Original Article

Access this article online

Quick Response Code:

Website: www.jfcmonline.com

DOI: 10.4103/jfcm.JFCM_21_18

Effect of Zamzam water on blood methemoglobin level in young rats

Ahmed Badar, Abdullah O. Bamosa, Mohammed Salahuddin[1], Abdullah Al Meheithif[2]

Abstract:

BACKGROUND: Methemoglobin (MetHb) level in blood indicates exposure to nitrogenous compounds. Acquired methemoglobinemia as a result of exposure to nitrates in drinking water is primarily an issue for infants. The amount of nitrates in Zamzam water is said to be on the high side. This study was designed to determine the effect of prolonged use of Zamzam water on MetHb in rat pups.

MATERIALS AND METHODS: Wistar rat pups (n = 52, 3 weeks old) were divided into four equal groups. All of them were given normal laboratory chow. The groups differed only in the exclusive source of water, that is ordinary bottled water, standardized mineral water, old Zamzam water (stored since 2008) or fresh Zamzam water. MetHb level was checked (using Avoximeter 4000) at the baseline, and then every week for 4 weeks from blood obtained from retro-orbital sinus. Other parameters tested were total haemoglobin, oxyhemoglobin and carboxyhemoglobin. ANOVA was used to compare the means between the groups.

RESULTS: None of the rats in any of the four groups showed any sign of methemoglobinemia or toxicity. Both groups on Zamzam water showed higher increments in their total hemoglobin by the end of the study compared to their baseline (22%) than the ordinary water (9%) and the mineral water (5%) groups. None of the groups showed any significant difference in MetHb levels on intergroup comparison at any of the weekly readings and at the end of the study.

CONCLUSION: Prolonged use of Zamzam water did not induce any significant difference in MetHb concentration in rat pups, which might indicate that it is safe for infants.

Keywords:

Methemoglobin, nitrate, water, zamzam

Introduction

Hemoglobin (Hb), the oxygen carrier protein of red blood cells (RBCs), is made up of 4 heme groups.[1] The heme group has an iron molecule in ferrous form (Fe^{2+}). Fe^{2+} combines with oxygen to form oxyhemoglobin by sharing an electron that is returned when oxyhemoglobin releases oxygen to the tissues.[2] Hb can accept and transport oxygen only when iron is in the Fe^{2+} form. If iron in Hb loses an electron and becomes oxidized, it is converted to the ferric state (Fe^{3+}) and the resulting Hb is called methemoglobin (MetHb).[2] MetHb does not have the electron needed to make a bond with oxygen and is therefore incapable of transporting oxygen.[3]

Normally RBCs contain about 1% of MetHb that is generated by the exposure of RBCs to different types of oxidative stresses.[4] Two important mechanisms maintain this low level of MetHb. The first is the hexose-monophosphate shunt pathway within the erythrocyte that reduces the oxidizing agents by glutathione prior to the formation of MetHb. The second mechanism against MetHb formation uses 2 enzyme systems, diaphorase I and diaphorase II that reduce MetHb to its original ferrous state.[2,5]

Department of Physiology, College of Medicine, Imam Abdulrahman Bin Faisal University, [1]Institute for Research and Medical Consultation, Imam Abdulrahman Bin Faisal University, Dammam, [2]Ministry of Water and Electricity, Riyadh, KSA

Address for correspondence:
Dr. Abdullah Omar Bamosa, Department of Physiology, College of Medicine, Imam Abdulrahman Bin Faisal University, P.O. Box 2114, Dammam 31441, KSA.
E-mail: bamosa@iau.edu.sa

How to cite this article: Badar A, Bamosa AO, Salahuddin M, Al Meheithif A. Effect of Zamzam water on blood methemoglobin level in young rats. J Fam Community Med 2019;26:30-5.

