Brazilian Journal of Biology®

ISSN 1519-6984 (Print)
ISSN 1678-4375 (Online)

Original Article

Physical characterization and wound healing properties of Zamzam water

Caracterização física e propriedades cicatrizantes da água de Zamzam

S. S. Moni[a*], M. H. Sultan[a], S. Alshahrani[b], P. Tripathi[b], A. Assiri[c], S. S. Alqahtani[d,e], M. A. Bakkari[a], O. A. Madkhali[a], M. F. Alam[b], A. H. Alqahtani[f], R. Tripathi[d], S. S. Pancholi[a,g], M. Ashafaq[b] and M. E. Elmobark[a]

[a]Jazan University, College of Pharmacy, Department of Pharmaceutics, Jazan, Kingdom of Saudi Arabia
[b]Jazan University, College of Pharmacy, Department of Pharmacology and Toxicology, Jazan, Kingdom of Saudi Arabia
[c]King Khalid University, College of Pharmacy, Department of Clinical Pharmacy, Abha, Kingdom of Saudi Arabia
[d]Jazan University, College of Pharmacy, Department of Pharmacy Practice, Jazan, Kingdom of Saudi Arabia
[e]Jazan University, College of Pharmacy, Pharmacy Practice Research Unit, Jazan, Kingdom of Saudi Arabia
[f]Al- Dawaa Medical Services, Co Ltd, Riyadh, Kingdom of Saudi Arabia
[g]Ganpat University, S. K. Patel College of Pharmaceutical Education and Research, Mahesana, Gujarat, India

Abstract

The objective of the study was to evaluate the quality of Zamzam water, holy water for Muslims and consumed for its medicinal value. The present study demonstrates the physicochemical characterization and wound healing property of Zamzam water. The physicochemical characterization of Zamzam water samples was analyzed for dissolved oxygen, pH, conductivity, total dissolved solids, redox potential, zeta potential, polydispersity index, and zeta size. The microbial quality of Zamzam water was also assessed by exposing water samples to open air. In this work, Zamzam water was also screened for the medicinal value through wound healing properties in Wistar rats. Zamzam water exhibited a unique physicochemical characterization with high levels of dissolved oxygen, zeta potential, polydispersity index, redox potential, total dissolved solids, and conductivity before exposure to open air. After open air exposure, Zamzam water resisted the growth of bacteria. The wound healing properties of Zamzam water *in vivo* showed a 96% of healing effect on 12th day observation. The wound healing was achieved by modulating pro-inflammatory cytokine such as interleukin -1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor -α (TNF-α). Followed by the level of apoptosis markers caspase-9 and caspase-3 were reduced. The present study proved that Zamzam water is a good-quality water and showed excellent wound healing property. Therefore, Zamzam water can be used for pharmaceutical formulations.

Keywords: Zamzam water, water quality, physicochemical parameters, bacterial growth, wound healing property.

Resumo

O objetivo do estudo foi avaliar a qualidade da água de Zamzam, água benta para os muçulmanos e consumida pelo seu valor medicinal. O presente estudo demonstra a caracterização físico-química e a propriedade cicatrizante da água de Zamzam. A caracterização físico-química das amostras de água de Zamzam foi analisada para oxigênio dissolvido, pH, condutividade, sólidos totais dissolvidos, potencial redox, potencial zeta, índice de polidispersidade e tamanho zeta. A qualidade microbiana da água de Zamzam também foi avaliada expondo amostras de água ao ar livre. Neste trabalho, a água de Zamzam também foi avaliada quanto ao valor medicinal através de propriedades cicatrizantes em ratos Wistar. A água de Zamzam exibiu uma caracterização físico-química única com altos níveis de oxigênio dissolvido, potencial zeta, índice de polidispersidade, potencial redox, sólidos totais dissolvidos e condutividade antes da exposição ao ar livre. Após a exposição ao ar livre, a água de Zamzam resistiu ao crescimento de bactérias. As propriedades de cicatrização de feridas da água de Zamzam in vivo mostraram um efeito de cicatrização de 96% no 12º dia de observação. A cicatrização da ferida foi alcançada pela modulação de citocinas pró-inflamatórias como a interleucina-1β (IL-1β), interleucina-6 (IL-6) e fator de necrose tumoral -α (TNF-α). Seguido pelo nível de marcadores de apoptose caspase-9 e caspase-3, estes foram reduzidos. O presente estudo provou que a água de Zamzam é uma água de melhor qualidade e mostrou excelente propriedade de cicatrização de feridas. Portanto, a água de Zamzam pode ser usada para formulações farmacêuticas.

Palavras-chave: água de Zamzam, qualidade da água, parâmetros físico-químicos, crescimento bacteriano, propriedade cicatrizante.

*e-mail: drsmsivakumar@gmail.com; smoni@jazanu.edu.sa
Received: April 5, 2022 – Accepted: July 23, 2022

1. Introduction

Water is one of the most valuable natural resources on the earth planet. Water is a necessary nutrient for the survival of cells, and it first serves as a construction ingredient. It controls and regulates our body temperature through sweating and respiration required to function many physiological functions within the human body properly. Water accounts for 75% of an infant's body weight and 55% of the bodyweight of an elderly person, and it is necessary for cellular balance and survival. Water quality impacts human health and is greatly influenced by topography, geological formations, and climatic condition (Balasubramanian et al., 2015; Zhu et al., 2012; Zhu et al., 2011; Vidyasagar, 2007; Zhu and Yang, 2007; Subramani et al., 2005; Cazier and Gekas, 2001; Helena et al., 2000). Human activities, such as industrialization and urbanization, produce effluents that affect natural water quality (Zhu, 2016). Balasubramanian et al., 2015; Zhu et al., 2012). One of the most important resources used by the pharmaceutical industry is water. It may be used as an excipient or used to reconstitute products during synthesis, manufacturing, or as a cleaning agent for vessels and equipment. Different levels of water quality are required for various medicinal applications. Water quality control, particularly microbiological quality control, is a significant problem, and the pharmaceutical sector invests significant resources in the development and operation of water purification systems. Zamzam is holy water considered a wonderful gift from God referred to as Allah in the Quran. Zamzam water is a natural treasure for Muslims in Saudi Arabia and across the globe. They believe in its importance either religiously or for its holistic medicinal value (Shomar, 2012). The holy water is drawn from the well of Zamzam, which is located near Kaaba, Masjid Al-Haram, Mecca, Saudi Arabia. Based on the medicinal and cultural factors, we were curious to study the quality and medicinal benefits of Zamzam water. In this study, we used physicochemical parameters, microbial load study to determine the purity of Zamzam water, and wound healing property to demonstrate the medicinal value.

2. Materials and Methods

2.1. Determination of quality of Zamzam water

Zamzam water was purchased from the King Abdullah Zamzam Project, Mecca, Saudi Arabia. The water samples were analysed immediately after the collection and after the exposed to atmospheric air. The exposure of Zamzam water to air was performed by placing 30 ml of water in individual Petri dishes for two weeks and their bacteriological load was determined by the spread plate technique on Muller Hinton (MH) agar media. The physicochemical characterization study of Zamzam water samples was performed before and after exposure to air. However, the bacteriological study was performed only after the exposure to air.

