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COMPARATIVE *INVIVO* STUDY BETWEEN ZAMZAM WATER AND MAGNETIC WATER IN CARCINOID AND NORMAL CELLS

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ABSTRACT
A study (*in vivo*) was conducted evalute the efficacy of zamzam water and magnetic water on carcinod and normal cells. A total of 40 mice were used included 20 mice bearing tumor and 20 normal mice with 5 replicates each. The carcinoid and normal groups where divided as follows: the first group was recived with tap water, the second group recived zmzam water, the third group was recived magnetic water, and the last group treated mix of zamzam and magnetic water (Z + M) . Tissue sample were collected after the end of the experiment period (12 days) from all groups to measure the Glutathione (GSH) and Malondialdehyde (MDA). Tumor volume and the percentage inhibition of tumor growth were maesured every three days during all the length of the experiment period. The Histopathological changes for tumor mass and liver were studied. The results showed that the best inhibition of cancer cell growth in the three types of water was zamzam and magnetic water. It showed a significant ($P < 0.05$) different of atrophy in tumor volume compared with the tumors in control group mice while the group received the mix water showed incomplete atrophy in tumor volume . Moreover, the histopatholigical changes in zamzam and magnetic groups showed necrosis of cancer cells, which are surrounded by fibrous and disappearance of normal shape of tissues with proliferation of connective tissue which replaced the cancer cells. Clusters of mono nuclear cells have been found (lymphocyte and macrophage) in liver tissue and central vein, while in mix groups, less necrosis areas have shown with areas of tumor cell. The maesurement of GSH and MDA in carcinoid and normal mice for liver and tumor mass showed a significant increase ($p < 0.05$) in GSH with a zamzam and magnetic treatment groups. In conclousion: the results of the present study confirmed that the best and safest therapies in the treatment of mammary gland could be achieved by using zamzam water, beside that the magnetic water seem to have asimilar but lesser effect on cancer cells .

KEYWORDS: zamzam water, magnetic water, GSH, MDA, carcinoid cells, tumor mass.

INTRODUCTION

Water is the main element for all animal cells, tissues and organization of all biological operations from digestion, absorbtion and nutritional transportation; to remove the toxins and the excrements (Sahin *et al*., 2016). The researchers confirmed that the treatment of water by magnetic zone causing changes in its specifications by making it more absorbing from the cells, after being noticed that water molecules are disassembly much more quick , due to that water consists from high number of clusters molecules (Davis, 2004) . This molecule disassembly is accompanied by the hydrogen: oxygen connectivel. Disassembly causing changing in some water properties like pH, Surface tention, viscosity, electrical conduction (Alkhazan and Saddiq, 2010). Using magnetic water in animal drinking which characterized by alkalinity in nature will give a positive results , like increase in growth rate of the animal, milk production , wool production and play an important role to treatment of many diseases (Barnstable, 2014), as well as cancer cells because these cells do not live in alkaline environment (Mohammed, 2009). Magnetic water tastes sweeter and has more clarity (Kobe *et al*., 2001). It has a positive effect on plant growth and on the microbial content. Magnetized water reduces the surface tension of water thus making it softer (Amiri and Ali, 2006). The increasing production of electric and magnetic fields (EF & MFs) due to the expanding use of electronic devices in normal life is encouraging studies on the effects of these fields on living organisms with a view to better protecting human health against their probable unfavorable effects (Rozumova *et al*., 2016). Physics showed that water changes under influence of magnetic fields as more hydroxyl (OH-) ions are created to form alkaline molecules , and reduce acidity . Increasing both the electric conductivity and dielectric constant of water was also documented (Ibrahim, 2006). Despite that several studies were carried out during the last years to evaluate the cytotoxic activity of several materials and extracts in vitro as well as in vivo but no study was conducted to investigate the effect of zamzam water on cancer cells. Therefore , it is the first time in Iraq , to study the effect of zamzam water and magnetic water on the cancer cells.

MATERIALS AND METHODS

Preparation of water

Preparation of zamzam water

Zamzam water was obtained from inside the Holy Mosque in Mecca Al-Mukarramah, Saudi Arabia. It has been analyzed chemically and physically in the Ministry of Science and Technology/Iraq.

Preparation of magnetic water

Magnetic water was prepared by using tap water then this water was passed to water magnetic device (magnetrone)

under high intensity (8000 Gauss). The intensity of magnetic water was reduced after about 15-18 hrs so in *invivo* tests, the magnetic water must be prepared twice daily, morning and evening, but in cytotoxicity tests, it has been equipped and used immediately.