Methemoglobinemia is mostly due to excessive production of MetHb following exposure to oxidant drugs, chemicals, or toxins. This increased production of MetHb overwhelms the physiologic regulatory mechanisms. These agents can cause an increase in MetHb levels either by ingestion or by absorption through the skin. Such agents fall into 2 general categories, nitrites and aromatic amines.[4] Abnormal Hb types can also cause methemoglobinemia. These abnormal Hbs are called Hb M because they are associated with methemoglobinemia.[6]

Methemoglobinemia occurs when RBCs contain >1% MetHb. Problems arise when levels increase, as MetHb does not bind with oxygen, leading to functional anemia. The result of methemoglobinemia is that oxygen delivery to tissues is impaired and the oxygen Hb dissociation curve shifts to the left.[2] Organs with high oxygen demand such as the central nervous system and the cardiovascular system are usually the first to manifest toxicity.[5] Methemoglobinemia is considered an acquired disorder although a few congenital cases have also been reported.[7,8]

Acquired methemoglobinemia can be caused by a variety of substances including inorganic as well as organic nitrates and nitrites. Nitrate, a relatively nontoxic substance, occurs naturally as part of the nitrogen cycle. However, bacteria can convert nitrate to nitrite in the environment, in foods and in the human body.[9] The nitrite can come from nitrate in drinking water, from food, some drugs or other sources.[10] Once in the blood, nitrite oxidizes iron in the Hb of RBCs to form MetHb. As MetHb levels increase, patients demonstrate evidence of cellular hypoxia. Death occurs when MetHb approaches around 70%, but it can occur at lower levels in patients with significant comorbidities.[11]

Nitrate level in ground and surface water is normally low. However, it can increase as a result of leaching, runoff from agricultural land or contamination from human or animal wastes. Likewise, anaerobic conditions may result in the formation and persistence of nitrite. The U.S. environmental protection agency set the maximum contaminant level (MCL) for nitrates in public drinking water at 10-mg/L nitrate-nitrogen (NO_3-N) to protect infants from methemoglobinemia.[12] Private drinking water systems are not regulated and may be more vulnerable to nitrate contamination, particularly in areas of intense agricultural activities.[13,14]

Acquired methemoglobinemia resulting from exposure to nitrates in drinking water is considered primarily an issue for infants <6 months old.[15] Pregnant women are also considered vulnerable to the effects from exposure to high levels of nitrate in drinking water,[11,16] and reports suggest an association between environmental nitrate exposure with adverse consequences both during pregnancy and delivery.[8,17]

Various types of drinking water are available in Saudi Arabia: Tap water, spring water, bottled water and mineral water. The water from wells in Saudi Arabia is often high in mineral content.[18]

Saudi Arabia is also the home of "Zamzam water" which according to the Islamic history was revealed to Hagar (*Hajira*), the wife of Prophet Ibrahim (Abraham) and mother of Ismail, around the year 2000 BC when she was desperately seeking water for her infant son, but couldn't find any.[19] The Zamzam well is located approximately 20 m (66 ft) East of the Kaaba (the building at the center of the holy mosque in Makkah).[20] The Saudi Geological Survey has a "Zamzam Studies and Research Centre" which analyses the technical properties of the well in detail. Water levels are monitored by a digital system that tracks the water level, electric conductivity, pH and temperature. All of this information is made continuously available via the Internet.[21]

Zamzam water has no colour or smell, but has a distinct taste, with its pH at 7.9–8.0, indicating that it is alkaline to some extent. Minerals mass concentration of zamzam is 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.[18]

MethHb level in the blood is an accepted biomarker used in research for assessing exposure to nitrogen compounds or other substances that can oxidize Hb. If exposure to an oxidizer such as nitrate raises MetHb level, it can be readily reduced back to Hb once the oxidizer is removed. We designed this study to test the effect of presumably high nitrate content of Zamzam water on MetHb in young rats.

Materials and Methods

This Quasi-experimental study was carried out from January 2015 to December 2016. Approval for the project was granted by the Institution Review Board of Imam Abdulrahman Bin Faisal University, Dammam, KSA, after certifying compliance to guiding principles in the care and use of animals adapted from National Institute of Health. A total of 52 Wistar Rat pups acquired from the university animal house, and weaned at the 3rd week from the mother's milk, were used. The intervention began after 1 week of quarantine, during which all animals were given ordinary bottled water to drink.