2.2. Physicochemical characterization of water samples

The physicochemical characterization of Zamzam water sample was analyzed for dissolved oxygen (DO), pH, oxidation-reduction or redox potential (ORP), total dissolved solids (TDS), zeta potential (ZP), electrical conductivity (EC), polydispersity index (PDI), and zeta size. DO is molecular oxygen that is dissolved in water, and was determined using the dissolved oxygen meter, Lutron DO-5509, Taiwan. The instrument was calibrated according to the manufacturer's instructions, and the DO value was expressed in milligrams per liter (mg/L). The pH is the measure of acidity or alkalinity of water-based samples. Both pH and ORP were measured using the Oakton pH 700 Benchtop Meter, USA. The ORP is expressed in millivolt (mV). TDS is the measurement of dissolved inorganic salts and organic matters in drinking water. The TDS value is expressed in parts-per-million (ppm).

ZP is the electrical charge that develops at the interface between a solid surface and its liquid medium. It is a measurement to monitor the water clarification capabilities by measuring the repulsive interaction between the dissolved particles, which migrate toward the electrodes of an opposite electric charge at a specific velocity. The ZP is expressed in millivolts (mV). The electric conductivity was also measured and expressed in milli siemens per centimeter (mS /cm). The PDI is the breadth of the molecular weight distribution, and it measures the deviation from the uniformity of dispersion. Particle size analysis is the measure to understand the size of the particle's composition of water. ZP, PDI, and zeta size analysis were performed by using Zeta nano sizer ns, Malvern Instruments, UK.

2.3. Bacteriological quality in water samples

The atmospheric air exposed Zamzam water sample was screened for bacteriological load. 100 µl of water samples were placed on MH agar plates and were spread thoroughly on the agar surface. After spreading the water samples, the plates were incubated at 37 °C in a bacteriological incubator for 24 hrs. After incubation, the growth of bacteria was expressed as a colony-forming unit per milliliter (CFU/ ML) of water.

2.4. In vivo wound healing property

After screening the physicochemical and microbial load studies, we conducted an experiment to explore potential medicinal properties of Zamzam water through wound healing efficacy *in vivo*. Healthy male Wistar rats weighing 170 – 200 g were obtained from the Central Animal Laboratories, Jazan University, Jazan. They were acclimatized to laboratory conditions and handled in strict compliance with the guidelines of the International standard and Institutional Animal Care and Use committee (IACUC). The study was approved by Institutional Research Review and Ethics Committee (IRREC), Reference number 908/1012/1441. The animal room temperature was maintained at 22 ± 08° C and the humidity was about 56 ± 6%, achieved by exposing with an alternating 12 h light/ dark cycle. The rats were divided into four groups, each group with 3 rats, into cages (54 cm × 29 cm × 15 cm). The experimental groups were as follows:

Group 1: Normal control group

Wounds were not induced, and they did not receive any drug or Zamzam water treatment. All the animals received a standard palette diet and were supplied with fresh water that they could access freely.

Group 2: Disease group

Wounds were created according to the protocol established by Sivakumar et al. (2018). In this process, the experimental rats were anesthetized with an intraperitoneal injection of xylazine hydrochloride (10 mg/kg) and ketamine hydrochloride (25 mg/kg). Then, the hair was removed by using commercially available hair remover on the dorsal surface of the abdominal region. An area of 1 × 1 cm of skin was cleaned with ethanol swab, 10% povidone iodine solution, and a 10 mm diameter section of skin was removed with a sterile surgical blade to create a circular wound. The diseased group did not receive any drug for the treatment of wound. All the animals received a standard palette diet and were supplied with fresh water that they could access freely.

Group 3: Standard drug treatment group

In the standard drug treatment group, wounds were created as described in Group 2. After wounds were created, animals received povidone iodine cream via spreading on the wound area once day for 12 days. All the animals received a standard palette diet and were supplied with fresh water that they could access freely.

Group 4: Zamzam water treatment group

In the Zamzam water treatment group, wounds were created as described in Group 2. After wounds were created, animals received 100 µl of Zamzam water, which was poured on the wound site and spread fully on the wound area once a day for 12 days. All the animals received a standard palette diet and were supplied with Zamzam water that they could access freely.

2.5. Measurement of wound contraction

Wound size was measured by with the help of a transparent ruler on days 0, 2, 4, 6, 8, and 12 for Groups 2, 3, and 4. Wound healing was measured as described by Nagar et al. (2016) by using the following formula (Equation 1)

$$\text{Percentage of wound healing} = \frac{\text{Initial wound area} - \text{wound area on specific day}}{\text{Initial wound area}} \times 100 \quad (1)$$

2.6. Collection of serum

On the 6th day of treatment, blood was collected from all the animals with the tail-vein method, pooled, and stored in individual Flacon blood collecting tubes without an anticoagulant.The blood samples were allowed to clot by placing the tubes in a slanting position and were centrifuged at 1,000–2,000 × g for 10 minutes in a refrigerated centrifuge. The serum was obtained by collecting the supernatant. The serum was kept in a refrigerator at 2 – 8 °C and used for further assays to determine the cytokine levels. The pro-inflammatory cytokines IL-1β, IL-6, and TNF-α levels were estimated by the enzyme linked immunosorbent assay. Apoptosis markers caspase-9 and caspase-3 was determined by a UV/VIS spectrophotometer assay.

2.7. Estimation of pro inflammatory cytokines

Pro-inflammatory cytokines IL-1β, IL-6, and TNF-α were determined by sandwich enzyme linked immune sorbent assay (ELISA) in the serum sample using Abcam kits, USA. The end reaction was determined by the density of color development and measured at 450 nm using an ELISA reader (Biotek ELX 800).

2.8. Determination of apoptosis markers

Apoptosis markers caspase-9 and caspase-3 were determined in the serum sample by using the spectrophotometric technique, according to the protocol described in Abcam kits, USA. The end reaction for caspase-9 and caspase-3 were quantified by pNA light emission, which was measured at 405 nm using an ELISA reader (Biotek ELX 800). The concentration of caspase-9 and caspase-3 were calculated by extrapolating on the standard curve.

3. Results and Discussion

The present research revealed the physicochemical characterization of Zamzam water and wound healing property. The first quality study of Zamzam water was performed by an Egyptian commission to Hijaz in 1935. In 1971 Zamzam water was analysed by the Ministry of Agriculture and its consultants, Riyadh, Saudi Arabia. Later studies were performed by analyzing the Zamzam water chemically as well as physiochemically (Khalid et al., 2014). The present study was designed to analyze various physicochemical parameters including DO, pH, conductivity, TDS, electric potential, ZP, PDI before and after open air exposure (Tables 1-2).

3.1. DO analysis

Earlier reports have shown that the DO level was 5.8 mg/L in freshly collected Zamzam water from the Zamzam well (Abu-Samn, 1982) but, water samples from Dawooddiyah and Masfalah (wells located adjacent to the Zamzam well) did not show any DO level (Graumlich et al., 1986). Contrastingly the present study demonstrated the dissolved oxygen level in Zamzam water was showing

Table 1. Physical characterization of Zamzam water before exposure to open-air.

