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ORIGINAL ARTICLE

# Effects of perinatal exposure to Zamzam water on the teratological studies of the mice offspring



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## KEYWORDS

Zamzam water;  
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Mice offspring;  
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Locomotor behavior

**Abstract** Zamzam water is well documented for plenty of medicinal value for curing illness. In the present study, the effects of perinatal consumption of Zamzam and normal drinking water by the pregnant mice on their offspring's physical parameters, early sensory motor reflexes, locomotor activities, acetylcholinesterase (AChE) activity in the homogenize brain tissue and blood parameters were compared. To achieve that; Zamzam water was given to female Swiss-Webster strain mice as the only source of drinking fluid and the control animals were administered plain tap water. Treatment started from the first day of pregnancy and continued until the postnatal day fifteen of delivery. All offspring were subjected to various tests. The rate of body weight gain remained relatively unaffected until the second week of weaning period, however; in the last week the offspring exposed to Zamzam water gained significant body weight as compared to their control offspring. Furthermore, the opening of eyes and appearance of body hairs in Zamzam exposed pups remained unaffected as compared to the controls. The sensory motor reflexes in Zamzam exposed pups after birth and during the first two weeks of weaning period were significantly increased. Locomotor Activity Test performed in the male and female offspring after weaning period showed a significant decrease in the male and increase in the female on most of the elements of this test due to Zamzam exposure. AChE activity in the homogenized brain tissue and blood parameters were unaffected as compared to the controls, the present Zamzam effects in the offspring are possibly via in utero action and/or via mother's milk.

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## 1. Introduction

Water is a basic need and is essential in carrying out various physiological functions in the human body (Cazier and Gekas, 2001). Humans can survive without food for a month, but they can survive without water for only seven days (Vidyasagar, 2007). Only 2.8% of the total water on earth is freshwater; the rest is saltwater that is hard to use (Khalid et al., 2014). Although the world's water sources are limited, and existing sources are depleting rapidly, there is a source

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of water called Zamzam that is providing water to billions of people (Khalid et al., 2014; Naeem et al., 1983).

This source of water is located in the Mecca area, one of the most sacred cities for Muslims. This city is located in the western part of Saudi Arabia about 70 km south of the city of Jeddah on the coast of the Red Sea. Geographically, it can be located at latitude 21° 26' 48" N, longitude 39° 53' 46" E, with an elevation of about 1399 ft. above mean sea level (Khalid et al., 2014).

Brief anecdotes that mention Zamzam are described in the holy books of various religions, including the Torah (Old Testament/Torah), the Bible, and the Quran. As narrated in these holy books, Zamzam is the holy water and is termed as a great gift from God (referred to as Allah in the Quran). It is allegedly an offshoot from a holy spring (currently present in the form of a well) in the barren desert surrounding Mecca. Muslims use Zamzam water to recover from diseases, according to the speech of the Prophet Muhammad, peace be upon him, who said: "Water of Zamzam is good for whatever (purpose) it has been drank." In another Hadith, the Prophet said: "Water of Zamzam is a healer of every disease. It is the place where angel Jibril indicated it and dug it, and it is the water of Allah that made Ismail (to be the first) to drink it" (Ahmad and Ibrahim, 1996).

Khalid et al. (2014) pointed in their review article that Zamzam water can be used to recover from some diseases. Also, it has unique analytic properties and has a strong anti-inflammatory effect. Zamzam water has a strong antitumor necrosis factor (TNF $\alpha$ ) and effect on interleukin 1 (IL1). Zamzam water has analytic action through an indirect influence on endocrine immunology and the growth system of the body (Ali et al., 2009c). Clinical examination revealed that, in the mixed dentition group, no statistically significant differences were detected, whereas among the permanent dentition group, the mean decayed, missing, filled teeth (DMFT) score was the lowest in all the children using Zamzam water (Al-Zuhair and Khounganian, 2006).

It was used in the treatment of implantation failure, for stimulation of endometrial prolactin, defense, luteinizing hormone (LH), endometrial vascular endothelial growth factor (VEGF), and angiopoietin receptors (Ali et al., 2009b). Furthermore, Zamzam water causes upregulation of gap junctional inter-cellular communication and connexin 43 antibodies in endometrium. Ali et al. (2009a) demonstrated that Zamzam water stimulates stem cells' differentiation in the endometrium. This phenomenon is triggered due to the high calcium and magnesium content of Zamzam. Also, the water provides more support to many other biochemical processes in the endometrium, including its vital role as a coenzyme during the formation of immunoglobulin (Ali et al., 2009b,c).