The rats were divided into the following groups:

- Group-M (n = 13): This group, given mineral water of verified composition as the only source of water, served as the main control. The mineral water selected had a certified quantity of nitrite and nitrate according to the World Health Organization (WHO) guidelines (i.e., the sum of the ratios of the concentration (C) of nitrate and nitrite to its guideline value (GV) not exceeding 1, Cnitrate/GVnitrate + Cnitrite/GVnitrite $\leq$1)[22]
- Group-B (n = 13): This group which continued to drink ordinary bottled water as the only source of water, served as the second control
- Group-OZ (n = 13): This group was given Zamzam water stored since 2008 with a certified Nitrate content of 125 ppm as the only source of water
- Group-NZ (n = 13): This group was given Fresh Zamzam water as the only source of water. Its Nitrate content was 35.48 ± 4.47 mg/L^{-1}, based on means of 33 samples as reported by Al-Barakah.[23]

The rats were housed in polycarbonate cages kept at 70 to 79°F on a daily 12-h light/dark cycle. Each cage was marked with the group name. Normal laboratory chow was provided *ad libitum*. A record of water consumption was kept.

At the start of the experiment a baseline value of blood MetHb was noted. MetHb was later on measured at weekly intervals for 4 weeks at the end of 1st, 2nd, 3rd and 4th week. The values of total haemoglobin, Oxyhemoglobin and carboxyhemoglobin were noted as supplementary data to estimate the proportion of MetHb.

Blood was obtained from retro-orbital sinus, observing the ethical methods for the procedure comprising gentle handling, unhostile environment, being blinded from procedure on other animals, sterilization and mild sedation.

The testing was performed immediately after the collection of blood using the portable Avoximeter 4000 (Avox Systems, Inc., San Antonio, TX) whole blood oximeter device that measures the total haemoglobin, and the percentage in the form of oxyhemoglobin, carboxyhemoglobin, or MetHb. The manufacturer affirms an accuracy of ±0.5% and precision of ±0.7%. However, calibration of the instrument was carried out at the beginning of each sampling day using optical standards and controls provided by the manufacturer. MetHb was reported as a percentage of total Hb.

IBM SPSS Statistics version 21 (IBM Inc.) was used for data analysis. Descriptive statistics were used to calculate mean and standard deviation of MetHb, total haemoglobin, oxyhemoglobin and carboxyhemoglobin. Mixed-Design ("Split-Plot") ANOVA was used to compare repeated measures between the groups. The dependent variable for the Mixed-Design ANOVA was the respective haemoglobin level that is MetHb, total haemoglobin, oxyhemoglobin and carboxyhemoglobin. The within-subjects factor was the 5 time points when measurements were carried out (baseline, weeks 1–4). The between-subjects factor was the treatment of the four types of water (bottled, mineral, old zamzam and new zamzam) given to the respective group of the rats. Assuming equal variance, Tukey's *post-hoc* test was used to carry out pair-wise analysis in order to determine the group with significant difference. A $P < 0.05$ was considered statistically significant.

Results

Most rats tolerated the procedure well. None of the rats showed any of the known signs or symptoms of methemoglobinemia. In addition, none of the rats showed any signs of toxicity. The number of samples obtained in certain readings was less because of inability to extract blood from the rats from retro-orbital sinus on those occasions.

Tables 1-4 show comparisons of total haemoglobin, oxyhemoglobin, carboxyhemoglobin and MetHb (respectively) at the baseline (day 0, that is after 7 days of quarantine) and weekly after that till the end of study on the 28th day (that are days 7, 14, 21 and 28).