DO (mg/L)	pH	EC (mS /cm)	TDS ppm	ORP (mV)	ZP (mV)	PDI
3.4	8	0.718	371	-50.6	-8.72	0.267

DO: Dissolved oxygen; pH: Scale used to specify how acidic or basic; EC: Electrical conductivity; TDS: Total dissolved solids; ORP: Redox potential; ZP: Zeta potential; PDI: Polydispersity index.

Table 2. Physical characterization water after exposure to open-air.

DO (mg/L)	pH	EC (mS /cm)	TDS ppm	ORP (mV)
4.6	8.3	2.26	210	-58.7

DO: Dissolved oxygen; pH: Scale used to specify how acidic or basic; EC: Electrical conductivity; TDS: Total dissolved solids; ORP: Redox potential.

3.4 mg/L. An earlier study suggesting that the concentration of DO reduce drastically in packed concentrated orange juices and reported that the oxygen was consumed due to oxidative reactions (WHO, 1996). The concentration of DO was increased when Zamzam water exposed to open-air for two weeks. Furthermore, there is no more published literature regarding the concentration of dissolved oxygen in drinking water before and after the packaging of water.

3.2. pH analysis

pH is the measurement of the potential activity of hydrogen ion (H^+) concentration. pH is an important factor that influences water quality. The pH of water regulates the solubility of chemical constituents, such as phosphorus, nitrogen, carbon, and heavy metals.The pH of Zamzam water was 8, which is a little high than what was found in an earlier report that found a pH of 6.9 in Zamzam water (Al-Gamal, 2008). A similar report published by Shomar in 2012 stated that the pH of sterile water is between 5 and 7 according to United States Pharmacopeia (USP, 2005). Zamzam water was alkaline with a pH of 8 that contains lithium, arsenic, calcium, potassium, nitrites etc. The alkalinity of water from natural resources is determined by the quality of soil and rock through which it passes, including the carbonate, bicarbonate, and hydroxide compounds contained therein. The pH of the Zamzam water samples was increased slightly (they became more alkaline) after exposure to the open air. The hardness of water is attained at the pH level above 8.5. If the Zamzam water processed for further purification through distillation, reverse osmosis and ultrafiltration may lead to pharmaceutical grade water that could comply with Pharmacopeia standards.

3.3. TDS analysis

The TDS is the presence of inorganic salts and/or organic matter in water. In this study, the Zamzam water showed a TDS value of 371 ppm. According to the WHO's TDS report (WHO, 1996) on the quality of water, water with less than 300 ppm is an excellent grade of water. One earlier report showed that Zamzam water contains 1011 ppm (Al-Gamal, 2008). Al-Badaii et al. (2013) reported that the TDS value of Semenyih river water, Selangor, Malaysia ranged from 17.66 to 80 mg/L during the rainy season (WHO, 2017). In the present study, TDS was increased when the water samples were exposed to air, which is probably due to dust particle accumulation. The turbidity of water caused by suspended chemicals and the presence of pathogenic microorganisms can have both water safety and aesthetic implications and can be an effective indicator of hazardous water (Bahteria and Jain, 2016). Total suspended solids imitate the number of sediments suspended in water, which indicates water clarity.

3.4. EC analysis

EC is the conduction ability of water, which is also a measure of the number of dissolved mineral salts or ions in water. The conductivity of water is highly influenced by the geological area through which the water flows. A water sample has less conductivity when it passes through granite bedrocks because of the presence of inert materials. However, a natural perennial stream that passes through clay soils show higher conductivity due to the presence of ionizing materials (Matsiyevska, 2017; WHO, 2017; Al-Badaii et al., 2013; Bahteria and Jain, 2016; Gupta and Paul, 2013; El-Zaiat, 2007). In this study, Zamzam water showed the EC of 0.718 mS /cm, which is equivalent to 718 micro siemens per centimeter.In 2007, El-Zaiat et al. reported that the EC of Zamzam water was 1390 micro siemens. According to European Pharmacopeia standards, the conductivity of water for pharmaceutical use should be < 4.3 µS/cm at 20º C, whereas the conduction of water for pharmaceutical use as per US pharmacopeia should be < 1.3 µS/cm at 25 ºC. The EC of Zamzam water was very high compared to these standards, demonstrating that Zamzam contains many minerals. The conductivity of Zamzam water was further increased when exposed to open air.

3.5. ORP analysis

ORP is the measure of electric potential energy per unit of charge. ORP is an important parameter in analyzing the quality of drinking water (Goncharuk et al., 2010).Redox reaction indicates the transfer of electrons between atoms and the availability of electrons is directly proportional to the oxidation and reduction potential of water. The ORP value of tap water, packaged drinking water, and rainwater generally have a positive ORP between 200-400 mV (Kim et al., 2000). In this study, the ORP of Zamzam water was -50.6 mV before open air exposure. Table 2 shows that the ORP values of Zamzam water increased to -58.7 mV, probably redox potential varied with type of microbial contamination of water (Okouchi et al., 2002). Certain studies have demonstrated that the ORP value of healthy human biological fluids, such as oral fluid and mother's milk, have an ORP of -7 (Nobbmann et al., 2010; Rael et al., 2009; USP, 2005; Okouchi et al., 2002). Therefore, a negative ORP value helps in the maintenance of healthy body physiology. The WHO recommends that the ORP of drinking water should not be more than 60 mV (Kim et al., 2000). Therefore, Zamzam water can be used as drinking water. On the other hand, the redox potential was fluctuated tremendously indicating the contamination of Zamzam water sample after open-air exposure which was reflected in microbial studies.

3.6. ZP analysis

ZP analysis of water provides measurable value to help monitor optimal water clarification capabilities. In this study, the ZP of Zamzam water (Figure 1A) was -8.72 mV before exposure to open air. An earlier study reported that the ZP ranged between -10 to +5 mV indicating minimal contaminants present in water (Sun et al., 2018). The ZP increased to above -14 mV (Figure 1B) when the Zamzam water was exposed to open air, indicating contamination. In ZP analysis, the electrophoretic mobility is determined by particles in dispersion or molecules in solution where frequency shift of the light scattered by the charged particles or organisms that are stirring in an external electric field. The electrical current of Zamzam water was determined directly from electron donors of organic matters of bacterial colonization, which led to the production of electrons. This mobility of the Zamzam water was reflected in Table 3 under experimental conditions.The PDI value of Zamzam water before air exposure was 0.267, which showed the uniformity of particle distribution. However, after exposure to the open air the PDI value of Zamzam water was increased to 0.623 (78.9%).

3.7. Bacteriological quality analysis

The microbial contamination of water samples determines the quality of water for drinking purposes and pharmaceutical applications. To a greater or lesser extent, natural waters come into touch with the surrounding air. If the pure water is allowed to come into contact with air, its purity will swiftly deteriorate. The present study demonstrated that the quality of Zamzam water sample before and after exposure to open air. This was directly linked to the microbial colonization that means microbial contamination. The bacterial contamination of water can be easily accessed by ORP values since the redox reaction depends on the biodegradation process. After exposure of Zamzam water to the open air for two weeks, minimal bacterial growth was occurred, which was reflected in ORP values (Table 3).

