ulcers, effectively reducing the risk of gangrenous complications(Taha, Elazab, Qutub, et al. 2023).

The recent study illustrated that co-administration of Zamzam water (ZW) with subcutaneous injection of CsA (Cyclosporine A) mitigated CsA-induced nephrotoxicity. This was evidenced by the restoration of renal function parameters and subsequent improvement in histological architecture. Additionally, ZW counteracted CsA-induced renal tubular apoptosis by reducing the protein levels of apoptotic markers and increasing the expression of the antiapoptotic protein Bcl-2. Furthermore, it promoted the autophagy process by upregulating LC3, Beclin-1, and Atg5, while downregulating the P62 autophagic marker proteins. This modulation of the AMPK/mTOR/ULK-1 pathway resulted in the inhibition of renal tubular cell apoptosis by enhancing the autophagic process (Taha, Elazab, Baokbah, et al. 2023). Our results revealed that there is increase of creatinine in case of starved rats when compared with normal rats, but there are no significant changes in urea.

The pH indicates that the Zamzam water is alkaline. That might explain the low concentrations of Al, Ag, Co, Cr, Cu, Cd, Zn, Ni, Pb, Fe, and Mn in Zamzam water. The low concentrations of Al in the alkaline Zamzam water do not agree with other research findings(Nguyen et al. 2004). Where Al was high in alkaline water. This disparity could be attributed to the elevated levels of humic acids present in his samples, whereas Zamzam water exhibited very low concentrations of total organic carbon (TOC)(Shomar 2012). The alkaline nature of the Zamzam water could explain its healing properties. Numerous studies have delved into the mechanisms through which alkaline water facilitates healing processes. For example, it was reported that alkaline drinking water plays an important part in ridding the body of mercury and other toxins(Kellas 2019). All these differences between ZW and normal water mean that ZW has special characteristics, and further investigation is needed to know and explain the different effects of ZW.

In our study, we observed a significant increase in HDL levels in the Zamzam water group, while there was no significant change in HDL levels between the two starved groups. However, in another study, certain parameters were notably reduced in the Zamzam water group compared to the tap water group: fasting blood sugar (96.5 vs. 147.1 mg/dl), serum insulin (0.44 vs. 1.31 mU/l), and insulin resistance (1.89 vs. 8.40). LDL cholesterol, HDL cholesterol, superoxide dismutase, lipid peroxidation, body weight, fat pads, carcass weight, as well as residual body weight (both absolute and relative), showed no significant changes (Ghadeer F. AlJuwaie et al. 2020). This difference in the case of the ZW group may be due two that our study used starved rate, but in the other study, they used normal feeding. Rats fed a ketogenic diet (KD) along with Zamzam water exhibited significantly reduced fasting blood glucose (FBG) and LDL cholesterol levels compared to rats fed a regular chow diet with ordinary water (p-values 0.001), as well as compared to those fed KD with ordinary water (p-values 0.004 and 0.006, respectively). However, there were no significant differences observed in serum insulin, insulin resistance, HDL cholesterol, superoxide dismutase, and malondialdehyde levels (Ghadeer Fouad AlJuwaie et al. 2023).

## CONCLUSION

Severe starvation causes biochemical changes, as evidenced by an increase in GPT, GOT, and creatine, particularly in the Zamzam water group. We observed a decrease in phosphorus, amylase, and LDL. In conclusion, the effect of the Zamzam water on the rat's biochemical reactions is not clear. So. we need further investigation, and also we need to push the rats to drink the normal amount of water during severe starvation because we observed that rats drink very small amounts of water during severe starvation.

REFERENCES

Abd-Allah, Ghada E. 2022. “Zamzam Water May Act as Pesticide with and without Globe Artichoke Extract against the Cotton Mealy Bug, Phenacoccus Solenopsis.” *The Journal of Basic and Applied Zoology* 83(1). https://doi.org/10.1186/s41936-022-00285-8.

Abdullah, A, E Abdelsalam, B Abdullah, and A Khaled. 2012. “Antioxidant Effects of Zamzam Water in Normal Rats and Those under Induced-Oxidative Stress.” *Journal of Medicinal Plants Research* 6(42): 5507–12.

Al-Shihri, Z. 2005. “Makkah Al-Mukarramah in the Old Period: A Reading in History and Poetry.” *Sci J King Faisal Univ* 2: 473–79.

Al-Zuhair, A, H Al-Ghamdi, and M Noorwali. 2005. “Analytical Report of Zamzam Water during the Ramadan and Hajj Seasons 1425H.” *The Institute of the Custodian of the Two Holy Mosques for Al Hajj Research Centre, Om Al Qura University, Makkah*.

Alfadul, Sulaiman M, and Mujahid A Khan. 2011. “Water Quality of Bottled Water in the Kingdom of Saudi Arabia: A Comparative Study with Riyadh Municipal and Zamzam Water.” *Journal of Environmental Science and Health, Part A* 46(13): 1519–28.