An elevation in total haemoglobin was observed in all the groups. However, this increase was more pronounced (statistically non-significant) by the end of the 4th week in both zamzam groups (OZ and NZ) as shown in Table 1. The percentage of elevation in total haemoglobin by the end of the study was almost the same in both groups, 22% of their corresponding baseline values. On the other hand, the bottled water and mineral groups showed only 9% and 5% increments in their total haemoglobin,

Table 1: Comparison of Total Hemoglobin between the four groups

Sampling Day	Bottled	Mineral	Old Zamzam	New Zamzam	*P*
Day-0	12.20±1.58 (*n*=12)	11.55±1.49 (*n*=13)	11.35±1.66 (*n*=13)	10.58±1.68 (*n*=13)	0.12
Day-07	12.66±1.43 (*n*=11)	11.16±1.56 (*n*=12)	12.09±1.19 (*n*=13)	11.27±2.28 (*n*=13)	0.11
Day-14	12.92±1.90 (*n*=11)	10.98±2.17 (*n*=12)	12.16±1.62 (*n*=12)	11.29±1.72 (*n*=11)	0.07
Day-21	12.83±2.46 (*n*=11)	11.58±2.11 (*n*=12)	12.60±1.28 (*n*=12)	12.35±1.34 (*n*=11)	0.41
Day-28	13.29±1.73 (*n*=10)	12.10±1.54 (*n*=12)	13.83±1.34 (*n*=13)	12.87±1.39 (*n*=11)	0.06

None of the differences was statistically significant on Mixed-Design Anova

Table 2: Comparison of Oxyhemoglobin between the four groups

Sampling Day	Bottled Water Mean±SD	Mineral Water Mean±SD	Old Zamzam Mean±SD	New Zamzam Mean±SD	*p*-Value
Day-0	54.20±12.31 (*n*=12)	52.65±10.65 (*n*=13)	53.68±14.47 (*n*=13)	55.05±15.70 (*n*=13)	0.97
Day-07	58.96±12.55 (*n*=11)	54.24±7.54 (*n*=12)	53.87±12.04 (*n*=13)	58.56±10.67 (*n*=13)	0.52
Day-14	53.40±12.18 (*n*=11)	55.72±11.53 (*n*=12)	54.13±11.53 (*n*=13)	57.07±14.17 (*n*=11)	0.9
Day-21	56.02±9.64 (*n*=11)	55.53±9.60 (*n*=12)	55.26±10.31 (*n*=13)	56.15±12.98 (*n*=11)	0.1
Day-28	53.93±12.61 (*n*=10)	62.97±12.62 (*n*=12)	55.59±11.80 (*n*=13)	57.07±18.49 (*n*=11)	0.45

None of the differences was statistically significant on Mixed-Design Anova

Table 3: Comparison of Carboxyhemoglobin between the four groups

Sampling Day	Bottled water Mean±SD	Mineral Water Mean±SD	Old Zamzam Mean±SD	New Zamzam Mean±SD	*p*-Value
Day-0	0.98±0.93 (*n*=13)	1.35±1.11 (*n*=13)	1.25±1.55 (*n*=13)	1.84±2.12 (*n*=13)	0.56
Day-07	1.35±1.31 (*n*=11)	1.63±1.53 (*n*=12)	0.97±1.31 (*n*=13)	1.46±1.57 (*n*=13)	0.7
Day-14	0.98±0.66 (*n*=11)	1.51±1.46 (*n*=12)	1.02±1.39 (*n*=13)	1.59±1.44 (*n*=11)	0.55
Day-21	1.01±1.24 (*n*=11)	0.52±0.56 (*n*=12)	0.55±0.90 (*n*=12)	0.60±1.12 (*n*=11)	0.6
Day-28	0.79±0.93 (*n*=10)	1.46±1.06 (*n*=12)	1.20±1.18 (*n*=13)	1.12±1.01 (*n*=11)	0.54