The ZP value gives an understanding of the electrophoretic behavior of bacteria in the aqueous system, which can directly indicate bacterial colonization. Therefore, the measurement of ZP indicates bacterial growth. The present study demonstrated that the ZP value of Zamzam water was -8 mV before open-air exposure, which increased to -14.6 mV after open-air exposure. The bacterial growth in Zamzam water was observed as 1×10^{-3} CFU/mL, proving the purity of water. The CFU value in Zamzam water was less probably due to the presence of a limited amount of arsenic and lithium (Shomar, 2012). According to the United States Pharmacopoeia (USP, 2005) water for injection should be sterile and prevent microbial contamination thereafter. Based on the standards of USP NF 24, Zamzam water is bacteriostatic and can be considered as pharmaceutical grade water. However, the presence of trace levels of arsenic can be considered as medicinally significant to heal many ailments.

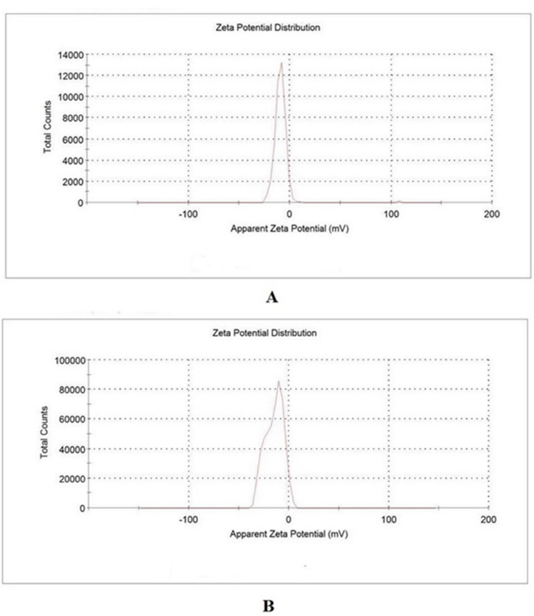

Figure 1. Zeta potential analysis of Zamzam water. (A) Before exposure to open-air; (B) After exposure to open-air.

3.8. Wound healing properties

Wound healing is comprised of various phases involving overlapping mechanisms that are comprised of many types of cells, signaling molecules, and extracellular matrices. The various phases are inflammation, angiogenesis, epithelization progression, matrix deposition, and remodeling (DeClue and Shornick, 2015; Diegelmann and Evans, 2004; Rippon et al., 2016; Atkin and Ousey, 2016). During the course of treatment, the effect of Zamzam water on wound healing was observed from the 3rd day onwards. By the 12th day of treatment, almost 96% of the wound healing process was complete in Group 4 (Table 4). Rippon et al., 2016 demonstrated a counter-intuitive model about healing wounds using the "hyper-hydration" of tissue, in which tissue is hydrated beyond the normally acceptable therapeutic level. Another study has reported that hydrotherapy provides an optimal healing environment through autolytic debridement and absorption of wound fluid, which enables re-epithelialization to occur in an unrestricted fashion (Pinto et al., 2018; Lin et al., 2017). However, in this work, Zamzam water showed potent wound healing properties, which may be due to the presence of a trace level of many minerals. Pinto et al. (2018) reported that arsenic contaminants in water inhibited wound healing process because of decreased the rate of cellular migration. Zamzam water has trace level of arsenic and enriched with other minerals, including zinc, which

Table 3. Zeta analysis and bacterial load after exposure to open-air.

ZP (mV)	PDI	% PDI	ZS (d.nm)	MOB (mS/cm)	BL (CFU/ML)
-14.6	0.623	78.9	1589	-1.441	1×10^{-3}

ZP: Zeta potential; PDI: Polydispersity index; ZS: Zeta size; MOB: Mobility of particles in a colloidal system; BL: Bacterial load.

Table 4. Comparative percentage wound healing of various treatment groups.

Day	Group 2	Group 3	Group 4
3	17.92 ± 0.27	24 ± 0.49**	30.53 ± 1.02**,##
5	40.7 ± 0.55	44.84 ± 1.24**	44.5 ± 1.3**,ns
8	57.2 ± 0.61	62.2 ± 0.94**	65.3 ± 0.2**,##
12	72.52 ± 1.11	93.3 ± 1.08**	96.26 ± 0.87**,#

Values are the mean ± SD, n=3; data were analyzed by Dunnett test (Sample vs Control), post hoc test. **Extremely significant when compared to Group 2 at $p < 0.01$ significant level. ##Extremely significant when compared to Group 3 at $p < 0.01$ significant level. #Significant when compared to Group 3 at $p < 0.05$ level. ns Nonsignificant when compared to Group 3. Group 2: Disease control (wound without treatment); Group 3: Standard control (treatment with povidone iodine cream); Group 4: Zamzam water treatment group.

plays an important role in protein and collagen synthesis and in angiogenesis process that leads to wound healing (Banerjee et al., 2015; Dangleben et al., 2013). Therefore, the present study showed a faster healing process with Zamzam water than povidone cream.

Cytokines are groups of lower molecular protein functional intracellular mediators that regulate immune cell homeostasis and harmonize immune responses. The results of this study on cytokine networks expressed as individual figures which is self-exemplary. Cytokine IL-1β is also known as lymphocyte activating factor, which is an important mediator of inflammatory responses (Lopez-Castejon and Brough, 2011; Tanaka et al., 2014).

Figure 2 depicts the IL-1β levels of the four treatment groups. The study demonstrates that after creating wounds in Group 2, the animals' IL-1β levels were increased by 44.26%, which is significant (p < 0.01) compared to Group 1. In the treatment Group 3 with povidone iodine cream decreased the IL-1β levels level to 21.5% have been reduced after the treatment in group 3. However, the wound treatment with Zamzam water was observed 38% reduction in group 4. The values were almost equivalent to the normal value of control group representing with 2% elevation when compared to Group 1. Studies suggesting that IL-1β is highly correlated with human surgical wound and plays a key role in human wound paradigms (Lopez-Castejon and Brough, 2011; Hu et al., 2010; McFarland-Mancini et al., 2010). The IL-1β expression and increased secretion due to induction of pro-IL-1β expression, an initial step encountered pathogen associated molecular patterns or may be due to danger associated molecular pattern. Danger associated molecular patterns are endogenous molecules released from necrotic dead cells (Takeuchi and Akira, 2010). On comparing the results between Group 3 and Group 4, treatment with Zamzam water exhibited a better effect in controlling IL-1 β. This might be due to the presence of arsenic in Zamzam water, as earlier studies suggest that arsenic was associated with the down regulation of both Th_1 and Th_2 cytokines (Ambrosch et al., 2008; Yang et al., 2016).