Furthermore, Zamzam water may be used in planting, Mutwally et al. (2015) reported that sole use of Zamzam water or a combination of Zamzam, with either treated water or tap water resulted in pronounced increases in the percentage of seed germination, shoot length, and the fresh and dry weights of the shoots. Similarly, the percentage of flowers in broad bean plants watered with Zamzam was considerably higher in comparison with other water treatments.

Researches that have examined the effect of drinking Zamzam water by mother during pregnancy and its impact on its offspring's growth and behavior was very little or almost

non-existent. Therefore, the current study was designed to investigate the effect of prenatal exposure to Zamzam water on the growth, behavior, some blood indicators and enzyme of acetylcholinesterase in laboratory mice offspring.

## 2. Materials and methods

### 2.1. Experimental animals

Male and female Swiss-Webster strain mice (8–9 weeks old) were housed in opaque plastic cages (three females to one male in each cage) measuring 30 × 12 × 11 cm, in the animal facility of the Zoology Department, King Saud University, Riyadh, Saudi Arabia. Animals were kept under reversed lighting conditions with white lights on from 22.30 to 10.30 h local time. The ambient temperature was regulated between 18 and *noe*. After pregnancy (appearance of vaginal plug was considered as day one of pregnancy), the males were removed from the cages and the females were subjected to experimental treatments. Food (Pillsbury's Diet) and water were available *ad libitum*, unless otherwise indicated.

### 2.2. Zamzam water administration

The animals were divided on the first day of pregnancy (the appearance of vaginal plug) into two groups, the first is the control which was given tap water, while the second group was given Zamzam water (purchased from the local market). Treatment of mothers was started after the delivery of offspring from day 1 (PD1) and was continued until postnatal day 15 (PD15) and thereafter the mothers were switched to tap water.

### 2.3. Physical assessment during weaning period

On the day of birth (postnatal day 0, PDO) the pups were culled to only eight per dam and were left with their mothers until PD22. During this weaning period, three pups in each litter were color marked from the others and were subjected to various behavioral tests (described below) under dim lighting (ca 8 lux). In all, 21 pups belonging to seven litters from each treatment category were considered. All observations were recorded on PD 1 and repeated every other day until PD21 in the same three color marked pups of each litter. These observations were used to measure the early development of sensory motor coordination reflexes together with morphological development in the pups. For statistical analysis, the mean of all three color marked pups per litter was considered as a single score. Thus, seven replicates from each treatment category were considered in these observations.

#### 2.3.1. Body weight

Weight is a useful indicator of development. Thus, the pups were weighed every alternate day from PD 1 to PD21.

#### 2.3.2. Eye opening and hair appearance

The day at which the body hair fuzz appeared, and the eyes opened was also recorded. These two parameters are also useful morphological indicators of development.

## 2.4. Neuromotor maturation assessment during weaning period

### 2.4.1. Righting reflex

The time taken by a pup placed on its back to turn over and place all four paws on the substrate was recorded. An upper limit of 2 min was set for this test.

### 2.4.2. Cliff avoidance

Pups were placed on the edge of a table top with the forepaws and face over the edge. The time taken by the pup to back away and turn from the "cliff" was recorded. Again an upper limit of 2 min was chosen. A latency of 2 min was attributed when the animal fell from the "cliff".

### 2.4.3. Rotating reflex

The surface used to measure the rotating reflex was the same as that used for righting reflex, except that it was inclined at an angle of 30°. The pups were placed on this surface with their heads pointed downward. The time elapsed until the pup rotates its body through 180° generatively and faces its head upward, was recorded as the rotating time. The upper limit of this test was also set at 2 min.

## 2.5. Behavioral assessment during post-weaning period (locomotory tests of young adult males and females)

The offspring were weaned on PD21 and thereafter, the males and females were isolated and kept in groups of two or three, for 14 days. Subsequently, 10 males and females from Zamzam and control group (including representatives from each 7 litters) were subjected to locomotor activity tests. The young adult males and females were placed in an experimental wooden arena measuring 80 × 80 × 30 cm and the floor was divided into 64 equal sized squares. Various behavioral elements were observed as described by Ajarem (1987). Elements of locomotory activity included the number of squares crossed and the number of wall rears as well as the duration of locomotion and immobility. The visual observations in the arena lasted 300 s for each animal.

## 2.6. Biochemical studies

In PD36, the brain of some animals (male and female) were removed and gently rinsed in physiological saline (0.9% NaCl), and then blotted on Whatman filter paper. Their fresh weights were recorded, and organs were then frozen.

