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Radioprotective effect of Zamzam (alkaline) water: A cytogenetic study

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ABSTRACT

Background: Radioprotectors are useful compounds to reduce radiation toxicity of normal cells. Many natural radioprotectors have antioxidant power and display fewer toxicity and side effects than the chemical ones. Alkaline waters such as Zamzam have antioxidant power potentially. This study aimed to investigate the radioprotective effect of Zamzam water in mice bone marrow exposed to gamma radiation by micronuclei test.

Method: Five study groups including control group which was fed by ordinary water, the second group was fed by Zamzam water, and radiation groups were received 2Gy gamma with ordinary and Zamzam water for 10 days and another for 20 days. The frequency of micronuclei and polychromatic erythrocytes to normochromatic erythrocytes ratio were calculated by micronuclei test.

Result: In the absence of radiation, no significant difference was found between Zamzam group and control in the number of micronuclei in normochromatic erythrocytes, micronuclei in polychromatic erythrocytes, and the polychromatic erythrocyte to polychromatic erythrocyte plus normochromatic erythrocyte ratio. But all of these indices were significantly different between irradiated and non-irradiated groups. The frequency of micronuclei in polychromatic erythrocytes was not significantly different between 10 and 20 days Zamzam irradiated groups, but the reduction in micronuclei in normochromatic erythrocytes and an increase in the polychromatic erythrocyte to polychromatic erythrocyte plus normochromatic erythrocyte ratio compared to ordinary water were seen in 20 days Zamzam group. Dose reduction factor was 1.36 and 2 for Zamzam water groups of 10 days and 20 days, respectively.

Conclusion: The results demonstrated that Zamzam alkaline water could reduce clastogenic and cytotoxic effects of gamma irradiation.

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1. Introduction

Due to serious damages of ionizing radiation on biological systems, application of a radioprotector is an important way to reduce radiation toxicity in normal cells (Nadi et al., 2016). Finding appropriate radioprotector agents is one of the most important concerns of radiation biologists and radiation oncologists (Johnke et al., 2014). Several chemical compounds were suggested as radioprotectors but usually, high toxicity in optimal doses prevents their extensive clinical uses (Tavassoli and Ghasemi, 2015; Heidari et al., 2016).

Nowadays, there is considerable interest to study the natural protection agents which have an antioxidant effect and less toxicity (Yamini and Gopal, 2010). One of the most important features of radioprotectors is their antioxidative effect, which can directly sweep the free radicals or activate antioxidant system indirectly (Varanda and Tavares, 1998; Naeeji et al., 2016; Rafat et al., 2016). Radiation is one of the most important sources of free radicals in living cells (Riley, 1994). Critical damages associated with free radicals include vital molecules such as DNA damage which have an important role in the development of more serious diseases

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(Mozdarani et al., 2007).

Antioxidants can suppress free radicals (Weiss and Landauer, 2000). These days more researchers are trying to find natural antioxidant agents like some plants, herbals, fruits, vegetables (Yamini and Gopal, 2010). Recently, some studies reported that alkaline natural water can potentially have antioxidant activity in animals and could suppress free radicals (Nassini et al., 2010) and could protect DNA (Shirahata et al., 1997).

A type of alkaline water, with antioxidant effect, is Zamzam water (Shomar 2012a). Zamzam wells are located in the Sacred Mosque in Mecca. It is holy water for Muslims. They believed Zamzam is a miracle and have healing effects. The unique Characteristics of Zamzam spring is that it is still flowing from 2000 BC and leads the human life in the arid desert of Saudi Arabia. It is known that it could cure and be useful for many diseases (Nauman et al., 2014) an example is a research which claimed Zamzam beneficial effects for cancerous patients as a complementary medicine in Saudi Arabia (Jazieh et al., 2012). One of the wonders of Zamzam water is that its composition is strongly stable (Shomar 2012b) which no changes was observed in its mineral compositions and Alkaline PH after two years (Shomar 2012a,b). Previous studies confirmed the antioxidant effects of Zamzam water in mice which reduce oxidative stress. Safety and non-toxic nature of Zamzam water were approved in animals (Abdelsalam et al., 2012) and it was studied as onco-preventative (Mohammed Ali and CosemiA, 2009). The healthy and curative effects of Zamzam water can be due to its alkaline nature, mineral and trace elements (Shomar 2012a,b). Previous studies recommended conducting more serious scientific researches to further evaluate the comprehensive health benefits and healing effect of Zamzam water. So, this experiment tried to find out whether antioxidant effects of Zamzam water can protect the normal cell against irradiation damage. The radioprotective effect can be measured by dose reduction factor (DRF). In animal studies, DRFs are usually assessed by irradiating mice with or without administering the radioprotector. The study aimed to evaluate the effect of Zamzam water in modulating the clastogenic and cytotoxicity damage induced by gamma irradiation.