Interleukin–6 (IL-6) is a pleotropic cytokine with a key role in the amalgamated immune response network against various infections (Lin et al., 2003). IL-6 plays a pathological effect on tissue injuries, chronic inflammation, autoimmunity, and the level of bacterial infection of wounds (Rose-John et al., 2017; Lin et al., 2003; Sun et al., 2018). However, studies also suggest that IL-6 modulates the

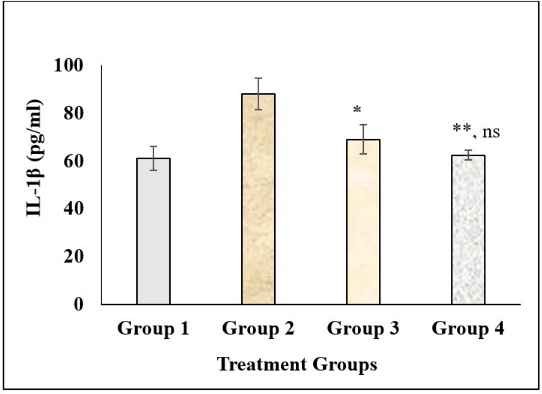

Figure 2. Serum IL-1β level of treatment groups. *Significantly lesser at $p < 0.05$ on comparing with Group 2; **Highly significant lesser at $p < 0.01$ on comparing with group 2; ns: nonsignificant when compared to Group 3. Group 1: Normal control; Group 2: Disease control (wound without treatment); Group 3: Standard control (treatment with povidone-iodine cream); Group 4: Zamzam water treatment group.

immune response, which has important roles in wound healing, probably by regulating leukocyte infiltration, angiogenesis, and collagen accumulation. The IL-6 levels of the four treatment groups are presented in Figure 3. IL-6 levels were increased 117% in Group 2 (p <0.001 level), showing a high significance. In the Groups 3 and 4 the levels were decreased. Group 3 showed a reduction of 33.46% in IL-6 levels. However, IL-6 levels in Group 4 reduced 53.93%. There was no significant difference between Groups 3 and 4 (p < 0.05 level). In 2018, Sun et al. (2018) reported that filtered water exhibited high concentration of hydrogen and played a major role in suppressing IL-6 and TNF-α levels due to the decreased expression of mRNA.

TNF-α is a cytokine chemically low molecular weight protein derived mainly from macrophages, which is involved in inflammatory mechanism (Ashcroft et al., 2012). TNF-α is the predominant pro-inflammatory cytokine involved in inflammatory reactions. The TNF-α levels of the four treatment groups are presented in Figure 4. In this study, the TNF-α increase in Group 2 had a high significance (p <0.001). The percent release of TNF-α in Group-2 was 141.56%. The level of TNF-α was decreased after the treatment with standard povidone iodine cream

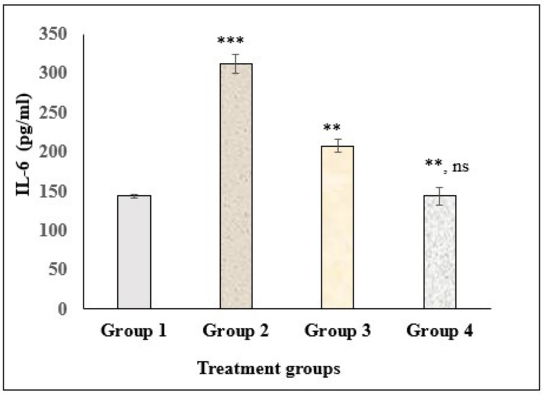

Figure 3. Serum IL-6 level of treatment groups. ***Extremely high significant at $p < 0.001$; **Extremely significant at $p < 0.01$ on comparing with Group 2; ns: nonsignificant when compared to Group 3. Group 1: Normal control; Group 2: Disease control (wound without treatment); Group 3: Standard control (treatment with povidone iodine cream); Group 4: Zamzam water treatment group.

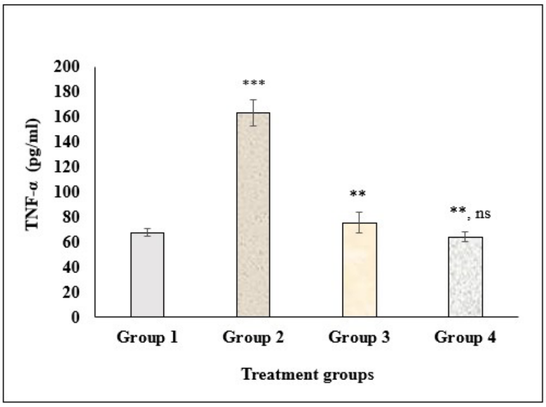

Figure 4. TNF-α level of treatment groups. ***Extremely high significant at $p < 0.001$; **Extremely significant at $p < 0.01$ on comparing with Group 2; ns: nonsignificant when compared to Group 3. Group 1: Normal control; Group 2: Disease control (wound without treatment); Group 3: Standard control (treatment with povidone iodine cream); Group 4: Zamzam water treatment group.

and with Zamzam water. The percent reduction of TNF-α in Group 3 was 53.76%, which was significant (p < 0.01). However, the level of TNF-α was reduced by 60.6%, which was significant (p < 0.01). The efficacy of Zamzam was better than the povidone iodine cream, but it was non-significant (p < 0.05). Furthermore, the level of TNF-α in Group 4 was about 4.9% high when compared to normal Group 1.Earlier reports showed that the TNF-α quickly and drastically releases and induces inflammation at the site wound (Ritsu et al., 2017). However, the wound healing mechanism is a complicated, since TNF-α induces vessel growth, a key step in the wound healing processes (Frank et al., 2003).

Yang et al. (2016) reported that hydrogen water treatment reduces the IL-6 and TNF-α levels in the neonatal hypoxic-ischemic encephalopathic condition. Earlier reports have suggested that oral zinc supplements reduce the TNF-α in mice with *E. coli*-LPS-induced diarrhea (Yusuf et al., 2019). Khalid et al. (2014) reported that the mineral composition in Zamzam water serves to benefit and regulate health and that Zamzam water is effective against cancer. Programmed cell death is termed as apoptosis process due to the cascade system that comprises various characteristic cell changes via cell shrinkage, nuclear fragmentation, chromatin condensation, and chromosomal DNA fragmentation. Caspase-9 is a protease enzyme that plays a key role in mitochondrial damage which is due to various environmental factors such as stimuli, chemotherapies, radiation, and stress (Li et al., 2017). The levels caspase 9 of the four treatment groups are presented in Figure 5. In the present study the caspase-9 was increased exponentially in Group 2 (p <0.001). In the Group 3 and group the reduced level was observed on the 8th day. In Group 3, caspase-9 levels were reduced about 29.87% (p < 0.01) whereas in Group 4, they were reduced by 38.40%. Caspase-3 is a death protease an important executioner caspase and is activated by caspase-8, 9, or 10. Caspase-3 involved in catalyzing and

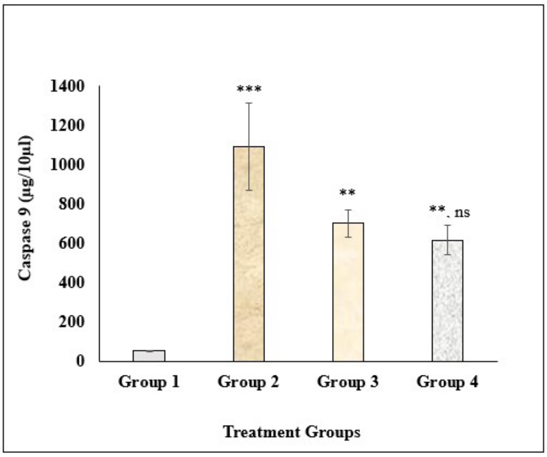

Figure 5. Caspase 9 levels of treatment groups. ***Extremely high significant at p <0.001 when compared to Group 1; **Significantly lesser at $p < 0.01$ on comparing with Group 2; ns: nonsignificant on comparing with group 3 at $p < 0.05$. Group 1: Normal control; Group 2: Disease control (wound without treatment); Group 3: Standard control (treatment with povidone-iodine cream); Group 4: Zamzam water treatment group.

in the cleavage of many key cellular proteins (Enari et al., 1998; Brentnall et al., 2013).