### 2.6.1. Tissue homogenate preparation

A 10% (w/v) homogenate of each frozen tissue was prepared in Teflon-glass homogenizer at 4 ± 1 °C, centrifuged at 1000g for 10 min. to remove cell debris and the supernatant was used for enzyme assays. The brain homogenate was prepared in an ice-cold phosphate buffer, (0.067M, pH7.2) solution.

### 2.6.2. Estimation of acetylcholinesterase

The acetylcholinesterase (AChE) activity in the homogenized brain tissue was estimated by the method of Ellman et al. (1961), utilizing acetylthiocholine iodide (ATCI) as a substrate. The rate of production of thiocholine is determined by the continuous reaction of the thiol with 5,5-dithiobis-2-

nitrobenzoate (DTNB) ion to produce the yellow anion of 5-thio-2-nitrobenzoic acid. Spectrophotometric assay of enzyme activity was performed by adding 0.4 ml of the supernatant to a cuvette containing phosphate buffer (2.6 ml, pH 8) and 0.2 ml of DTNB (5,5-dithio-bis(2-nitrobenzoic acid), Sigma Chem. Co., St. Louis, MO, USA). After adjusting the absorbance to zero, 0.02 ml of the substrate acetylthiocholine iodide (Sigma Chem. Comp., St. Louis, MO, USA) was added and change in absorbance over 5 m was recorded. The specific activity of AChE was expressed as μ moles of acetylthiocholine iodide hydrolyzed/min/g of wet tissue.

### 2.6.3. Blood parameters

In PD36, blood was collected from animals (male and female) within heparinized tubes at the end of the experiments. Blood parameters namely, red blood count, packed cell volume, hemoglobin content, total white blood count and blood platelets were measured using the automated parameter hematology analyzer (T 450, USA).

## 2.7. Statistical analysis

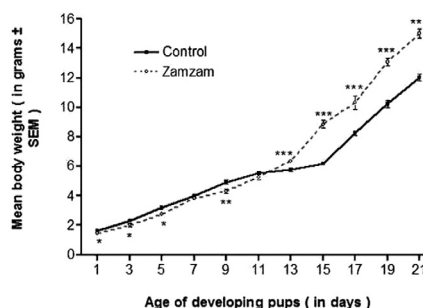
The data of body weight, dates of morphological developments, data of sensory motor reflexes and data of biochemical analyses were compared within the experimental groups by analysis of variance (ANOVA) using Minitab computer program, and were subsequently analyzed by Student's *t*-test (Yamane, 1973). Data of locomotory test were compared within the experimental groups by analysis of variance (ANOVA) and were subsequently analyzed using Mann-Whitney *U*-tests (Sokal and Rohlf, 1981).

## 3. Results

### 3.1. Physical assessment during weaning period

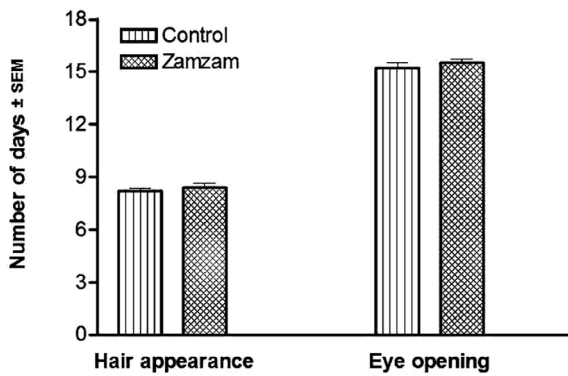
The results in the present study showed that the body of pups (Fig. 1), whose mothers drank Zamzam water, was decreased in PD9 ( $p < 0.01$ ), PD1, PD3, PD5 ( $p < 0.05$ ) while was increased from PD13 to PD21 compared to the control group.

Other morphological developments such as the opening of the eyes and appearance of body hair fuzz were not significant differences between Zamzam water pups and their controls (Fig. 2).



\*, \*\* and \*\*\* shows statistically significant at  $P < 0.05$ ,  $P < 0.01$  and  $P < 0.001$  respectively from the control by ANOVA and Student's *t*-test.

