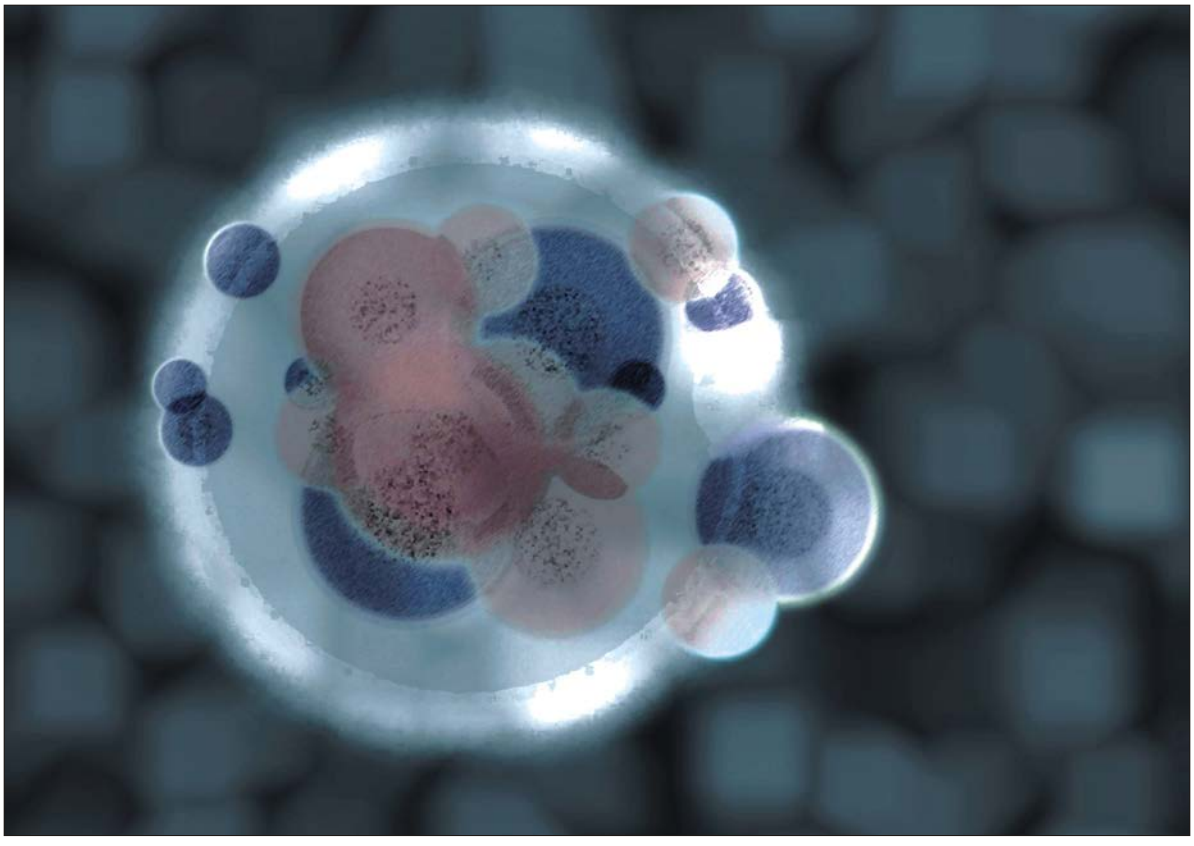

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Research Article
Influence of pH and Sterilization Conditions on the Inhibitory Effects of Zamzam Water on the Cell Viability of Cancer Cells

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Abstract

Background: The extracellular pH (pH_{ex}) of malignant tumors is acidic whereas, the pH_{ex} of tissues is more alkaline. Hence, slight changes in pH_{ex} may have an effect on the phenotype of cells. **Objective:** The aim of the current study is to illustrate the influence of pH and sterilization conditions of Zamzam Water (ZW) on the cell viability of human breast adenocarcinoma cell line (MCF-7) and human colon cancer cell line (HCT-116). **Materials and Methods:** The cell viability of cancer cells was determined using MTT assay after the treatment with ZW under different preparation conditions. **Results:** The results of this study revealed that ZW has a selective inhibitory effect on cancer cells. Only the natural non-adjusted pH (alkaline) ZW treatment reduces the growth of both MCF-7 ($p \leq 0.00001$) and HCT-116 cells ($p \leq 0.0001$). **Conclusion:** The selective cytotoxic effects of different ZW treatments on cancer cells are influenced by the pH value and sterilization conditions. Therefore, it is suggested that the high alkaline pH value and the presence of certain elements in ZW play a role in inhibiting the growth of cancer cells.