2. Materials and methods

2.1. Water samples

We have provided the Zamzam water in the packed bottles which were available for pilgrims of Mecca.

2.2. Animal preparation

25 Male NMRI Mice (28–35gr, 8–10 weeks old) were obtained from Babol animal lab. They were preserved in standard condition and given standard pallets and two kinds of water (Tab and Zamzam water). They were kept in a controlled lighting condition (light: dark, 12:12 h) and divided into five groups, with five samples size. Simple random sampling was applied by random number table.

2.3. Irradiation

The control group received ordinary water and tolerated all the stress expect radiation (sham procedure), the second group received Zamzam water while did not receive radiation, the third group received ordinary water with 2 Gy of gamma rays, the fourth group received Zamzam water for ten days and 2 Gy gamma rays, the fifth group received Zamzam water for 20 days with 2 Gy gamma rays. For irradiations, mice were put in Plexiglas cages and received a total dose of 2 Gy for the whole body from a source of cobalt-60 (gamma ray). Source to skin distance was 80 cm, and the dose rate was 76.92 cGy/min.

2.4. Micronucleus assay

Micronuclei test was carried out according to Schmid study (Schmid, 1975). Briefly, 24h after irradiation, mice were sacrificed by cervical dislocation and then both femurs of animals were removed. Bone marrows were flushed with fetal calf serum (FCS) into a centrifuge tube. Cell sediments obtained using centrifugation at 1000 rpm for 7 min, then the solution over the deposited substances in each tube was discharged and about 100 μl of the serum remained in the tube and the smear was provided by one drop of solution. The slides were kept for 24 h at room temperature in the laboratory then were fixed with methanol and subsequently after one more 24 h, they are stained with May-Grünwald and Giemsa.

2.5. Analyzing the slides

Cells of slides were counted by light microscopy with a magnification of 100 times. For each sample, 1000 polychromatic erythrocyte (PCE) and all the normochromatic erythrocyte (NCE) cells around the PCEs were counted. The quantities of micronuclei in 1000 PCEs (MnPCEs) and micronuclei in 1000 NCEs (MnNCE) were measured. Also, polychromatic erythrocyte to polychromatic erythrocyte plus normochromatic erythrocyte (PCE/PCE + NCE) ratio was calculated. Radioprotective effect of agents was measured by a dose reduction factor (DRF) which was calculated by the PCE/PCE + NCE percent in absence of Zamzam water along with radiation dividing to the PCE/PCE + NCE percent in presence of Zamzam water along with radiation. The data were analyzed by the one-way ANOVA and Tukey's multiple comparisons test using the SPSS16 software. The study was approved by institute ethical committee.

3. Results

The MnPCEs, MnNCEs, and PCE/PCE + NCE ratio were not significantly different between the control and Zamzam groups, but, all of these indices were significantly different in all irradiated groups compared to the control group (Figs. 1–3).

The comparison of Zamzam water effects on the PCE/PCE + NCE ratio in mice bone marrow exposed to 2Gy gamma radiation were shown in Fig. 1. Regarding PCE/PCE + NCE ratio among irradiated groups, Both Zamzam water (10, 20 days) had a reduction compared to ordinary water group, but, this difference was only significant for the group which was fed for 20 days.

The comparison of Zamzam water Effects on the radiation-induced MnPCE/PCE (%) and MnNCE/NCE (%) in mice bone marrow exposed to 2Gy gamma radiation were shown in Figs. 2 and 3 respectively.

There was no significant difference in all three indices between two irradiated Zamzam groups (10 and 20 days) but our results showed that cytotoxicity was reduced by a DRF factor of 1.36 for 10 days and 2 for 20 days with Zamzam water.

4. Discussion

In this study, we have evaluated the capability of Zamzam water as a radioprotector, by micronuclei assay, as an effective method to examine the cytotoxicity of the agents such as ionizing radiation, chemical materials in mammalian (Jin et al., 2006). Clastogenic effect of 2 Gy gamma irradiations was quite visible by increasing the MnPCE and MnNCE and the cytotoxic effects were observed by decreasing the PCE/PCE + NCE ratio in mice bone marrow in comparison to the control group. This was is in agreement with

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