Malaysian Journal of Applied Sciences 2016, **Vol 1 (1)**: 68-72

eISSN 0127-9246 (Online)

UniSZA Universiti Sultan Zainal Abidin

Malaysian Journal of Applied Sciences

ORIGINAL ARTICLE

Zamzam Water: Influence of Containers on Ionic Concentration and In-vitro Cytotoxic Effects on U87 Cell Line

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Received: 14/02/2016, Accepted: 09/04/2016

Abstract

Zamzam is holy water believed by Muslim to have remedial power for all kinds of diseases. It contains many electrolytes and the concentration of the electrolytes may be affected by the types of container used for its storage. This study was carried out to determine the difference in ions concentration of Zamzam water stored in plastic and glass containers, and to determine cytotoxicity effects of Zamzam water against U-87 cell line (human primary glioblastoma cell line). Ion Chromatography (IC) was used to analyze the concentration. The analyzed anions in the Zamzam water include bromide, chloride, phosphate, nitrite, nitrate, sulfate and fluoride whereas the cations were ammonium, lithium, potassium, sodium, calcium and magnesium. Subsequently, MTT assay was used to determine the cytotoxicity of Zamzam water on U-87 cell line. This study reveals that Zamzam water anions and cations concentration was not statistically significant neither in plastic nor glass container. In addition, the Zamzam water did not cause any toxicity on the U87 cell line. We postulate that types of container do not have much influence on the ion concentration of Zamzam water and it is non-toxic on U87 cell line.

Keywords: Zamzam water; ion concentration; plastic; glass; U87 cell line.

Introduction

Zamzam water is consumed by millions of Muslims worldwide. It is natural water originated from Zamzam well, founded by Siti Hajar and her son, Prophet Ismail a.s (Ahmad and Ibrahim, 1996) which is one of the miracles from the almighty Allah s.w.t. The Muslims believe that Zamzam water is divinely blessed, able to cure illness and satisfy both thirst and hunger depending on the consumers' intention and prayers (Shomar, 2012). The Well of Zamzam water is located in the holy mosque (Haram) at about 20m east of Ka'abah in Makkah, which is known as western province of the Kingdom of Saudi Arabia (Shomar, 2012). The water is very popular among the Muslims, especially during the Haj seasons in which it is consumed extensively to replace the mineral loss in the body caused by high perspiration rate under the scorching desert conditions (Saad et al., 1998).The pilgrims also tend to take Zamzam water to their home countries for their relatives as a gift. Zamzam water has been reported to have higher ion concentration of Ca, Mg, K, Na, Cl, Br and F (Shomar, 2012; Alfadul and Khan,

2011) compared to normal mineral water. Earlier studies mainly focus on the ion concentration of Zamzam water without associating it with the effects of types of container used for its storage on ions concentration. Hence, this study was carried out to analyse the influence of different types of container on ions concentration of Zamzam water. In addition, the study also aimed to determine the toxicity effects of Zamzam water on U87 cell line.

Material and Methods

Sample Collection

Raw Zamzam water was obtained from Makkah, Saudi Arabia. It was stored in two different containers: plastic container (Group-A) and glass container (Group-B). For the controlled group in MTT assay, distilled water was obtained from Chemistry Laboratory of Universiti Sultan Zainal Abidin (UniSZA), Terengganu, Malaysia.

Major Cations and Anions

Ion analysis was carried out using ion chromatography (METROHM / 881 COMPACT IC PRO Model) and it was run for one hour to reach stable conditions. Standards of different concentrations were prepared to calibrate the IC using the calibration standards with certified METROHM for required parameters for lithium (Li), sodium (Na), ammonium (NH_4), potassium (K), calcium (Ca), magnesium (Mg), fluoride (F), chloride (Cl), nitrite (NO_2), nitrate (NO_3), bromide (Br), phosphate (PO_4^3) and sulphate (SO_4). After calibrating the water, samples were run and the concentrations of the samples were recorded as mg/l. The injection volume was 10 µL.

Cell Culture

U-87 cells were grown in Roswell Park Memorial Institute (RPMI) medium (Sigma, St. Louis, USA) containing 15% foetal bovine serum, 2 mM L-glutamine and 100 U/mL penicillin/streptomycin at 37^0C in 5% CO_2. The cells were used at passages between six and nine.