Key words: Zamzam water, MCF-7 cell line, HCT-116 cell line, MTT assay, alkaline pH

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The extracellular pH (pH_{ex}) of malignant tumors is acidic (pH_{ex} 6.5-6.9) due to the increased metabolism of glucose and poor perfusion, whereas, the pH_{ex} of normal tissues is more alkaline (pH_{ex} 7.2-7.5)[1-3]. Although, tumor acidity is thought to play a critical role in metastasis and cell invasion, most of the *in vitro* experiments of cancer cells are still carried out at the relatively alkaline pH_{ex} ranges (7.2-7.4)[4,5]. Some researchers studied the effect of alkaline pH_{ex} on cancer cells and they found that slight changes in pH_{ex} may have an effect on the cell phenotype. *In vivo* study on breast cancer showed that oral sodium bicarbonate increases extracellular pH of tumors and reduces the formation of spontaneous metastases[6]. Moreover, it has been found that extracellular alkaline pH inhibited the growth of the cancer cells[5]. Another *in vitro* and *in vivo* researches studied the effect of alkaline water such as Alkaline Reduced Water (ARW) and Electrolyzed Reduced Water (ERW) on different cells. These alkaline waters were prepared with either high pH or in combination of minerals and high pH[7,8]. In addition, *in vitro* research showed that ERW reduced the cell viability of human (HL-60) leukaemia cells[9].

Zamzam Water (ZW) is an alkaline water that is found naturally in Makkah and never been chemically treated or chlorinated. The miraculous feature of this holy water is its continuous flow from the well since 4000 years and never dried[10]. This unique water has been found to be naturally carbonated and mineralized[11]. The ZW is consumed by millions of Muslims around the world due to the religious belief in its healing power for a wide range of illnesses[12,13]. It was reported that the intake of ZW diminished the size of colon tumor in rats[14]. Therefore, this study was designed to illustrate the influence of pH and sterilization conditions of ZW on the cell viability of human breast adenocarcinoma (MCF-7) and human colon cancer (HCT-116) cell lines.

MATERIALS AND METHODS

Cell lines: The three human cell lines used in this study were breast adenocarcinoma (MCF-7), colon cancer (HCT-116) and skin fibroblasts cells (HSF). The cell lines were obtained from Alzamil chair for cancer research in King Fahad Medical Research Centre (KFMRC) at King Abdulaziz University (KAU), Jeddah, Kingdom of Saudi Arabia (KSA).

ZW source: The ZW samples were provided by the General Presidency of the Affairs of the Two Holy Mosques. Water samples were obtained directly from the well which is located in the holly mosque, Makkah, Kingdom of Saudi Arabia (KSA). The GPS coordinates of the well are 21°25'19.2"N, 39°49'33.6"E. The pH of ZW was naturally alkaline (pH 8) and the major chemical composition of ZW was previously described[11]. One large patch of ZW was stored in sterilized glass bottles at room temperature until it was used.

Preparation of culture medium: One liter of high glucose growth medium DMEM (Hyclone, USA) was prepared by dissolving the content of one vial of DMEM in deionized water and supplemented with 10% Fetal Bovine Serum (FBS) (Hyclone, USA), 1% L-glutamine (Sigma-Aldrich chemical Co, UK), 1% penicillin/streptomycin (Gibco, UK), 1% non-essential amino acids (Gibco, Canada), 1% HEPES buffer (Gibco, Canada) and sodium bicarbonate ($NaHCO_3$) (Sigma Co. Limited, USA). The pH of medium was adjusted to 7.2. The medium was filtered by using 0.22 μm filter and used to culture the cells. After reaching the desired confluence at 70%, the growth medium was removed and treatments of ZW were tested for 24 h. This medium was used as a control medium.