The levels 3 of the four treatment groups are depicted in Figure 6. In this study, the caspase-3 level was increased exponentially in Group 2 like caspase-9 at p <0.001 significant level. In Groups 3 and 4, the level of caspase-3 was reduced proportionately. In Group 3 the level of caspase-3 was reduced 28.4% (p <0.01). However, in Group 4 it was reduced 36% (p <0.01). Caspase-9 and caspase-3 can stimulate the intrinsic cell death through increased ROS production, which leads to cell damage (Brentnall et al., 2013). However, both the standard

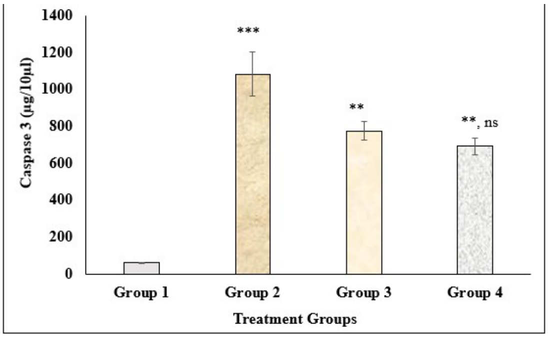

Figure 6. Caspase 3 levels of treatment groups. ***Extremely high significant at p <0.001 when compared to Group 1; **Highly significant lesser at p < 0.01 on comparing with Group 2; ns: nonsignificant when compared to group 3 at p < 0.05. Group 1: Normal control; Group 2: Disease control (wound without treatment); Group 3: Standard control (treatment with povidone-iodine cream); Group 4: Zamzam water treatment group.

drug and Zamzam water treatment protected from cell death, which was reflected in wound healing through tissue regeneration, which was observed on 12th day of treatment (Table 4). Both caspase-9 and caspase-3 were not significantly reduced much compared to cytokine networks. Therefore, the understanding of wound healing is a complex phenomenon by altering the inflammatory markers level, imitating angiogenesis and remodeling is highly challenging (Takeuchi and Akira, 2010). Figure 7 demonstrates the stepwise *in vivo* wound healing property of Zamzam water and povidone iodine cream as self-exemplary. It is obvious that Zamzam water was having wound healing property when compared to povidone iodine cream. In the Zamzam water treatment group the animals developed hairs and cover total wound on the 12th day obviously the effect was better than povidone iodine cream. The present study proved that Zamzam water showed a better immunomodulation effect and exhibited excellent wound healing property.

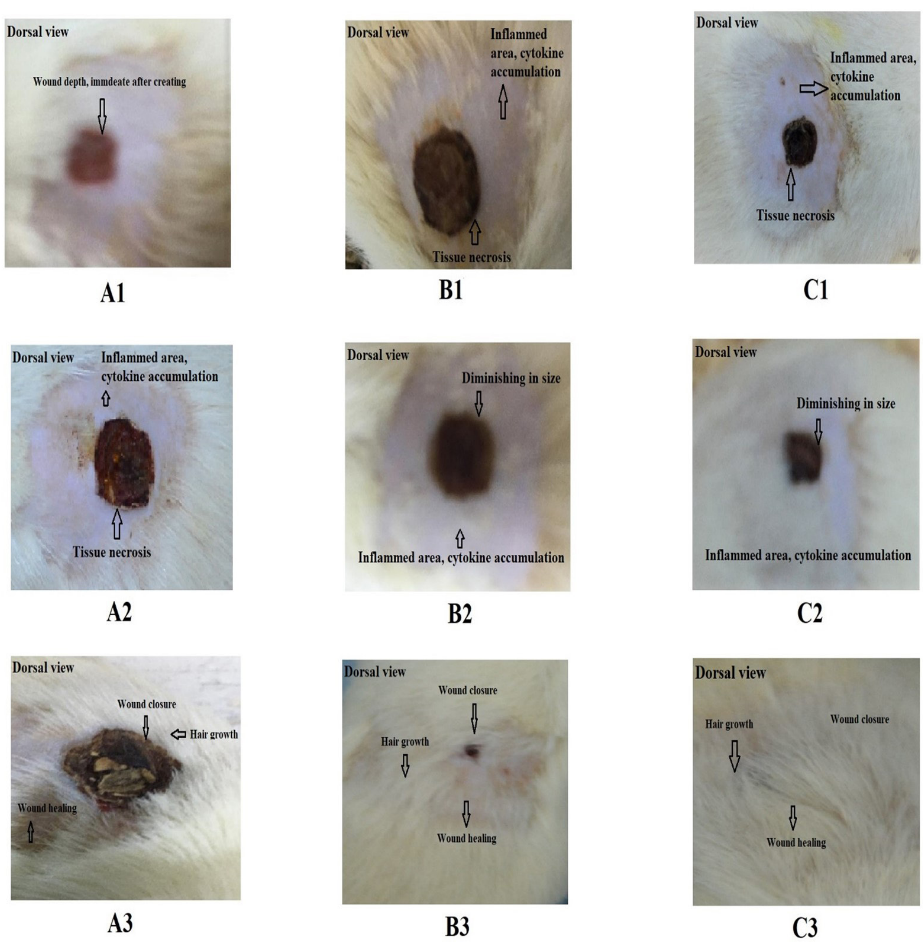

Figure 7. A comparative wound healing study. A1: Control animals, dorsal view of wound soon after creating on the 1st day; B1: Dorsal view of the wound after treating with povidone-iodine cream on 3rd day; C1: Dorsal view of the wound after treating with Zamzam water on 3rd day; A2: Control animals, dorsal view of the wound on 6th day; B2: Dorsal view of the wound after treating with povidone-iodine cream on 6th day; C2: Dorsal view of the wound after treating with Zamzam water on 6th day; A3: Control animals, dorsal view of the wound on 12th day; B3: Dorsal view of the wound after treating with povidone-iodine cream on 12th day; C3: dorsal view of the wound after treating with Zamzam water on 12th day.

4. Conclusion

Despite the controversial report on Zamzam water, the present study showed that the Zamzam water can be used as drinking water since it has unique physicochemical parameters that comply with the quality of good drinking water as reported in the manual of WHO. Furthermore, Zamzam water can be used as pharmaceutical grade water for various drug formulations since it has bacteriostatic quality. However, the parameters such as pH were little high, and conductivity was higher to comply with Pharmacopoeia standards since Zamzam water is highly rich in minerals. In the in vivo wound healing model, both the standard drug and Zamzam water showed a good spectrum of activity. However, the level of efficacy was higher in Zamzam water when compared to standard drug treatment. The study proved that Zamzam water has medicinal properties. Further studies should be conducted for the pharmaceutical characterization of Zamzam water to ascertain the quality of various drug formulations and their pharmaceutical significance.

References

ABU-SAMN, R.H.A., 1982. Scientific overview on sacred water of Zamzam well: some qualitative chemical and biological analysis. *Pakistan Journal of Scientific Research*, vol. 34, no. 3-4, pp. 7-12.