Cytotoxicity Assay (MTT)

The cytotoxicity of the water samples was determined by 3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) as proposed by Zegura et al., (2009). This assay measures the conversion of MTT to insoluble formazan by dehydrogenase enzymes of the intact mitochondria of living cells. U-87 cells were seeded at a density of $1x10^4$ cells/well into 96-well microtiter plates in five replicates. After 4 hour incubation at 37^0C to allow attachment of the cells, the growth medium was replaced with fresh medium containing 40 vol. % of water samples and the cells were incubated for 20 hours. After treatment, MTT was added at a final concentration of 0.5 mg mL1 and the cells were further incubated for 3 hours at 37^0C. The medium was removed and the formazan crystals were dissolved in DMSO. The amount of formazan crystals directly correlates to the number of viable cells. The optical density (OD) was measured at 570 nm (reference filter 690 nm) using an ELISA mircoplate reader (Tecan, Genios). Cell survival (viability) was determined by comparing the OD of the wells containing cells treated with water samples to cells exposed to 40 vol. % distilled water in growth medium. A 30% reduction of the viability by the sample is considered a cytotoxic response.

Results and Discussion

Table 1 shows the origin, types of container and pH value of the Zamzam water samples. The pH value can be defined as a measure of the concentration of hydrogen ions in the water and its value indicates the acidity or alkalinity of the water. The pH of pure water is 7 which is neutral. In general, water with a pH lower than 7 is considered acidic meanwhile a pH greater than 7 is basic. The pH of the water is vital because it affects the availability and solubility of nutrients, and how it can be utilized by aquatic organisms (Kelly et al., 2004). Geology of catchments area and buffering capacity of water are the two main factors that can influence the pH of water (Muhamad et al., 2011).

Table 1. Origin, types of container and pH value of the Zamzam water

Group	Sample	Origin	Container	pH
A	Zamzam water	Makkah	Plastic	7.91
B	Zamzam water	Makkah	Glass	8.31

According to International Bottled Water Association (IBWA), the limit of pH value for drinking water is specified as 6.5 to 8.5. The pH value of Zamzam water sample stored in glass container shows slightly alkaline trend (pH 8.31) compared to Zamzam water sample stored in plastic container (pH 7.91). Zamzam water stored in plastic container is less alkaline since it may be exposed to the sunlight thus causing the oxidation of organic compounds through the photodegradation which produces compounds, such as phthalate ester (Monarca et al., 1994) haloacetic (Begley & Hollifield, 1989) acids and acetaldehyde (Lo Russo et al., 1985).

The quality of bottled water varies and it may be affected by the source, treatment type, container type and length of storage (Alabdula'aly and Khan, 1995). Furthermore, sun light exposure and temperature of storing may cause changes in all physicochemical properties of water in the plastic bottle (Muhamad et al., 2011; Toma et al., 2013). In this study, there was a difference in the ion concentration stored in plastic and glass bottles (Tables 2 and 3) but it is not statistically significant. Most plastics are relatively permeable and let atmospheric air easily through, possibly setting off oxidation reactions, and liquids may easily evaporate through them causing concentration of constituents and possible oversaturation (Ármannsson and Ólafsson, 2006).

Table 2. Types of container and analysis of cation concentration of the Zamzam water

Group	Li (mg/L)	Na (mg/L)	NH_4 (mg/L)	K (mg/L)	Ca (mg/L)	Mg (mg/L)
A	0.059	4.43	0.12	41.16	65.85	12.93
B	0.059	1.73	0.11	41.16	65.73	12.67

Table 3. Types of container and analysis of anion concentration of the Zamzam water

Group	F (mg/L)	Cl (mg/L)	NO_2 (mg/L)	NO_3 (mg/L)	Br (mg/L)	PO_4^3 (mg/L)	SO_4^2 (mg/L)
A	0.41	76.08	0.23	36.23	0.77	0.62	88.28
B	0.42	69.11	0.24	33.60	0.64	ND	95.44

ND= Not Detected

The effect on the cells after exposure to Zamzam and mineral water was particularly non-toxic, after 20 hours of exposure (Table 4). Only 4.76% reduction of the viability was recorded for Zamzam water and 1.59% reduction for mineral water when compared to the distilled water. A 30% reduction of the viability by the sample is considered a cytotoxic response. Toxicity of the water is a common result of microbial activity and possible chemicals. This result supports the hypothesis proposed by Mashat (2010) that there is no sign of biological growth in the well of Zamzam water. Water from well usually will change in taste and odor due to the growth of algae, however Zamzam water does not change in taste, smell, color or even mouldy since its existence (Koshak, 1983).