Optimization strategy for ZW medium preparation

Filtered Zamzam medium: One liter of filtered Zamzam medium was prepared by dissolving the content of one vial of high glucose DMEM in ZW and supplemented with 10% Fetal Bovine Serum (FBS), 1% L-glutamine, 1% penicillin/streptomycin, 1% non-essential amino acids, 1% HEPES buffer and $NaHCO_3$. The medium was filtered with 0.22 μm filter in sterilized bottles. The medium was adjusted to 7.2 (F1) or left with natural alkalinity 8 (F2).

Auto-cleaved Zamzam medium: In sterilized glass bottle, 1 L of auto-cleaved Zamzam medium was prepared by dissolving the content of one vial of high glucose DMEM in auto-cleaved ZW and supplemented with 10% Fetal Bovine Serum (FBS), 1% L-glutamine, 1% penicillin/streptomycin, 1% non-essential amino acids, 1% HEPES buffer and $NaHCO_3$. The pH was either adjusted to 7.2 (A1) or left with natural alkalinity 8 (A2).

Natural Zamzam medium: In sterilized glass bottle, one liter of natural Zamzam medium was prepared by dissolving the content of one vial of high glucose DMEM in ZW and supplemented with 10% Fetal Bovine Serum (FBS), 1% L-glutamine, 1% penicillin/streptomycin, 1% non-essential amino acids, 1% HEPES buffer and $NaHCO_3$. The pH was adjusted to 7.2 (N1) or left with natural alkalinity 8 (N2).

Determination of the cell viability: The cytotoxic effect of ZW on MCF-7 and HCT-116 cells was determined with MTT assay. MCF-7 and HCT-116 cells were seeded into 96-well tissue culture plates at cell density of 1×10^4 cells well^{-1}. Cells in each well were allowed to attach for 24 h at 37°C and 5% CO_2. After 24 h, the culture medium was replaced with 200 µL of Zamzam treatments (F1, F2, A1, A2, N1 and N2) or with 200 µL of culture medium as control. In the following day, MTT assay was carried out by adding 50 µL of 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium Bromide (MTT) reagent (Sigma-Aldrich chemical Co, UK) to each well[15]. Cells were incubated with MTT for 2 h at 37°C in 5% CO_2. The produced formazan crystals were dissolved by adding 200 µL of isopropyl (BDH, England). The intensity of the colour was measured at 490 nm using a BioTek microplate reader. Three independent experiments were performed to ensure reproducibility.

Statistical analysis: The data were analyzed using GraphPad Prism software version 6. The significant differences were evaluated using p-values of one-way analysis of variance (ANOVA) followed by Bonferroni's test. The cut-off level for significance was $p\leq0.05$.

RESULTS

Effect of different ZW preparations on the viability of cancer cells

Cell viability of MCF-7 cells: The cell viability of MCF-7 for each preparation of ZW medium was measured using MTT assay. The cell viability of MCF-7 was significantly decreased in the three ZW preparations compared to control. For filtered ZW preparations, the percentage of MCF-7 viability was reduced to 88.65±2.546% and 79.45±2.357% after the treatment with ZW F1 (filtered and adjusted pH) and F2 (filtered and non-adjusted pH), respectively compared to the control (Fig. 1a). When the same cells exposed to ZW A1 (auto-cleaved adjusted pH) and A2 (auto-cleaved non-adjusted pH), the viability of the cells as shown in Fig. 1b was also significantly decreased to 78.79±2.243% and