**Figure 1** Effect of Zamzam water on body weight of laboratory mice.

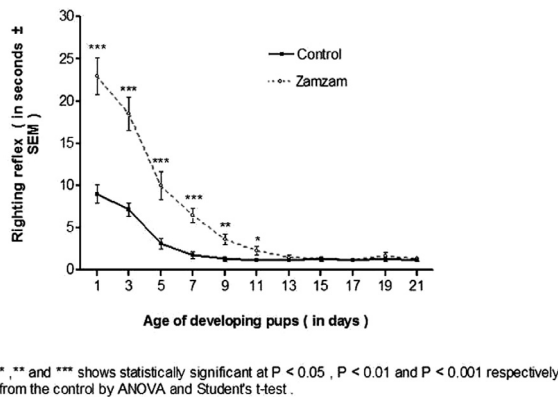


**Figure 2** Effect of Zamzam water on eye opening and hair appearance of laboratory mice.

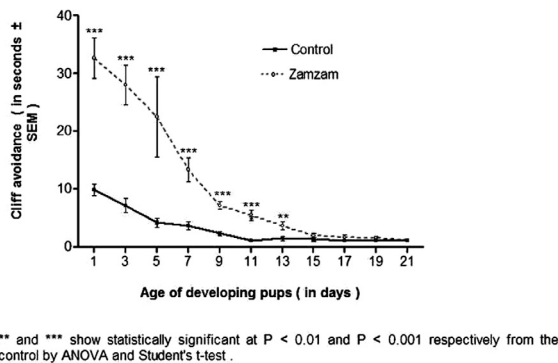
3.2. Neuromotor maturation assessment during weaning period

Righting reflex: Zamzam water exposed pups which were placed on their back delayed to turn over and place all four paws on the substrate compared to the control group (Fig. 3). The significant difference was  $p < 0.001$  in PD1, PD3, PD5 and PD7, while  $p < 0.01$  in PD9 and ( $p < 0.05$ ) in PD11. There was no significant difference in PD13–PD21.

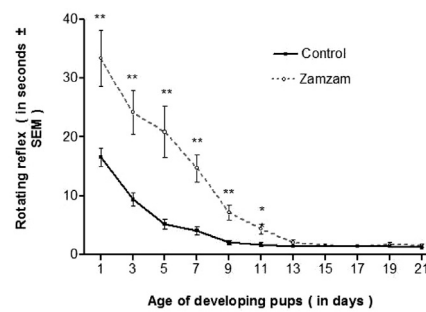
Cliff avoidance: The pups placed on the edge who were given Zamzam water delayed (Fig. 4) to back away and turn



**Figure 3** Effect of Zamzam water on righting reflex of laboratory mice.



**Figure 4** Effect of Zamzam water on cliff avoidance of laboratory mice.



\* and \*\* show statistically significant at  $P < 0.05$  and  $P < 0.01$  respectively from the control by ANOVA and Student's t-test.

**Figure 5** Effect of Zamzam water on rotating reflex of laboratory mice.

from the cliff. It was extremely significant ( $p < 0.001$ ) in PD1–PD11 and ( $p < 0.01$ ) and was not significant in PD15–PD21 when compared to control.

Rotating reflex: Zamzam water exposed pups which were placed on the surface with their heads pointed downward took more time to rotate their bodies through  $180^\circ$  geonegatively and face their heads upward (Fig. 5). The significant difference in PD1–PD9 was  $p < 0.01$ , and Pd11  $p < 0.05$  while no significance was seen in PD13–PD21.

3.3. Locomotory tests of young adult males and females

In males (Table 1), it is indicated that Zamzam water decreased motor activity, the number of squares crossed ( $p < 0.001$ ), wall rears ( $p < 0.001$ ), rear ( $p < 0.01$ ) and locomotion duration ( $p < 0.001$ ) while the number of body wash and immobility duration were increased ( $p < 0.05$ ,  $p < 0.001$ ) respectively when compared to the control.

Table 2 shows that the motor activity of female mice was increased, like the number of Squares crossed ( $p < 0.05$ ), number of wall rears ( $p < 0.001$ ), rear ( $p < 0.05$ ), the number of body wash ( $p < 0.05$ ) and locomotion duration ( $p < 0.001$ ) while immobility duration was decreased ( $p < 0.05$ ) when compared to the control.

3.4. Biochemical Studies

3.4.1. Acetylcholinesterase

Fig. 5 for males and Fig. 6 for females showed that there are no significant differences between Zamzam group and its control.

3.4.2. Blood parameters

Figs. 7–16 for males and females showed that Zamzam water did not affect the blood parameters, namely, red blood count, packed cell volume, hemoglobin content, total white blood count and blood platelets compared to their controls (see Fig. 17).