- Preparation of mix water: - equal amounts of (Zamzam water + Magnetic water).
- Measurement of tumor volume according to Grote *et al.* (2001)
- Measurement of percentage of tumor growth inhibition (TGI) or specific growth rate (SGR) according to (Mehrara, 2009).
- Histopathological test
- Measurement of tissue Glutathione (Ellman *et al*., 1959).
- Measurement of tissue Malondialdehyde (Gilbert *et al*., 1984).

Statistical analysis

SGR was estimated using SAS (Statistical Analysis System-version 9.1). "Two-way ANOVA and Least significant differences (LSD) post hoc test was performed (multiple comparisons), to assess significant difference among means. P< 0.05 was considered statistically significant.

RESULTS

The *invivo* study was designed by using 40 laboratory mice, 20 mice bearing tumor and 20 normal mice, they are randomly divided into 8 equal groups each made up of 5 mice. Each of the carcinod and normal groups where divided as follows: the first group was recived with tap water, while second group recived zmzam water , the third group was recived magnetic water, and the last group treated mix water (Z + M) .

Tissue sample were collected after the end of the experiment period (12 days). from all groups to measure GSH, MDA. Tumor volume and the percentage inhibition of tumor growth were maesured every three days during all the length of the experiment period.

Tumor growth inhibition (TGI) or specific growth rate (SGR).

The tumor volume after three days represents the initial volume for all groups. Results showed that the differences in tumor volume were not significant for all groups. The initial tumor volume considered as the zero time when we estimated the GSR.

TABLE 1: Comparison of initial tumor volume among groups of mice transplanted with mammary adenocarcinoma

Group	Initial tumor volume (mm^3)
Control	0.04±0.006
Zamzam	0.05±0.009
Magnetic	0.05±0.01
Mix	0.05±0.008

n = 5 mice for each group

Results showed that the SGR significantly (P< 0.05) increased after 6, 9 and 12 days in control group (0.58, 0.90, and 1.21 respectively). Whereas, the SGR significantly (P< 0.05) decreased in zamzam and magnetic groups. On the other hand, the trend in mix group was stable throughout the various periods. Comparison among groups within each period revealed that zamzam and magnetic groups were differed significantly (P<0.05) from control for the three periods. While mix group was differed from control only in the two periods (after 9 and 12 days)

TABLE 2: Mean ± SE of Specific Growth Rate (SGR) for all groups of mice transplanted with mammary adenocarcinoma.

	Specific Growth Rate (SGR)		
Group	After 6 days	After 9 days	After 12 days
Control	B 0.58±0.12^{a}	AB 0.90±0.16^{a}	A 1.21±0.17^{a}
Zamzam	A -0.57±0.07^{b}	B -1.52±0.04^{c}	B -1.55±0.05^{c}
Magnetic	A -0.33±0.04^{b}	B -1.47±0.09^{c}	B -1.54±0.08^{c}
Mix	A 0.51±0.10^{a}	A 0.39±0.21^{b}	A 0.42±0.19^{b}

LSD= 0.3649

Different small letters in the same column indicate significant differences (P<0.05)

Different capital letters in the same row indicate significant differences (P<0.05)

(n = 5 mice for each group)

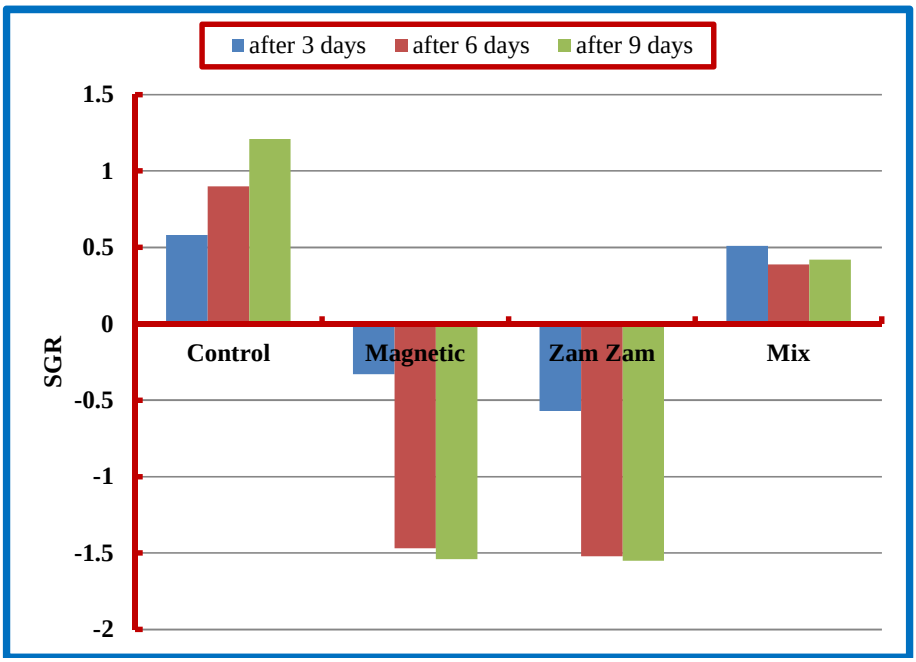

FIGURE 1: Trend of changes in SGR values across three periods for all groups.