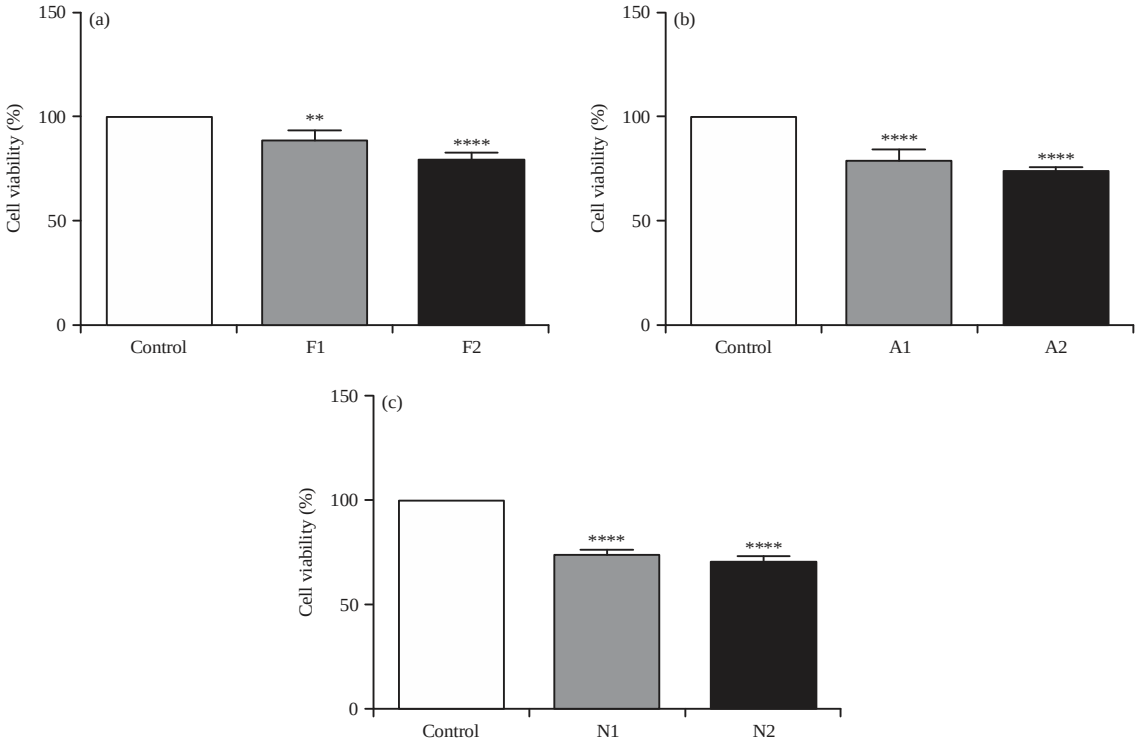

Fig. 1(a-c): Effect of different ZW medium preparations on the cell viability of MCF-7 cells measured by MTT assay, (a) Cell viability of MCF-7 cells treated with filtered ZW medium (F1 and F2) compared to the control, (b) Cell viability of MCF-7 cells treated with auto-cleaved ZW medium (A1 and A2) compared to the control and (c) Cell viability of MCF-7 cells treated with natural ZW medium (N1 and N2) compared to the control. The data represents the mean of three independent experiments (n = 3±SEM). Comparison of means was made using one-way ANOVA followed by Bonferroni's test (**$p\leq0.01$ and ****$p\leq0.0001$)