Ali, Ali Farid Mohammed et al. 2009. “Treatment of Cervical Insufficiency Abortion by Zamzam Water Activated Autologeous Human Peripheral Blood Mononuclear Cell, Modern Trend.” In *13th International Water Technology Conference, IWTC: Hurghada, Egypt*, , 1533–41.

AlJuwaie, Ghadeer F. et al. 2020. “Effects of Zamzam Water on Glycemic Status, Lipid Profile, Redox Homeostasis, and Body Composition in Rats.” *Journal of Taibah University Medical Sciences* 15(1): 14–18. https://doi.org/10.1016/j.jtumed.2019.12.008.

AlJuwaie, Ghadeer Fouad et al. 2023. “Additive Effects of Zamzam Water in Reducing Fasting Blood Glucose and LDL-Cholesterol in Rats Fed on a Ketogenic Diet.” *Arab Gulf Journal of Scientific Research* 41(2): 134–40.

Allison, Simon P. 1980. “Effect of Insulin on Metabolic Response to Injury.” *Journal of Parenteral and Enteral Nutrition* 4(2): 175–79.

Aziz, Rukhsanda et al. 2014. “Impact Assessment of Cadmium Toxicity and Its Bioavailability in Human Cell Lines (Caco-2 and HL-7702).” *BioMed research international* 2014.

Balasubramanian, N et al. 2015. “Groundwater Classification and Its Suitability in Kadaladi, Ramanathapuram, India Using GIS Techniques.” *Environmental Earth Sciences* 74: 3263–85.

Bamosa, A et al. 2013. “Zamzam Water Ameliorates Oxidative Stress and Reduces Hemoglobina1c in Type 2 Diabetic Patients.” *J Diabetes Metab* 4(03): 2–6.

Bassam H. H. Mashat. 2010. “The Microbiological Quality of Sabil (Free) Drinking Water in Makkah Al-Mukarramah during Ramadan 2007.” 142(587): 1–27.

Cahill Jr, George F. 2006. "Fuel Metabolism in Starvation." *Annu. Rev. Nutr.* 26: 1–22.

Careem, S. 2005. "The Miracle of Zamzam. Sunday Observer. Retrieved on 5th June, 2005. Provides a Brief History of the Well and Some Information on the Health Benefits of Zamzam Water."

Cazier, Jean-Baptiste, and Vassilis Gekas. 2001. "Water Activity and Its Prediction: A Review." *International Journal of Food Properties* 4(1): 35–43.

Crook, M A, V Hally, and J V Panteli. 2001. "The Importance of the Refeeding Syndrome." *Nutrition* 17(7–8): 632–37.

Dai, Qi et al. 2007. "The Relation of Magnesium and Calcium Intakes and a Genetic Polymorphism in the Magnesium Transporter to Colorectal Neoplasia Risk." *The American journal of clinical nutrition* 86(3): 743–51.

Dufour, D Robert et al. 2000. "Diagnosis and Monitoring of Hepatic Injury. I. Performance Characteristics of Laboratory Tests." *Clinical chemistry* 46(12): 2027–49.

El-Zaiat, S Y. 2005. "Group Refractive Index Measurement by Fringes of Equal Chromatic Order." *Optics & Laser Technology* 37(3): 181–86.

El- Kashef, Hassan. 1994. "Optical and Electrical Properties of Materials." *Review of scientific instruments* 65(6): 2056–61.

Fuentebella, Judy, and John A Kerner. 2009. "Refeeding Syndrome." *Pediatric Clinics* 56(5): 1201–10.

Hanaoka, Kokichi et al. 2004. "The Mechanism of the Enhanced Antioxidant Effects against Superoxide Anion Radicals of Reduced Water Produced by Electrolysis." *Biophysical chemistry* 107(1): 71–82.

Helena, Beatriz et al. 2000. "Temporal Evolution of Groundwater Composition in an Alluvial Aquifer (Pisuerga River, Spain) by Principal Component Analysis." *Water research* 34(3): 807–16.

Hofer, Tim et al. 2008. "Increased Iron Content and RNA Oxidative Damage in Skeletal Muscle with Aging and Disuse Atrophy." *Experimental Gerontology* 43(6): 563–70.

Kellas, W., Dworkin, A., Kellas, W., Dworkin, A.,. 2019. *GCC Standardization Organization. Technical Committee: Labeling for Drinking Water and Bottled Natural Mineral Water*.

Khalid, Nauman et al. 2014a. "Mineral Composition and Health Functionality of Zamzam Water: A Review." *International journal of food properties* 17(3): 661–77.

———. 2014b. "Mineral Composition and Health Functionality of Zamzam Water: A Review." *International Journal of Food Properties* 17(3): 661–77.

Lee, Sung Ryul et al. 2015. "Influence of Starvation on Heart Contractility and Corticosterone Level in Rats." *Pflügers Archiv-European Journal of Physiology* 467: 2351–60.

Mashat, Bassam. 2010. "The Microbiological Quality of Sabil (Free) Drinking Water in Makkah Al-Mukarramah during Ramadan 2007." *Journal of King Abdulaziz University-Meteorology,* *Environment and Arid Land Agriculture Sciences* 21(2): 87–100.