4. Discussion

Zamzam water is in the Mecca area, in the city of Makkah, at the western province of the Kingdom of Saudi Arabia

**Table 1** Effect of Zamzam water on locomotor activity of laboratory male mice.

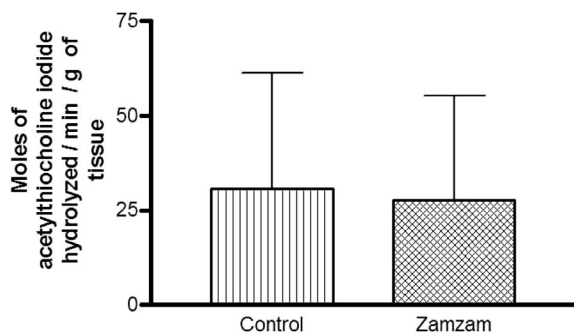
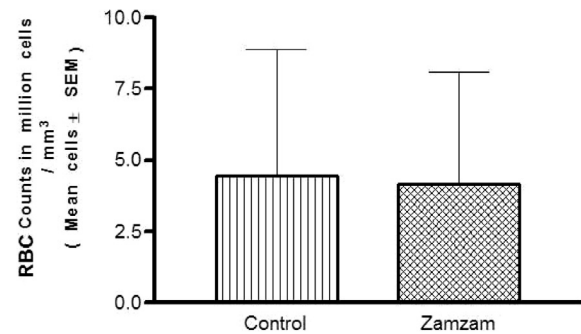
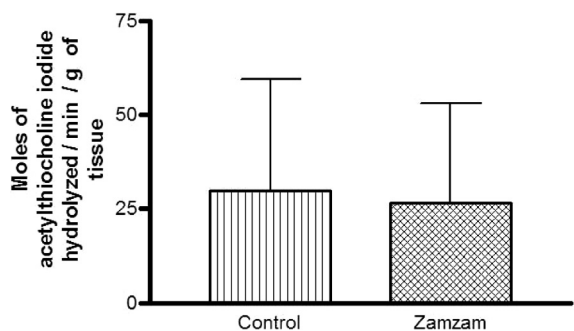
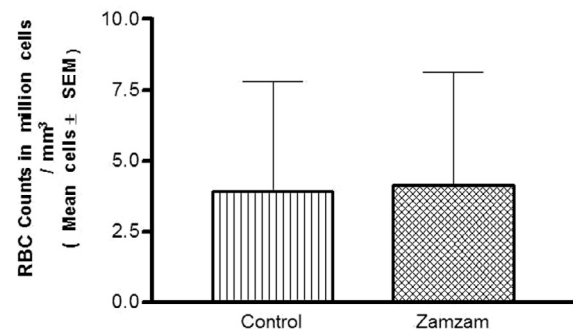
Treatment group	Median number (with ranges) of acts and postures					
	Number of squares crossed	Wall rears	Rears	Wash	Locomotion duration (s)	Immobility duration (s)
Control	248.0 (217.0–286.0)	42.0 (30.0–57.0)	20.0 (16.0–24.0)	11.0 (5.0–15.0)	182.75 (164.1–229.8)	122.05 (90.0–140.3)
Zamzam	134.0*** (105.0–175.0)	17.0*** (10.0–29.0)	7.0** (2.0–21)	15.0* (10.0–20.0)	115.05*** (70.2–135.9)	177.95*** (159.7–210.0)

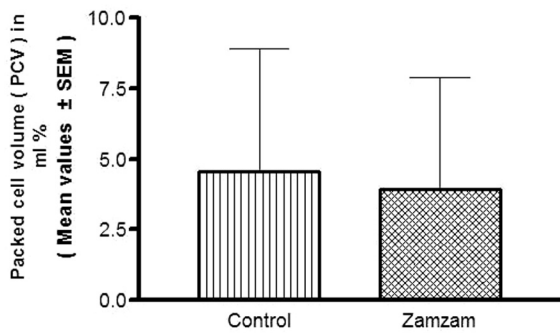
\*, \*\* and \*\*\* show statistically significant differences at  $p < 0.05$ ,  $p < 0.01$  and  $p < 0.001$ , respectively from the control by ANOVA and Mann–Whitney  $U$  test.