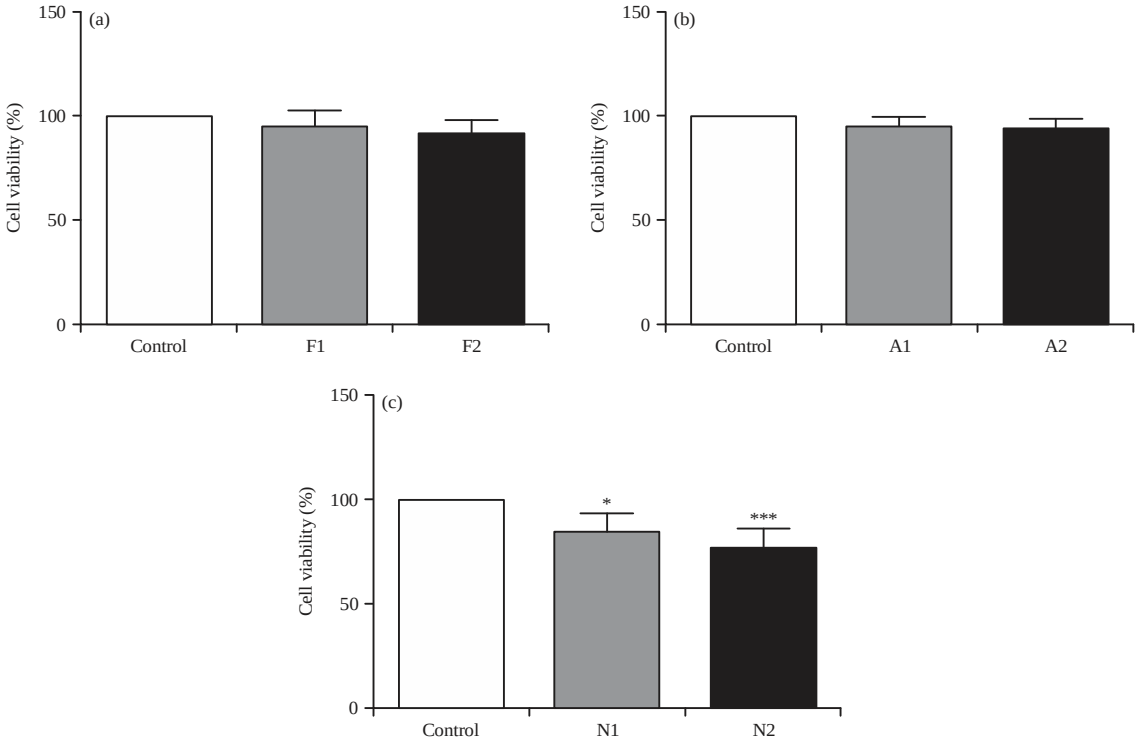

Fig. 2(a-c): Effect of different ZW medium preparations on the cell viability of HCT-116 cells measured by MTT assay, (a) Cell viability of HCT-116 cells treated with filtered ZW medium (F1 and F2) compared to the control, (b) Cell viability of HCT-116 cells treated with auto-cleaved ZW medium (A1 and A2) compared to the control and (c) Cell viability of HCT-116 cells treated with natural ZW medium (N1 and N2) compared to the control. The data represents the mean of three independent experiments (n = 3±SEM). Comparison of means was made using one-way ANOVA followed by Bonferroni's test (*$p \leq 0.05$ and ***$p \leq 0.001$)

74.80±2.115%, respectively compared to the control. Moreover, Fig. 1c showed that ZW N1 (natural adjusted pH) and N2 (natural non-adjusted pH) had significant decrease in the viability of MCF-7 cells to 74.15±1.485% and 70.61±1.485%, respectively compared to the control.

Cell viability of HCT-116 cells: The cell viability of HCT-116 for each preparation of ZW medium was measured using MTT assay. Interestingly, the viability of HCT-116 cells did not change significantly when they were exposed to ZW F1, F2, A1 and A2 treatments (Fig. 2a, b). The cell viability of colon cancer cells was 95.24±3.656%, 91.56±3.656, 95.68±2.356 and 94.46±2.356% after the treatment with F1, F2, A1 and A2, respectively compared to the control. However, when the same cells were exposed to ZW treatments, N1 and N2, the viability of the cells was significantly decreased to 84.96±4.522% and 77.23±4.330%, respectively compared to the control (Fig. 2c).

Effect of the non-adjusted pH ZW preparations on the cell viability of cancer cells: This comparison was made to determine the effect of the alkalinity of ZW in non-adjusted pH preparations on the reduction of the cancer cells growth. Figure 3a demonstrated a significant reduction effect of all F2, A2 and N2 treatments on the viability of MCF-7 cancer cells when compared to the control ($p \leq 0.00001$) while as shown in Fig. 3b, only the N2 treatment had a significant decrease on the cell viability of HCT-116 cells when compared to the control ($p \leq 0.0001$).