None of the differences was statistically significant on Mixed-Design Anova

Table 4: Comparison of Methemoglobin between the four groups

Sampling Day	Bottled water Mean±SD	Mineral Water Mean±SD	Old Zamzam Mean±SD	New Zamzam Mean±SD	*p*-Value
Day-0	1.08±1.11 (*n*=13)	0.55±0.63 (*n*=13)	0.62±0.52 (*n*=13)	0.69±0.53 (*n*=13)	0.30
Day-07	0.88±0.52 (*n*=11)	0.53±0.41 (*n*=12)	1.19±0.66 (*n*=13)	1.20±0.90 (*n*=13)	0.06
Day-14	1.34±1.15 (*n*=11)	1.01±0.64 (*n*=12)	2.00±1.42 (*n*=13)	2.06±1.67 (*n*=11)	0.14
Day-21	2.27±1.68 (*n*=11)	1.83±1.56 (*n*=12)	2.15±1.76 (*n*=12)	2.75±1.91 (*n*=12)	0.64
Day-28	2.04±2.06 (*n*=10)	1.48±1.34 (*n*=11)	1.93±1.61 (*n*=12)	2.55±1.91 (*n*=11)	0.53

None of the differences was statistically significant on Mixed-Design Anova

respectively. There were no significant differences in oxyhemoglobin or carboxyhemoglobin in any of the readings for any group [Tables 2 and 3].

MetHb had a faster trend towards elevation in both Zamzam groups; however the increase was statistically non-significant [Table 4].

Discussion

The results reported in this study indicate a similar effect for the four types of water on the levels of MetHb. Zamzam water of both high and low concentrations of nitrate showed similar levels of MetHb. Normal bottled water, mineral water and new Zamzam water had confirmed nitrate concentrations below WHO highest recommended level (50 mg/l).[22,23] Although the concentration of nitrate in the old Zamzam water was well above this level (>100 mg/l),[18,24] it did not alter the MetHb level in our study.

Conversion of haemoglobin to MetHb is due to oxidation of iron by oxidant agents.[4] Alkaline water is known to possess antioxidant power.[25,26] Being alkaline, Zamzam water is therefore expected to reduce the oxidative effect of nitrate/nitrite on haemoglobin. Indeed, Zamzam water is reported to have a strong antioxidant effect in patients with Type 2 diabetes mellitus.[27]

All other types of haemoglobin were not significantly different in the four water samples, which indicates the similarity in safety for these sources of water. An interesting finding in this study was the increase (statistically non-significant) in the level of haemoglobin in the two Zamzam water samples. This increase could be due to the lower starting baseline level of haemoglobin in both Zamzam groups that recovered with the growth of the young rats. However, the finding is interesting and deserves further investigation in anaemic animals and humans.

Comly was the first to propose an association of infantile methemoglobinemia to nitrate-contaminated water in 1945.[28] This suggestion led to a survey that reviewed 278 cases of infantile methemoglobinemia in the USA and proposed safe level of nitrates in the water. Subsequently, the USA and WHO established a MCL of 10 ppm for nitrate in drinking water.[29] As only Nitrites (NO_2^-) can react directly with haemoglobin to form methaemoglobin, Nitrates (NO_3^-) in drinking water must first be converted to nitrites (NO_2^-) within the digestive system of infants. It had been suggested earlier that the presence of a concomitant bacterial infection of the upper gastrointestinal tract caused this Nitrate to Nitrate conversion.[28] However, in the course of the next three decades it was established that some gastrointestinal disturbances actually led to infantile methemoglobinemia even in the absence of high nitrate content in drinking water.[30,31] Therefore, the current understanding based upon multiple evidence is that endogenous nitrite production and not exogenous nitrate contamination of drinking water

is the primary cause of methemoglobinemia. This has led to the thinking that restrictions on drinking water containing nitrates solely on account of infantile methemoglobinemia are unnecessarily strict.[32,33] In addition, the WHO has found convincing evidence that the risk of methemoglobinemia is related to concurrent gastrointestinal infections. They affirmed that water with nitrate of 50–100 mg/L can be used for bottle -fed infants if it is microbiologically safe.[22]

However, a relatively recent study in populations using well water in the mountains of Romania reported a direct correlation between the level of MetHb and the nitrate concentrations in water samples. Based upon their data they recommended that only well water with the maximum of 48 mg/L nitrates content should be used.[34]

Until a few years ago, the Nitrate content of Zamzam water was reported to be higher than the standard set by WHO.[24,35] However, recent studies undertaken after vigorous quality control measures undertaken by the Saudi government have shown that Zamzam water in its current form is microbiologically safe with its Nitrate content within the limits set by WHO.[23]

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 make every effort to drink this water during their pilgrimage, and carry it as a gift on their return to their respective countries. Those living nearby tend to drink the water more regularly. However, no population regularly uses this water as their "only source" of water. Our results indicate that Zamzam water might not pose any danger even for bottle-fed infants if is continuously used to make their feed for a few weeks.