AL-BADAII, F., SHUHAIMI-OTHMAN, M. and GASIM, M.B., 2013. Water quality assessment of the Semenyih river, Selangor, Malaysia. *Journal of Chemistry*, vol. 2013, p. 871056. http://dx.doi.org/10.1155/2013/871056.

AL-GAMAL, S.A., 2008. Remarks on the isotopic composition of holy water of Zamzam, Mecca area. *World Environment Magazine*, pp. 19-22.

AMBROSCH, A., LOBMANN, R., POTT, A. and PREISSLER, J., 2008. Interleukin-6 concentrations in wound fluids rather than serological markers are useful in assessing bacterial triggers of ulcer inflammation. *International Wound Journal*, vol. 5, no. 1, pp. 99-106. http://dx.doi.org/10.1111/j.1742-481X.2007.00347.x. PMid:18179556.

ASHCROFT, G.S., JEONG, M.J., ASHWORTH, J.J., HARDMAN, M., JIN, W., MOUTSOPOULOS, N., WILD, T., MCCARTNEY-FRANCIS, N., SIM, D., MCGRADY, G., SONG, X.Y. and WAHL, S.M., 2012. Tumor necrosis factor-alpha (TNF-α) is a therapeutic target for impaired cutaneous wound healing. *Wound Repair and Regeneration*, vol. 20, no. 1, pp. 38-49. http://dx.doi.org/10.1111/j.1524-475X.2011.00748.x. PMid:22151742.

ATKIN, L. and OUSEY, K., 2016. Wound bed preparation: a novel approach using hydrotherapy. *British Journal of Community Nursing*, vol. 21, suppl. 12, pp. S23-S28. http://dx.doi.org/10.12968/bjcn.2016.21.Sup12.S23. PMid:27922792.

BAHTERIA, R. and JAIN, D., 2016. Water quality assessment of lake water: a review. *Sustainable Water Resources Management*, vol. 2, no. 2, pp. 161-173. http://dx.doi.org/10.1007/s40899-015-0014-7.

BALASUBRAMANIAN, N., SIVASUBRAMANIAN, P., SOUNDRANAYAGAM, J., CHANDRASEKAR, N. and GOWTHAM, B., 2015. Groundwater classification and its suitability in Kadaladi, Ramanathapuram, India using GIS techniques. *Environmental Earth Sciences*, vol. 74, no. 4, pp. 3263-3285. http://dx.doi.org/10.1007/s12665-015-4394-7.

BANERJEE, S., MITRA, T., PUROHIT, G.K., MOHANTY, S. and MOHANTY, B.P., 2015. Immunomodulatory effect of arsenic on cytokine and HSP gene expression in Labeo rohita fingerlings. *Fish & Shellfish Immunology*, vol. 44, no. 1, pp. 43-49. http://dx.doi.org/10.1016/j.fsi.2015.01.029. PMid:25652292.

BRENTNALL, M., RODRIGUEZ-MENOCAL, L., GUEVARA, R.L., CEPERO, E. and BOISE, L.H., 2013. Caspase-9, caspase-3 and caspase-7 have distinct roles during intrinsic apoptosis. *BMC Cell Biology*, vol. 14, no. 1, p. 32. http://dx.doi.org/10.1186/1471-2121-14-32. PMid:23834359.

CAZIER, J.B. and GEKAS, V., 2001. Water activity and its prediction: a review. *International Journal of Food Properties*, vol. 4, no. 1, pp. 35-43. http://dx.doi.org/10.1081/JFP-100002187.

DANGLEBEN, N.L., SKIBOLA, C.F. and SMITH, M.T., 2013. Arsenic immunotoxicity: a review. *Environmental Health*, vol. 12, no. 1, p. 73. http://dx.doi.org/10.1186/1476-069X-12-73. PMid:24004508.

DECLUE, C.E. and SHORNICK, L.P., 2015. The cytokine milieu of diabetic wounds. *Diabetes Management*, vol. 5, no. 6, pp. 525-537. http://dx.doi.org/10.2217/dmt.15.44.

DIEGELMANN, R.F. and EVANS, M.C., 2004. Wound healing: an overview of acute, fibrotic and delayed healing. *Frontiers in Bioscience*, vol. 9, no. 1-3, pp. 283-289. http://dx.doi.org/10.2741/1184. PMid:14766366.

EL-ZAIAT, S.Y., 2007. Inherent optical properties of Zamzam water in the visible spectrum: dispersion analysis. *Arabian Journal for Science and Engineering*, vol. 32, no. 2A, pp. 171-180.

ENARI, M., SAKAHIRA, H., YOKOYAMA, H., OKAWA, K., IWAMATSU, A. and NAGATA, S., 1998. A caspase-activated DNase that degrades DNA during apoptosis, and its inhibitor ICAD. *Nature*, vol. 391, no. 6662, pp. 43-50. http://dx.doi.org/10.1038/34112. PMid:9422506.

FRANK, J., BORN, K., BARKER, J.H. and MARZI, I., 2003. *In vivo* effect of tumor necrosis factor alpha on wound angiogenesis and epithelialization. *European Journal of Trauma*, vol. 29, no. 4, pp. 208-219. http://dx.doi.org/10.1007/s00068-003-1284-6.

GONCHARUK, V.V., BAGRII, V.A., MEL'NIK, L.A., CHEBOTAREVA, R.D. and BASHTAN, S.Y., 2010. The use of redox potential in water treatment processes. *Journal of Water Chemistry and Technology*, vol. 32, no. 1, pp. 1-9. http://dx.doi.org/10.3103/S1063455X10010017.

GRAUMLICH, T.R., MARCY, J.E. and ADAMS, J.P., 1986. Aseptically packaged orange juice and concentrate: a review of the influence of processing and packaging conditions on quality. *Journal of Agricultural and Food Chemistry*, vol. 34, no. 3, pp. 402-405. http://dx.doi.org/10.1021/jf00069a004.

GUPTA, T. and PAUL, M., 2013. The seasonal variation in ionic composition of pond water of Lumding, Assam, India. *Current World Environment*, vol. 8, no. 1, pp. 127-131. http://dx.doi.org/10.12944/CWE.8.1.12.

HELENA, B., PARDO, R., VEGA, M., BARRADO, E. and FERNANDEZ, J., 2000. Temporal evolution of groundwater composition in an alluvial aquifer (Pisuerga River, Spain) by principal component analysis. *Water Research*, vol. 34, no. 3, pp. 807-816. http://dx.doi.org/10.1016/S0043-1354(99)00225-0.

HU, Y., LIANG, D., LI, X., LIU, H.H., ZHANG, X., ZHENG, M., DILL, D., SHI, X., QIAO, Y., YEOMANS, D., CARVALHO, B., ANGST, M.S., CLARK, J.D. and PELTZ, G., 2010. The role of interleukin-1 in wound biology. Part II: *in vivo* and human translational studies. *Anesthesia and Analgesia*, vol. 111, no. 6, pp. 1534-1542. http://dx.doi.org/10.1213/ANE.0b013e3181f691eb. PMid:20889944.

KHALID, N., AHMAD, A., KHALID, S., AHMED, A. and IRFAN, M., 2014. Mineral composition and health functionality of Zamzam water: a review. *International Journal of Food Properties*, vol. 17, no. 3, pp. 661-677. http://dx.doi.org/10.1080/10942912.2012.660721.