DISCUSSION

The ZW is naturally hard carbonated water and its pH ranges from 7.9-8.2, indicating that it is alkaline[16]. In this study, the influence of pH and different sterilization methods of ZW on the cell viability of MCF-7 and HCT-116 cell lines were investigated. The aim of this study is to make an optimization

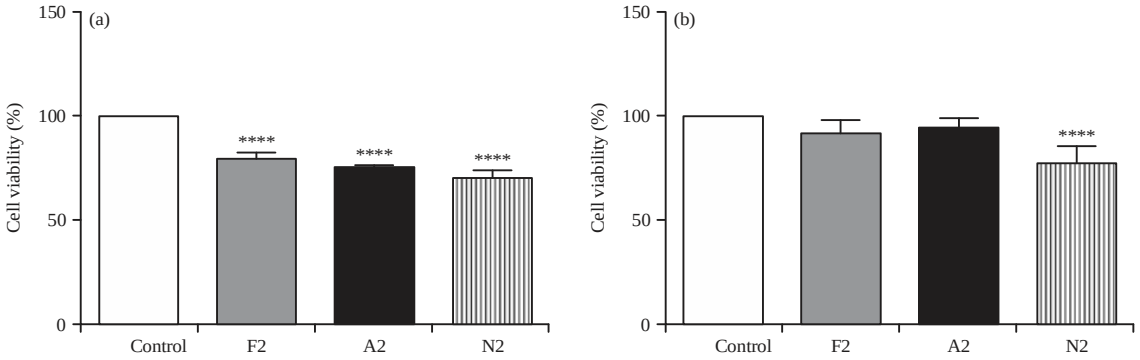

Fig. 3(a-b): Effect of non-adjusted pH medium filtered, auto-cleaved and natural (F2, A2 and N2) of ZW on the cell viability of MCF-7 and HCT-116 cells measured by MTT assay, (a) Cell viability of MCF-7 cells treated with non-adjusted pH medium ZW (F2, A2 and N2) compared to the control and (b) Cell viability of HCT-116 cells treated with non-adjusted pH medium ZW (F2, A2 and N2) compared to the control. The data represents the mean of three independent experiments (n = 3±SEM). Comparison of means was made using a one-way ANOVA followed by Bonferroni's test (***$p \leq 0.001$ and ****$p \leq 0.0001$)

strategy for ZW medium preparation to illustrate whether the natural alkalinity of ZW and/or sterilization methods play a role in the reduction of cancer cells growth or not.

The results of the current study provide evidence that ZW had an inhibitory effect on the cell proliferation and growth of MCF-7 and HCT-116 cancer cells. This effect may be due to the presence of low concentrations of some inorganic minerals such as arsenic, selenium and lithium[11,17,18] and/or the high concentrations of bicarbonate, calcium and potassium[11]. The alkalinity of this water is due to the presence of high concentrations of bicarbonate.

For MCF-7 cancer cells, the results showed a marked decreased in the cell viability for all ZW medium preparations. In addition, these effects have the same high significant level of the cell viability ($p \leq 0.00001$) for all ZW medium preparations (the adjusted pH and non-adjusted pH) but only the filtered adjusted pH medium (F1) has a less significant level than the other preparations ($p \leq 0.001$). The findings of the current *in vitro* study are in agreement with another *in vivo* study[19], which found that ZW significantly reduced the tumor mass in mice bearing tumor ($p<0.05$). Moreover, several researches studied the effect of the high pH (alkaline) and minerals on cancer cells. It has been reported that ERW inhibited the cell proliferation in lung A549 cancer cells[20]. In a study performed on breast cancer, the oral administration of sodium bicarbonate was found to increase the extracellular pH of tumors and reduces the formation of spontaneous metastases[6]. Another study reported that mineral-enriched Deep Sea Water (DSW) has inhibitory effects on breast cancer (MDA-MB-231 cells) invasion/metastasis[21].

Furthermore, the results showed that the effect of ZW medium preparations on colon cancer cell line (HCT-116) was selective. The results demonstrated that only ZW natural medium (N1 and N2) had a significant inhibition effect on the growth of HCT-116 cells with no significant effect of F1, F2, A1 and A2 treatments on the growth of the same cells. Moreover, the significant level of both natural mediums differs from ($p \leq 0.01$) for (N1) to ($p \leq 0.0001$) for (N2) in HCT-116 cells. The inhibitory effect of ZW on colon cancer cells in this study is in agreement with *in vivo* research, which found that ZW significantly decreased the size of colon tumor[14].