Conclusion

This study showed similar effects of Zamzam water, bottled water, and mineral water on MetHb concentration in young rats. Even the high levels of nitrate in samples of old Zamzam did not induce any difference in MetHb concentration. This indicates that the prolonged use of Zamzam water does not adversely affect the Hb of young rats.

Financial support and sponsorship

The work was supported by a grant from Imam Abdulrahman Bin Faisal University (Formerly University of Dammam).

Conflicts of interest

There are no conflicts of interest.

References

1. Steinberg MH, Benz EJ, Adewoye AH, Ebert BL. Pathobiology of the human erythrocyte and its hemoglobins. In: Hoffman R, Benz EJ, Silberstein LE, Heslop HE, Weitz JI, Anastasi J, *et al*., editors. Hematology. 7th ed. Philadelphia: Elsevier; 2017. p. 447-57.
2. Percy MJ, Barnes C, Crighton G, Leventer RJ, Wynn R, Lappin TR, *et al*. Methemoglobin reductase deficiency: Novel mutation is associated with a disease phenotype of intermediate severity. J Pediatr Hematol Oncol 2012;34:457-60.
3. Hamilton GC, Janz TG. Blood and blood components. In: Walls RM, Hockberger RS, Gausche-Hill M, editors. Rosen's Emergency Medicine: Concepts and Clinical Practice. Ch. 111. Philadelphia: Elsevier; 2017. p. 1789-801.
4. Skold A, Cosco DL, Klein R. Methemoglobinemia: Pathogenesis, diagnosis, and management. South Med J 2011;104:757-61.
5. Ozsoylu S. About methemoglobinemia/methemoglobinemia Hakkinda. Turk J Hematol 2012;29:104-6.
6. Cortazzo JA, Lichtman AD. Methemoglobinemia: A review and recommendations for management. J Cardiothorac Vasc Anesth 2014;28:1043-7.
7. McQueen CA. Hematopoietic systems toxicology. In: Comprehensive Toxicology. 3rd ed. Philadelphia: Elsevier; 2017. p. 462-87.
8. Sadler R, Maetam B, Edokpolo B, Connell D, Yu J, Stewart D, *et al*. Health risk assessment for exposure to nitrate in drinking water from village wells in Semarang, Indonesia. Environ Pollut 2016;216:738-45.
9. Pawełczyk A. Assessment of health hazard associated with nitrogen compounds in water. Water Sci Technol 2012;66:666-72.
10. Bedale W, Sindelar JJ, Milkowski AL. Dietary nitrate and nitrite: Benefits, risks, and evolving perceptions. Meat Sci 2016;120:85-92.
11. Schullehner J, Hansen B. Nitrate exposure from drinking water in Denmark over the last 35 years. Environ Res Lett 2014;9:095001.
12. Dubrovsky NM, Burow KR, Clark GM, Gronberg JM, Hamilton PA, Hitt KJ, *et al*. The Quality of Our Nation's Waters-Nutrients in the Nation's Streams and Groundwater, 1992-2004. US Geological Survey; 2010.
13. Francis A. Condensed Toxicity Summary for Nitrates. The Risk Assessment Information System. Available from: https://www.rais.ornl.gov/tox/profiles/nitrates_c_V1.html. [Last accessed on 2017 Nov 14].
14. Nolan BT, Ruddy BC, Hitt KJ, Helsel DR. National Look at Nitrate Contamination of Ground Water. National Water-Quality Assessment (NAWQA) Program. United States Geological Survey. Available from: https://www.water.usgs.gov/nawqa/nutrients/pubs/wcp_v39_no12/. [Last accessed on 2017 Nov 12].
15. Comly HH. Landmark article September 8, 1945: Cyanosis in infants caused by nitrates in well-water. By hunter H. Comly. JAMA 1987;257:2788-92.
16. Sindelar JJ, Milkowski AL. Human safety controversies surrounding nitrate and nitrite in the diet. Nitric Oxide 2012;26:259-66.
17. Tabacova S, Baird DD, Balabaeva L. Exposure to oxidized nitrogen: Lipid peroxidation and neonatal health risk. Arch Environ Health 1998;53:214-21.
18. Al Zuhair N, Khounganian R. A comparative study between the chemical composition of potable water and Zamzam water in Saudi Arabia. Saudi Dent J 2006;18:1-9.
19. Hawting GE. The disappearance and rediscovery of Zamzam and the 'well of the Ka'ba'. Bull Sch Orient Afr Stud 1980;43:44-54.
20. Shil MI, Abdul-Wahid AR. Historic Places: The Well of Zamzam. Available from: http://www.islamonline.net/English/hajj/2002/01/stories/article4.shtml/. [Last accessed on 2017 Oct 14].
21. Zamzam Studies and Research Centre. Saudi Geological Survey.