SGR was used to identify the best equation that describes the response curve of tumor volume. The equations could be used to predict the volume of tumor at a certain point of time, the best equation for control group is linear with R^2=0.99. This mean that a positive linear increasing during advanced age.

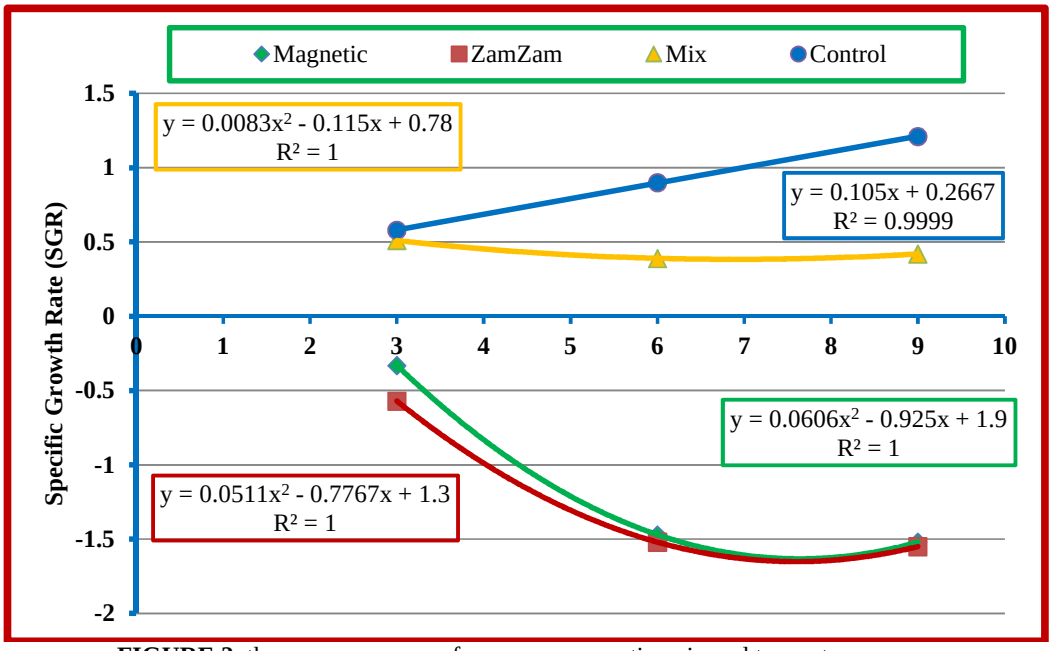

FIGURE 2: the response curve of zamzam, magnetic, mix and tap water groups.

Malondialdehyde (MDA) and Glutathione (GSH)

Malondialdehyde and glutathione were measured in liver and tumor mass of normal mice and treated groups, the results showed significant decrease ($P \leq 0.05$) in concentration of malondialdehyde in the previous tissue. While significant decrease in concentration of glutathione were observed in tap water inoculated tumor mice (P), Whereas, in treated groups, the inoculated tumor mice caused significant normalization of tissue glutathione ($P \leq 0.05$). Liver and tumor mass of treated mice (Z, M, X) showed a significant decline in malondialdehyde concentration as compared to the control group.

TABLE 3:- Concentration of Malondialdehyde (n / mole / gm.wet tissue)

Tissue / group	Liver	Tumor
Control (-ve)	0.69±0.03[c]	-
P	2.64±0.02[a]	2.15±0.28[a]
Z	1.57±0.03[b]	1.08±0.09[b]
M	1.56±0.04[b]	1.08±0.12[b]
X	1.77±0.03[b]	1.28±0.12[b]
LSD	0.0903	0.5146

The values are expressed as mean ± SE, n = 5 mice in each group.
Small letters denote deferent between groups ($p < 0.05$)
Control (- ve) / normal mice received tap water.
P / mice bearing mammary adenocarcinoma received tap water.
Z, M, X / mice bearing mammary adenocarcinoma recived zamzam, magnetice and mix water .