McCray, Stacey, Sherrie Walker, and Carol Rees Parrish. 2005. "Much Ado about Refeeding." *Pract Gastroenterol* 29(1): 26–44.

Moni, S. S. et al. 2022. "Physical Characterization and Wound Healing Properties of Zamzam Water." *Brazilian Journal of Biology* 82: 1–11.

Naeem, A, M Y Alsanussi, and A A Almohandis. 1983. "Multielemental and Hydrochemical Study of Holy Zamzam Water." *Journal of the New England Water Works Association* 97(2).

Nassini, Romina et al. 2010. "A Bicarbonate-Alkaline Mineral Water Protects from Ethanol-Induced Hemorrhagic Gastric Lesions in Mice." *Biological and Pharmaceutical Bulletin* 33(8): 1319–23.

Nguyen, K L, D M Lewis, M Jolly, and J Robinson. 2004. "Determination of Soluble Aluminium Concentration in Alkaline Humic Water Using Atomic Absorption Spectrophotometry." *Water research* 38(19): 4039–44.

Samy, S M, A K Khamis, M M Khalid, and others. 2017. "Prevalence and Predictors of Khat Chewing among Students of Jazan University, Jazan, Kingdom of Saudi Arabia." *Int J of Preventive and Public Health Sciences* 2. https://www.ijpphs.com/index.php/ijpphs/article/view/107 (July 3, 2023).

Shomar, Basem. 2012. "Zamzam Water: Concentration of Trace Elements and Other Characteristics." *Chemosphere* 86(6): 600–605.

Subramani, T, L Elango, and S R Damodarasamy. 2005. "Groundwater Quality and Its Suitability for Drinking and Agricultural Use in Chithar River Basin, Tamil Nadu, India." *Environmental Geology* 47: 1099–1110.

Taha, Medhat, Mohamed Ezzat Mahmoud, et al. 2023. "Anxiolytic and Antidepressant like Effects of Zamzam Water in STZ-Induced Diabetic Rats, Targeting Oxidative Stress, Neuroinflammation, BDNF/ERK/CREP Pathway with Modulation of Hypothalamo-Pituitary–Adrenal Axis." *Frontiers in Neuroscience* 17.

Taha, Medhat, Sara T. Elazab, Ammar Qutub, et al. 2023. "Novel Insights about Synergistic Effect of Zamzam Water with SGL2 Inhibitors on Wound Healing in STZ-Induced Diabetic Rats: The Role of Anti-Inflammatory and Proangiogenic Effects." *Journal of Investigative Surgery* 36(1). https://doi.org/10.1080/08941939.2023.2266736.

Taha, Medhat, Sara T Elazab, Tourki A S Baokbah, et al. 2023. "Palliative Role of Zamzam Water against Cyclosporine-Induced Nephrotoxicity through Modulating Autophagy and Apoptosis Crosstalk." *Toxics* 11(4): 377.

Taha, Medhat, Sara T. Elazab, Abdullah A. Saati, et al. 2023. "Zamzam Water Ameliorates Gentamicin-Induced Testicular Toxicity in a Rat Model via Targeting Sperm Parameters, Testicular Tissue Oxidative Insult, Inflammation, Apoptosis, and Pituitary-Gonadal Axis." *Toxics* 11(1).

Vidyasagar, D. 2007. "Global Minute: Water and Health–Walking for Water and Water Wars." *Journal* *of Perinatology* 27(1): 56–58.

Zhu, Bingqi et al. 2011. "Hydrogeochemistry of Three Watersheds (the Erlqis, Zhungarer and Yili) in Northern Xinjiang, NW China." *Applied Geochemistry* 26(8): 1535–48.

———. 2012. "Climatic and Geological Factors Contributing to the Natural Water Chemistry in an Arid Environment from Watersheds in Northern Xinjiang, China." *Geomorphology* 153: 102–14.

———. 2016. "Natural Water Quality and Its Suitability for Drinking and Irrigation Purposes in the Jungar Basin." *Central Asia. Journal of Civil & Environmental Engineering* 6: 232.

Zhu, BingQi, and XiaoPing Yang. 2007. "The Ion Chemistry of Surface and Ground Waters in the Taklimakan Desert of Tarim Basin, Western China." *Chinese Science Bulletin* 52(15): 2123–29.

Al Zuhair, Nour, and Rita Khounganian. 2006. "A Comparative Study between the Chemical Composition of Potable Water and Zamzam Water in Saudi Arabia." *Saudi Dental Journal* 18: 1–9.

## Figure Legends

**Figure -1:** Changes in the rats' weight (gram) under conditions of severe starvation and daring in Zamzam water and normal water were compared with the control group.

## Table Legends

**Table 1: Comparison of Biochemical Parameters between Control and Starved Groups**

**Table 2: Comparison of Biochemical Parameters between Control and Starved Groups with Zamzam Water Consumption**

**Table 3: Comparison of Biochemical Parameters between Starved Groups with Normal Water and Zamzam Water**

**Table 4: Results of One-Way ANOVA Test Comparing Biochemical Parameters among Three Groups**