The Comparison of non-adjusted pH ZW medium preparations (F2, A2 and N2) on the cell viability of MCF-7 and HCT-116 cancer cells indicated that MCF-7 cells are more sensitive when treated with ZW than HCT-116 cells, which are more aggressive. In particular, MCF-7 growth is significantly inhibited after the treatment with all ZW non-adjusted pH (F2, A2 and N2) ($p \leq 0.00001$), whereas, the growth of HCT-116 cells was inhibited only with N2 treatment. This explains the importance of the alkalinity on the inhibition of cancer proliferation[5]. It is assumed that the natural features of ZW might be lost during the filtration and auto-cleaving methods due to the exclusion or adsorption of ZW minerals through the filter content or due to the precipitation of ZW minerals through the auto-cleaving process. Because of these two reasons and due to the lack of studies on ZW, it was necessary to find an optimum preparation method for ZW medium.

Finally, the findings of this study indicated that both natural ZW mediums (N1 and N2) are the optimum treatment conditions after the comparison analysis between cancer cells

and the non-cancerous cells (HSF). The high level of nitrate as well as the presence of silver and copper in ZW might act as an antimicrobial agents[22]. High nitrate levels coupled with microbiological safety might be a favorable attribute of ZW and could be responsible for some of its benefits[23]. Numerous researchers have reported that silver is effective in killing bacteria[24]. Copper is also recognized as one of the best fungicides and pesticides[25]. However, it is not clear which component of ZW is affected by filtration and autoclave. Moreover, the results of this study indicated that ZW has no effect on normal HSF cells. The cell viability percentage of HSF cells was 91.3 and 91.2% for N1 and N2, respectively. These results are in agreement with a previous study that showed that ERW had no effect on the cell viability of normal peripheral blood mononuclear cells ($PBMC_S$)[9].

CONCLUSION

The ZW reduced the cell viability of MCF-7 and HCT-116 cancer cell lines under certain treatments and preparation conditions. It seems that both the natural alkaline pH of ZW and the amount of certain elements play a role in the inhibition of the cancer cell growth. Further researches are necessary in order to elucidate the exact mechanism of action of ZW against cancer cells.