TABLE 4- Concentration of Glutathione (M mole / gm . wet tissue)

Tissue / group	Liver	Tumor
Control(-ve)	15.10±0.10[a]	-
P	12.66±0.14[b]	0.76±0.04[b]
Z	16.48±0.16[a]	2.40±0.19[a]
M	16.18±0.31[a]	2.40±0.14[a]
X	16.02±0.18[a]	2.21±0.13[a]
LSD	1.3851	0.413

The values are expressed as mean ± Se. small letters denote different between groups.
Capital letters denote different between cells.
Control (- ve) / normal mice received tap water.
P / mice bearing mammary adenocarcinoma received tap water.
Z , M , X / mice bearing mammary adenocarcinoma received zamzam , magnetice , mix water

Histopathological Study

The changes of liver and tumor mass were studied in mice treated with zamzam, magnetic and mix water and control group which treated with tap water. The section of each organ gave the following microscopic changes:-

Liver

Histopathological study of liver in cancer inoculated mice showed degenerative changes in liver cells, with rupture cell and cytoplasm, compared with negative control group. This lesion was occurred as a result of oxidative damage by cancer, while treated mice by zamzam, magnetic and mix water showed increase in kupffer cells with presence of focal aggregation of mono nuclear cells (MNCs), lymphocyte and macrophage cells , around central veins and parenchymal liver. This is evident of the presence of cell–mediated immunity in treated groups, proliferation by megakaryocyte, enlargement of kupffer cells dueto increases phagocytic activity.

Tumor mass

Histopathological section of skin mammary adenocarcinoma for mice treated with tap water showed progressive stage of cancer manifested by presence of cancer cells with hyprochromatin and polymorphic in different size, with proliferation of nucleus and cancer cell aggregation at glandular structure. However, histopathological section of tumor mass for mice treated with zamzam, magnetic and mix water, showed presence of inflammatory cells like macrophage and lymphocyte with proliferation of fibrous connective tissue, and necrotized tissues that replaced cancer cells.

DISCUSSION

Reduced tumor Volume

Ali *et al.* (2009) documented the oncolytic action of zamzam water in their research due to the down regulation of oncogenic gene, and induction of apoptosis. This is evident in upregulation of genes which control apoptosis (Charles, 1996). Many reports clearly indicated down regulation of cyclin D1 gene (Arber *et al*., 1997, Tetsu, 1999; AlMoustafa, 2004), which cause regression of tumor. However, it was evident that zamzam water acts by stimulating downregulation of gene which facilitates tumor growth, and upregulation of gene which benefit for the tissues (El – Zaiat, 2005, Ali *et al.*, 2009). The increase of nitrate level in zamzam and magnetic water play an important role due to the antibacterial action (WHO, 2011). Thus, high nitrate levels coupled with microbiological safety might be a favorable attribute of zamzam water and could be responsible for some of its benefits (Al- Meheithif, 2012).

MDA & GSH

The increase in the tissue of GSH and the decrease in the tissue of MDA concentration could be due to the anti-oxidant effect of zamzam and magnetic water (Burtona and Jauniauxb, 2011, Al–Meheithif, 2012). The formation of MDA as bioproduct of lipid peroxidation (LPO) has been a simple and useful diagnostic tool for the measurement of LPO (Bamosa, 2013).

Histopathological study

The results of the current study showed disappearance of tumor mass which could be attributed to the anti-oxidant

effects of zamzam water and magnetic water (Al-Meheithif, 2012, Bamosa, 2013) and the oncolytic action of zamzam water (Ali *et al*., 2009) that showed a high reduction of tumor mass .

REFERENCES

Ali, F.M., Ermilando, C., Sayed, K. Sana, M., Maged, E., Laila, F., Samer, S. (2009) Oncolytic action of zamzam water on azoxyonethone (AOM) induced colon tumors in rats. Thirteenth Inernational water technology conference, IWTC 13, thurghada m Egypt 1521.

Alkhazan, M.M.K. & Amna Ali Nasser Saddiqm A. A. N. (2010) The effect of magnetic field on the physical, chemical and microbiological properties of the lake water in Saudi Arabia. Journal of Evolutionary Biology Research, 2 (1):7-14.

Amiri, M.C. & Ali, A. (2006) On reduction in the surface tension of water due to magnetic treatment. Colloids and surfaces A: Physiocochem. Eng. Aspects, 278:252–255.

Arber, N., Doki, Y., Han, E.K. – H , Sgambato, A., Zhou, P., Kim, N. – H , Delohery, T., Klein, M.G., Holt, P.R., Weinstein, I.B. (1997) Antisense To Cyclin D1 inhibitions the growth and tumorigenicity of human colon cancer cells. Cancer Res 57: 1569 – 1575.