Table 4. Cell viability of different types of water on U87 cell line

Sample	Optical density	% reduction
Distilled water (Control)	0.63	0
Zamzam water	0.60	4.76
Mineral water	0.62	1.59

Note: 30% reduction of the viability by the sample is considered a cytotoxic response

Conclusion

We conclude that types of container do not influence the ion concentration of Zamzam water and we also have demonstrated that Zamzam water is non-toxic to human primary glioblastoma cell line.

Acknowledgement

The authors would like to thank UniSZA/NRGS/2013/RR057-1 for funding this research.

References

Alabdula'aly, A.I., & Khan, M.A. (1995). Microbiological quality of bottled water in Saudi Arabia. *Journal of Environmental Science & Health Part A*, *30*(10), 2229-2241.

Alfadul, S.M. & Khan, M.A. (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.

Ahmad, A.A.A., & Ibrahim, S. (1996). The water of Zamzam water (349th ed.). London: Dar Al Taqwa.

Ármannsson, H., & Ólafsson, M. (2006). *Collection of geothermal fluids for chemical analysis*. Iceland: Geosurvey.

Begley, T.H. & Hollifield, H.C. (1989). Liquid chromatographic determination of residual reactants and reaction by-products in polyethylene terephthalate. *Journal - Association of Official Analytical Chemists*, 72(3), 468–470.

Kelly A.M.S., Linda Green M. S., Elizabeth Herron M. A. (2004). pH and Alkalinity, Retrived from http://web.uri.edu/watershedwatch/water-quality-factsheets/

Koshak, Y.H. (1983). *Zamzam*. Jeddah: Dar Alelm for Publication

Lo Russo, S., Gramiccioni, L., Di Marzio, S., Milana, M. R., Diprospero, P., & Papetta, A. (1985). Acetaldehyde migration from poly (ethyleneterephthalate)(pet) containers-gc-determination and toxicological assessment. *Annali di Chimica*, *75*(9-10), 403-414.

Mashat, B.H.H. (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 Siences*, *21*(2), 87–100.

Monarca, S., De Fusco, R., Biscardi, D., De Feo, V., Pasquini, R., Fatigoni, C., Moretti, M. & Zanardini, A. (1994). Studies of migration of potentially genotoxic compounds into water stored in pet bottles. *Food and Chemical Toxicology,* 32(9), 783–788.

Muhamad, S., Esmail, L. & Hasan, S. (2011). Effect of storage temperature and sunlight exposure on the physicochemical properties of bottled water in Kurdistan region-Iraq. *Journal of Applied Sciences and Environmental Management*, 15(1), 147-154.

Saad, B., Pok, F.W., Sujari, A.N.A. & Saleh, M.I. (1998). Analysis of anions and cations in drinking water samples by Capillary Ion Analysis. *Food Chemistry*, 61(1-2), 249–254.

Shomar, B. (2012). Zamzam water: concentration of trace elements and other characteristics. *Chemosphere*, 86(6), pp.600–605.

Toma, J. J., Hanna, N. S., & Mohammed, B.A. (2013). Effect of storage condition on some bottled water quality in Erbil city, Kurdistan region-Iraq. *Journal of International Association of Scientific Innovation and Research*, 1, 33-43.

Žegura, B., Heath, E., Černoša, A., & Filipič, M. (2009). Combination of in vitro bioassays for the determination of cytotoxic and genotoxic potential of wastewater, surface water and drinking water samples. *Chemosphere*, 75(11), 1453-1460.

How to cite this paper:
Halim, S., Mohamad, N., Toriman, M.E., Abu Bakar, N.H. & Latif, A.Z.A (2016). Zamzam water: influence of containers on ionic concentration and in-vitro cytotoxic effects on U87 cell line. *Malaysian Journal of Applied Sciences, 1*(1), 68-72.