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REFERENCES

1. Griffiths, J.R., 1991. Are cancer cells acidic? Br. J. Cancer, 64: 425-427.
2. Vaupel, P., F. Kallinowski and P. Okunieff, 1989. Blood flow, oxygen and nutrient supply and metabolic microenvironment of human tumors: A review. Cancer Res., 49: 6449-6465.
3. Wike-Hooley, J.L., J. Haveman and H.S. Reinhold, 1984. The relevance of tumour pH to the treatment of malignant disease. Radiother. Oncol., 2: 343-366.
4. Glunde, K., S.E. Guggino, M. Solaiyappan, A.P. Pathak, Y. Ichikawa and Z.M. Bhujwalla, 2003. Extracellular acidification alters lysosomal trafficking in human breast cancer cells. Neoplasia, 5: 533-545.
5. Khan, M.A., A. Misra, A. Trivedi and A.N. Srivastava, 2014. Effect of alkalinity on cancerous cells at different pH and morphological variations *in-vitro*. Int. J. Bioassays, 3: 3297-3302.
6. Robey, I.F., B.K. Baggett, N.D. Kirkpatrick, D.J. Roe and J. Dosescu *et al*., 2009. Bicarbonate increases tumor pH and inhibits spontaneous metastases. Cancer Res., 69: 2260-2268.
7. Lee, K.J., S.K. Park, J.W. Kim, G.Y. Kim and Y.S. Ryang *et al*., 2004. Anticancer effect of alkaline reduced water. J. Int. Soc. Life Inform. Sci., 22: 302-305.
8. Tsai, C.F., Y.W. Hsu, W.K. Chen, W.H. Chang, C.C. Yen, Y.C. Ho and F.J. Lu, 2009. Hepatoprotective effect of electrolyzed reduced water against carbon tetrachloride-induced liver damage in mice. Food Chem. Toxicol., 47: 2031-2036.
9. Tsai, C.F., Y.W. Hsu, W.K. Chen, Y.C. Ho and F.J. Lu, 2009. Enhanced induction of mitochondrial damage and apoptosis in human leukemia HL-60 cells due to electrolyzed-reduced water and glutathione. Biosci. Biotechnol. Biochem., 73: 280-287.
10. Khalid, N., A. Ahmad, S. Khalid, A. Ahmed and M. Irfan, 2014. Mineral composition and health functionality of Zamzam water: A review. Int J. Food Prop., 17: 661-677.
11. Shomar, B., 2012. Zamzam water: Concentration of trace elements and other characteristics. Chemosphere, 86: 600-605.
12. Elnour, A., A.O. Bamosa, A. Al Meheithif and K.A. Aleissa, 2013. Amelioration of severe carbon tetrachloride toxicity by Zamzam water in rats. J. Nutr. Food Sci., Vol. 3, No. 197. 10.4172/2155-9600.1000197
13. Munir, N., M. Shoaib, M.J. Asad, A.Y. Tanooli, N. Munir and S.K. Sherwani, 2015. Physio-chemical differences between Aab-E-ZamZam and mineral water effect of water intake on blood pressure. Am-Eur. J. Toxicol. Sci., 7: 83-87.
14. Ali, A.F.M., E. Cosemi, S. Kamel, S. Mohammed, M. Elhefnawy, L. Farid and S. Shaker, 2009. Oncolytic action of Zamzam water on azoxyonethone (AOM) induced colon tumors in rats. 19th Int. Water Technol. Conf., 13: 1521-1526.
15. Korashy, H.M., Z.H. Maayah, A.R. Abd-Allah, A.O. El-Kadi and A.A. Alhaider, 2012. Camel milk triggers apoptotic signaling pathways in human hepatoma HepG2 and breast cancer MCF7 cell lines through transcriptional mechanism. BioMed Res. Int., Vol. 2012. 10.1155/2012/593195
16. Ali, A.F.M., E. Cosemi, S. Kamel, S. Mohammed, M. Elhefnawy, L. Farid and S. Shaker, 2009. Zamzam water gene downregulation in uterine Fibrochondrosarcoma cell line. 19th Int. Water Technol. Conf., 13: 1543-1547.
17. Krsnjavi, H. and D. Beker, 1990. Selenium in serum as a possible parameter for assessment of breast disease. Breast Cancer Res. Treat., 16: 57-61.
18. Forkner, C.E. and T.F.M. Scott, 1931. Arsenic as a therapeutic agent in chronic myelogenous leukemia: Preliminary report. J. Am. Med. Assoc., 97: 3-5.
19. Abdullah, A.A.I. and T.S. Al-Azawi, 2016. Comparative *in vivo* study between Zamzam water and magnetic water in carcinoid and normal cells. Int. J. Adv. Biol. Res., 6: 333-337.
20. Ye, J., Y. Li, T. Hamasaki, N. Nakamichi and T. Komatsu *et al*., 2008. Inhibitory effect of electrolyzed reduced water on tumor angiogenesis. Biol. Pharma. Bull., 31: 19-26.

21. Kim, S., S.Y. Chun, D.H. Lee, K.S. Lee and K.S. Nam, 2013. Mineral-enriched deep-sea water inhibits the metastatic potential of human breast cancer cell lines. Int. J. Oncol., 43: 1691-1700.
22. Gorchev, H.G. and G. Ozolins, 1984. WHO guidelines for drinking-water quality. WHO Chron., 38: 104-108.
23. Al Meheithif, A., A. Elnour, A. Bamosa and K. Aleissa, 2012. Antioxidant effects of Zamzam water in normal rats and those under induced-oxidant stress. J. Med. Plants Res., 6: 5507-5512.
24. Morones, J.R., J.L. Elechiguerra, A. Camacho, K. Holt, J.B. Kouri, J.T. Ramirez and M.J. Yacaman, 2005. The bactericidal effect of silver nanoparticles. Nanotechnology, 16: 2346-2353.
25. Cioffi, N., L. Torsi, N. Ditaranto, G. Tantillo and L. Ghibelli *et al*., 2005. Copper nanoparticle/polymer composites with antifungal and bacteriostatic properties. Chem. Mater., 17: 5255-5262.