**Table 2** Effect of Zamzam water on locomotor activity of laboratory female mice.

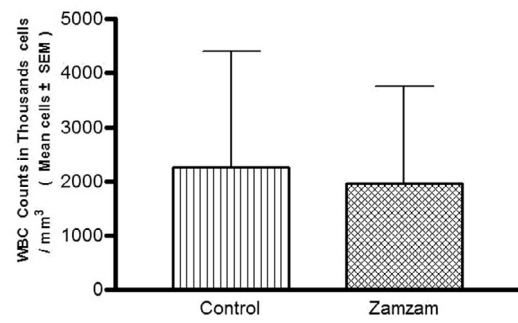
Treatment group	Median number (with ranges) of acts and postures					
	Number of squares crossed	Wall rears	Rears	Wash	Locomotion duration (s)	Immobility duration (s)
Control	148.0 (125.0–186.0)	17.0 (10.0–29.0)	12.0 (2.0–21.0)	8.0 (4.0–12.0)	120.95 (74.60–139.40)	175.30 (109.60–230.0)
Zamzam	200.0* (138.0–238.0)	34.0*** (30.0–44.0)	19.0* (11.0–24)	12.0* (9.0–17.0)	179.05*** (160.60–225.40)	124.70* (70.0–190.40)

\* and \*\*\* show statistically significant differences at  $p < 0.05$  and  $p < 0.001$ , respectively from the control by ANOVA and Mann–Whitney  $U$  test.

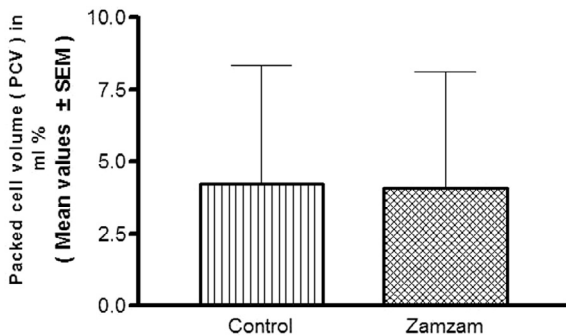
**Figure 6** Effect of Zamzam water on acetylcholinesterase of male laboratory mice.**Figure 8** Effect of Zamzam water on red blood cells of male laboratory mice.**Figure 7** Effect of Zamzam water on acetylcholinesterase of female laboratory mice.**Figure 9** Effect of Zamzam water on red blood cells of female laboratory mice.



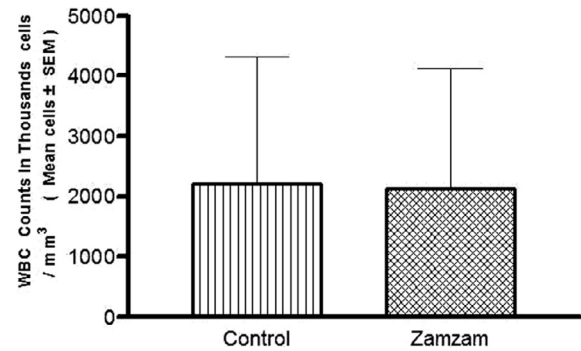
**Figure 10** Effect of Zamzam water on packed cell volume of male laboratory mice.



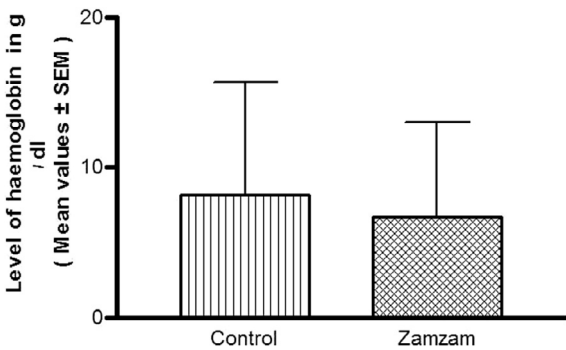
**Figure 14** Effect of Zamzam water on total white blood count of male laboratory mice.



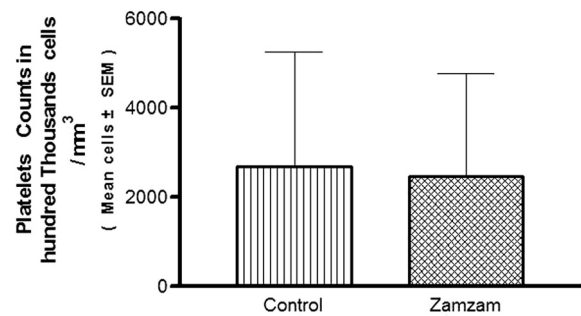
**Figure 11** Effect of Zamzam water on packed cell volume of female laboratory mice.