Al-Moustafa, A.E., Foulkes, W.D., Wong, A., Jallal, H., Batist, G., Yu, Q., Herlyn, M., Sicinski, P., Alaoui–Jamali, Ma (2004) Cyclin D1 is essential for neoplastic transformation induced by both E6/E7 and E6/E7/Erb B-2 coopearation in normal cells. Oncogene 23:5252 – 5256.

Al-Meheithif, A., Elnour, A., Bamosa, A., Khaled, A., (2012) Antioxidant effects of zamzam water in normal rats and those under induced–oxidative stress. J Medicinal Plants Research 6 (42): 5507 –5512 .

Bamosa, A., Elnour, A., Kaatabi, H., Al Meheithif, A., Aleissa, K. (2013) Zamzam water Ameliorates Oxidative Stress and Reduces Hemoglobin A1c in Type 2 Diabetic patients . J Diabetes Netab 4: 249. doi: 10.4172 / 2155 – 6156.1000249.

Barnstable, C.D. (2014) County Department of Health and Environment, Data and Statistics. Barnstable Country Septic Database . HYPERLINK "https://septic. barnstabe country Health.org/posts%20/"https://septic. Barnsta be country Health.org/posts / data- and statistics.

Burtona, G.J., Jauniauxb, E. (2011) Oxidative stress. Best pract. Res. Clin. Obstet. Gynaecol. 25: 287 – 289.

Charles, J.S. (1996) Cancer cells. Cycles Science 274: 1672 – 1677.

Davis, L.M. (2004) Structural is changing models larg water -molecule claster may be cracial to cellular processes:The Scientist LLC -.18(21)14-20.

Ellman, G.L. (1959) Tissue Sylfhydryl group, Arch. Bioch. Biophus, 82:70- 77.

El-Zaiat, S.Y. (2005) Group refractive index measurement by Frings of equal chromatic order. Opt. and Lasers Technol 376: 181.

Gilbert, H.S., Stump, D.D. & Roth, E.F. (1984) Amethod of correct errors caused by generation of interfering compounds during erythrocytes lipid peroxidation. Analyt. Bioch., 137: 282 – 286 .

Grote, D., Russel, S.J., Cornu, T.I., Cattanco, R., Vile, R., Poland, G.A. and Fiedding, A.K. (2001) Liver attenuated measles virus induces regression of human lymphoma Xenog in immune defficient mice blood, 97(12).

Ibrahim, H. (2006) Biophysical properties of magnetized Distilled water. Egypt J. Sol., 29 (2): 363-369.

Kobe, S. Drazic, G. McGuiness, P.J. & Strazisar, J. (2001) The influence of the magnetic field on the crystallization from calcium carbonate and the testing of a magnetic water-treatment device. Journal of Magnetic and Magnetic Materials, 236 : 71 – 76.

Mehrara, E., Forssell-Aronsson, E., Ahlman, H., & Bernhardt, P. (2009) Quantitative analysis of tumor growth rate and changes in tumor marker level: Specific growth rate versus doubling time. Acta Oncologica, 48: 591_597.

Mohammed, A. Farid, Cosemi E. and Kamel, S. (2009) Oncolytic action of zamzam water on azoxyonethone (AOM) induced colon tumor in rats. Thirteenth international water technology Conference, IWTC 13, 2009, Hurgada, Egypt.

HYPERLINK "http:// www. hindawi.com/ 70410537/" Şahin, M., HYPERLINK "http://www.hindawi. com/ 36540276/" Şahin, A., HYPERLINK "http://www. hindawi.com /62815708/" Elbey, B., HYPERLINK http:// www. hindawi.com /14856232/ Yüksel, A., HYPERLINK "http:// www.hindawi.com/ 89073138/" Türkcü, F. M., and HYPERLINK http://www. hindawi. Com/42486919/ Cingü, A.K., (2016) Mean platelet volume in patients with nonarteritic anterior ischemic optic neuropathy. Journal of Ophthalmology. Article ID 1051572:1-5.http://dx. doi. org/10.1155/2016/1051572

Rozumova, L., Zivotsky, O. and Safarikova, M. (2016) magnetically modified peanut husks as an effective sorbent of heavy metals. Journal of Enviromental chemical Engineering, 4 (1): 549 – 555.

SAS.2010.SAS/STAT Users Guide for Personal Computer. Release 9.1. SAS Institute, Inc., Cary, N.C., USA. Tetsu, O., Mc Cormick, F (1999) B–catenin requlates expression of cyclin D1 in colon Carcinoma cells, Nature, 398: 442 – 426.