Available from: http://www.sgs.org.sa/Arabic/zamzam/Pages/default.aspx . [Last accessed on 2017 Oct 07].
22. World Health Organization. Guidelines for Drinking-Water Quality. Geneva: World Health Organization; 2011.
23. Al-Barakah FN. Nitrate and arsenic concentration status in Zamzam water, Holly Mecca Almocarama, Saudi Arabia. Proc Int Acad Ecol Environ Sci 2016;6:110.
24. Shomar B. Zamzam water: Concentration of trace elements and other characteristics. Chemosphere 2012;86:600-5.
25. Davidson EA, David MB, Galloway JN, Goodale CL, Haeuber R, Harrison JA, *et al*. Excess nitrogen in the US environment: trends, risks, and solutions. Issues Ecol 2011. Available from: https://www.pubs.er.usgs.gov/publication/70032270. [Last accessed on 2017 Nov 01].
26. Balazs C, Morello-Frosch R, Hubbard A, Ray I. Social disparities in nitrate-contaminated drinking water in California's San Joaquin Valley. Environ Health Perspect 2011;119:1272-8.
27. Bamosa A, Elnour A, Kaatabi H, Al Meheithif A, Aleissa K, Al-Almaie S. Zamzam water ameliorates oxidative stress and reduces hemoglobina1c in type 2 diabetic patients. J Diabetes Metab 2013;4:2.
28. Comly HH. Cyanosis in infants caused by nitrates in well water. J Am Med Assoc 1945;129:112-6.
29. Walton G. Survey of literature relating to infant methemoglobinemia due to nitrate-contaminated water. Am J Public Health Nations Health 1951;41:986-96.
30. Lebby T, Roco JJ, Arcinue EL. Infantile methemoglobinemia associated with acute diarrheal illness. Am J Emerg Med 1993;11:471-2.
31. Gebara BM, Goetting MG. Life-threatening methemoglobinemia in infants with diarrhea and acidosis. Clin Pediatr (Phila) 1994;33:370-3.
32. Richard AM, Diaz JH, Kaye AD. Reexamining the risks of drinking-water nitrates on public health. Ochsner J 2014;14:392-8.
33. Zeman C, Beltz L, Linda M, Maddux J, Depken D, Orr J, *et al.* New questions and insights into nitrate/nitrite and human health effects: A retrospective cohort study of private well users' immunological and wellness status. J Environ Health 2011;74:8-18.
34. Niţuc E, Năstase V, Mihăilescu G, Chioveanu D. Researches of the nitrates and nitrites in some well waters from rural area in correlation with methoglobinemia morbidity. Rev Med Chir Soc Med Nat Iasi 2010;114:580-6.
35. Khalid N, Ahmad A, Khalid S, Ahmed A, Irfan M. Mineral composition and health functionality of Zamzam water: A review. Int J Food Properties 2014;17:661-77.