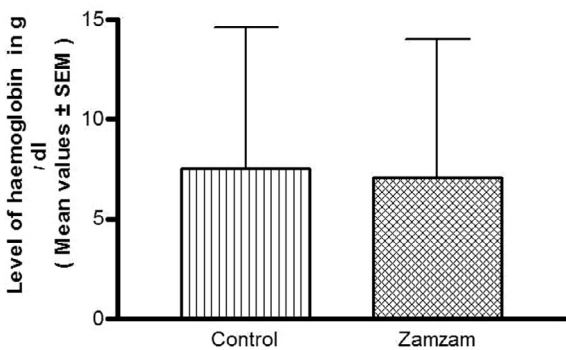
**Figure 15** Effect of Zamzam water on total white blood count of female laboratory mice.



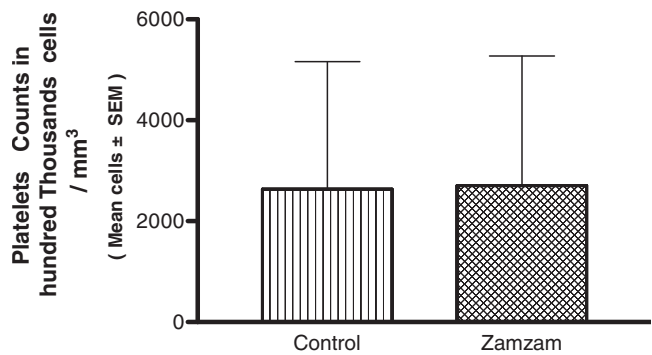
**Figure 12** Effect of Zamzam water on hemoglobin content of male laboratory mice.



**Figure 16** Effect of Zamzam water on blood platelets of male laboratory mice.



**Figure 13** Effect of Zamzam water on hemoglobin content of female laboratory mice.



**Figure 17** Effect of Zamzam water on blood platelets of female laboratory mice.

(Al-Ghamdi, 2012). Many Muslims believe that the water of the Zamzam well is divinely blessed, able to satisfy both hunger and thirst, as well as cure illness (Shomar, 2012). Zamzam water is different from other water in many ways: first no bacteria can form at its source. Second it doesn't go moldy nor does it change color, taste or smell (Alshikh, 2013). Biological growth and vegetation usually take place in most wells, however in Zamzam water well, there isn't any sign of biological growth (Mashat, 2010). The Chemical analysis of Zamzam water contains some inorganic elements such as sodium (Na), calcium (Ca), magnesium (Mg), and totally dissolved salts (TDS) (Al-Zuhair et al., 2005). Four toxic elements arsenic (As), cadmium (Cd), lead (Pb), and selenium (Se) have been found below the danger level for human consumption (Watanabe et al., 2000; El-Zaiat, 2007; Al-Rawi et al., 2009; Al Nouri et al., 2014). Table 3 shows ranges of some elements, salts and pH of Zamzam water (Al Meheithif et al., 2012).

The results of the present study indicated that perinatal exposure of dams to Zamzam water enhanced body weight gain of their offspring compared to the controls. Our results are in agreement with the study of Watanabe et al. (2000) and disagreement with that of Al Meheithif et al. (2012). It may be due to difference in species. Tayyeb et al. (2004) pointed that water contains macro elements such as Na, Ca and Mg which are necessary to sustain biological life and trace elements function chiefly as catalysts for enzymatic activity in human bodies. However, their accumulations cause acute or chronic poisoning and have to be removed from drinking water. We suggest that the high concentration of Ca, Mg, Na and K (Al Meheithif et al., 2012) may affect the homeostasis metabolism of the body and the hormones and enzymes which regulate it. Regulation of serum calcium concentration is complex and requires the integrated actions of PTH, vitamin D metabolites, and calcitonin. PTH and calcitriol (1,25-dihydroxyvitamin D3) are the main regulators of calcium homeostasis and have major regulatory effects on each other (Schenck et al., 2012). The high concentration of calcium in Zamzam water may interact with phosphate in the bone and alter the biological correlation between calcium and phosphorus. Calcium may be accumulated in the bones due to this alternation (Schenck et al., 2012). In general, abnormalities in response to teratogens may be due to several factors that cause alterations in normal cell metabolism, especially in

enzymes and their substrates (Wilson, 1973), or due to a combination of several factors (Coyle et al., 1976).

The sensory motor reflexes data in Zamzam exposed pups after birth and during the first two weeks of weaning periods were significantly increased as compared to their controls. It seems that it depends on the state of the brain when the behavioral tests were done (Al-Shanti, 1996). Calcium is required in the body for many vital intracellular and extracellular functions, as well as for skeletal support. Ionized calcium ( $iCa$  or  $Ca^{2+}$ ) is required for enzymatic reactions, membrane transport and stability, blood coagulation, nerve conduction, neuromuscular transmission, muscle contraction, vascular smooth muscle tone, hormone secretion, bone formation and resorption, control of hepatic glycogen metabolism, and cell growth and division (Schenck et al., 2012). The slow muscle response may rise from the increased calcium, which affects the level of natural element and hinders the entry of element to the cell, where most of the damage to the cells associated with calcium (Schenck et al., 2012). Na-K ATPase enzyme – which regulates the sodium pump- also plays a direct role in the regulation of calcium distribution and other ions, as the concentration of calcium stability on both sides of the membrane is the driving force responsible for the flow of calcium operations through ion exchange of both sodium and calcium systems, as the proportion of calcium in the charge may stimulate many cellular activities, such as muscle contraction and energy production processes (Konda and Sakamoto, 1991). The inhibition of the sodium pump – necessary for the occurrence of polarization in the cells and transfer impulses nerve – leads to a decrease in the effectiveness of the ion exchange based on the regulation of calcium influx operations (Satyavathi and Prabhakara Rao, 2000); which caused exposed pups to stay a long time on the back, edge or surface leaning compared to the control (Haefner et al., 1984).

Data on locomotor activity behavior showed a decrease in most ailments of test for males and an increase in seam test for females. The difference between males and females may be due to hormonal effects (Abu-Taweel et al., 2011). Locomotor behavior is the activity of animal in the open area, an individual without Group (Ajarem, 1999).

Elements of locomotor activity test such as number of scoured crossed, wall rears, rears, locomotion duration were decreased while immobility duration was increased significantly (Table 1). Jhulka and Gill (Julka and Gill, 1996) believed that the homeostasis change of calcium is responsible for damages that occur in the nervous or muscular systems, and noted that the increase in cellular calcium amounts causes an imbalance in the organization of proteins for metabolism and enzyme  $Ca^{+2}$  – ATPase and liquidity of the cell membrane, and this probably contributed to the interpretation of the lack of movement and reduced activity in the most testing periods. Munoz et al. (1989) pointed that low body weight increases the locomotor activity. In the present study, Fig. 1 shows that consumption of Zamzam water by mothers increased the body weight of their offspring. The heaviness of the body may suggest a possible relationship between it and reduced locomotor activity in treated pups. In general, Miu et al. (2003) thought that the bad psychological state plays a role in the lack of motor behavior.

Locomotor activity elements such as number of scoured crossed, wall rears, rears, locomotion duration were increased while immobility duration was decreased significantly

**Table 3** Ranges of some elements, salts and pH of Zamzam water.

No.	Parameter	Range
1	Calcium Carbonate (ppm)	300–340
2	Magnesium (ppm)	19–24
3	Chromium (ppb)	0.7–0.75
4	Manganese (ppb)	0.07–0.10
5	Cobalt (ppb)	0.3–0.4
6	Copper (ppb)	0.5–1.0
7	Zinc (ppb)	1–2
8	Arsenic (ppb)	19–26
9	Selenium (ppb)	3–4
10	Strontium (ppb)	700–800
11	Cadmium (ppb)	0.2–1.0
12	Lead (ppb)	0.05–0.1
13	Nitrate (ppb)	70–90
14	pH	7.75–8.0

(Table 2). Verity (1990) and Rodrigues et al. (1996) were restored the increasing of locomotor activity in rats to the damage that occurs in the hippocampus, where it leads to many neurological symptoms, activity and aggressive. Figs. 5 and 6 in males and females, respectively, show slight lack of acetylcholinesterase (AChE) activity of treated pups but the difference was not significant. Despite the uncertainty surrounding the relationship between neurotransmitters and behavior, many studies have attributed most of these changes in behavior to change in the activity of neurotransmitters (Brain, 1982). Found that serotonin affect mood and behavior (Badawy, 1981), and there is a relationship between lack of metabolic products of serotonin and aggressive and suicidal behavior (Asberg, 1986). Serotonin also plays an important role in stimulating the motor behavior (Al-Shanti, 1996). The study, conducted by Oskarsson et al. (1986) was linked between the increase in the activity of rats and the amount of the increase occurring in dopamine and serotonin. In general, the increase in locomotor activity may be due to the increase in dopamine, epinephrine and serotonin or deficiencies in GABA and amino acid Glutamate (Cutler, 1977; Shailesh- Kumar and Desiraju, 1990; Ramin and Porter, 1997).

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