KIM, C., HUNG, Y.-C. and BRACKETT, R.E., 2000. Roles of oxidation–reduction potential in electrolyzed oxidizing and chemically modified water for the inactivation of food-related pathogens. *Journal of Food Protection*, vol. 63, no. 1, pp. 19-24. http://dx.doi.org/10.4315/0362-028X-63.1.19. PMid:10643764.

LI, P., ZHOU, L., ZHAO, T., LIU, X., ZHANG, P., LIU, Y., ZHENG, X. and LI, Q., 2017. Caspase-9: structure, mechanisms and clinical application. *Oncotarget*, vol. 8, no. 14, pp. 23996-24008. http://dx.doi.org/10.18632/oncotarget.15098. PMid:28177918.

LIN, P.H., SERMERSHEIM, M., LI, H., LEE, P.H.U., STEINBERG, S.M. and MA, J., 2017. Zinc in wound healing modulation. *Nutrients*, vol. 10, no. 1, p. 16. http://dx.doi.org/10.3390/nu10010016. PMid:29295546.

LIN, Z.Q., KONDO, T., ISHIDA, Y., TAKAYASU, T. and MUKAIDA, N., 2003. Essential involvement of IL-6 in the skin wound-healing process as evidenced by delayed wound healing in IL-6-deficient mice. *Journal of Leukocyte Biology*, vol. 73, no. 6, pp. 713-721. http://dx.doi.org/10.1189/jlb.0802397. PMid:12773503.

LOPEZ-CASTEJON, G. and BROUGH, D., 2011. Understanding the mechanism of IL-1β secretion. *Cytokine & Growth Factor Reviews*, vol. 22, no. 4, pp. 189-195. http://dx.doi.org/10.1016/j.cytogfr.2011.10.001. PMid:22019906.

MATSIYEVSKA, O., 2017. Influence of redox potential of different water quality on the human blood. *Environmental Technology*, vol. 2017, pp. 34-38.

MCFARLAND-MANCINI, M.M., FUNK, H.M., PALUCH, A.M., ZHOU, M., GIRIDHAR, P.V., MERCER, C.A., KOZMA, S.C. and DREW, A.F., 2010. Differences in wound healing in mice with deficiency of IL-6 versus IL-6 Receptor. *Journal of Immunology*, vol. 184, no. 12, pp. 7219-7228. http://dx.doi.org/10.4049/jimmunol.0901929. PMid:20483735.

NAGAR, H.K., SRIVASTAVA, A.K., SRIVASTAVA, R., KURMI, M.L., CHANDEL, H.S. and RANAWAT, M.S., 2016. Pharmacological investigation of the wound healing activity of Cestrum nocturnum (L.) ointment in Wistar Albino Rats. *Journal of Pharmaceutics*, vol. 2016, p. 9249040. PMid:27018126.

NOBBMANN, U., MORFESIS, A., BILLICA, J. and GERTIG, K., 2010. The role of zeta potential in the optimization of water treatment. *NSTI-Nanotech*, vol., 3, pp. 605-607.

OKOUCHI, S., SUZUKI, M., SUGANO, K., KAGAMIMORI, S. and IKEDA, S., 2002. Water desirable for the human body in terms of oxidation-reduction potential (Orp) to pH relationship. *Journal of Food Science*, vol. 67, no. 5, pp. 1594-1598. http://dx.doi.org/10.1111/j.1365-2621.2002.tb08689.x.

PINTO, B.I., LUJAN, O.R., RAMOS, S.A., PROPPER, C.R. and KELLAR, R.S., 2018. Estrogen mitigates the negative effects of arsenic contamination in an *in vitro* wound model. *Applied In Vitro Toxicology*, vol. 4, no. 1, pp. 24-29. http://dx.doi.org/10.1089/aivt.2017.0020. PMid:30956995.

RAEL, L.T., BAR-OR, R., SALOTTOLO, K., MAINS, C.W., SLONE, D.S., OFFNER, P.J. and BAR-OR, D., 2009. Injury severity and serum amyloid a correlate with plasma oxidation-reduction potential in multi-trauma patients: a retrospective analysis. *Scandinavian Journal of Trauma, Resuscitation and Emergency Medicine*, vol. 17, p. 57. PMid:19925664.

RIPPON, M.G., OUSEY, K. and CUTTING, K.F., 2016. Wound healing and hyper-hydration: a counterintuitive model. *Journal of Wound Care*, vol. 25, no. 2, pp. 68-75. http://dx.doi.org/10.12968/jowc.2016.25.2.68. PMid:26878298.

RITSU, M., KAWAKAMI, K., KANNO, E., TANNO, H., ISHII, K., IMAI, Y., MARUYAMA, R. and TACHI, M., 2017. Critical role of tumor necrosis factor-α in the early process of wound healing in skin. *The Journal of Dermatologic Surgery*, vol. 21, no. 1, pp. 14-19. http://dx.doi.org/10.1016/j.jdds.2016.09.001.

ROSE-JOHN, S., WINTHROP, K. and CALABRESE, L., 2017. The role of IL-6 in host defence against infections: immunobiology and clinical implications. *Nature Reviews. Rheumatology*, vol. 13, no. 7, pp. 399-409. http://dx.doi.org/10.1038/nrrheum.2017.83. PMid:28615731.

SHOMAR, B., 2012. Zamzam water: concentration of trace elements and other characteristics. *Chemosphere*, vol. 86, no. 6, pp. 600-605. http://dx.doi.org/10.1016/j.chemosphere.2011.10.025. PMid:22138338.

SIVAKUMAR, S.M., MOHAMMAD, F.A., HAFIZ, A.M., AAMENA, J., SYEDA, S., RAHIMULLAH, S., REMESH, M. and SOLIMAN, F., 2018. Therapeutic potential of oleic acid nanovesicles prepared from petroleum ether extract of *Sargassum binderi* in streptozotocin–induced diabetic wound in Wistar rats. *Tropical Journal of Pharmaceutical Research*, vol. 17, no. 11, pp. 2123-2128.

SUBRAMANI, T., ELANGO, L. and DAMODARASAMY, S.R., 2005. Groundwater quality and its suitability for drinking and agricultural use in Chithar River Basin, Tamil Nadu, India. *Environmental Geology*, vol. 47, no. 8, pp. 1099-1110. http://dx.doi.org/10.1007/s00254-005-1243-0.

SUN, Q., XIN, F., WEN, X., LU, C., CHEN, R. and RUAN, G., 2018. Protective effects of different kinds of filtered water on hypertensive mouse by suppressing oxidative stress and inflammation. *Oxidative Medicine and Cellular Longevity*, vol. 2018, p. 2917387. http://dx.doi.org/10.1155/2018/2917387. PMid:30622665.

TAKEUCHI, O. and AKIRA, S., 2010. Pattern recognition receptors and inflammation. *Cell*, vol. 140, no. 6, pp. 805-820. http://dx.doi.org/10.1016/j.cell.2010.01.022. PMid:20303872.

TANAKA, T., NARAZAKI, M. and KISHIMOTO, T., 2014. IL-6 in inflammation, immunity, and disease. *Cold Spring Harbor Perspectives in Biology*, vol. 6, no. 10, p. a016295. http://dx.doi.org/10.1101/cshperspect.a016295. PMid:25190079.

THE UNITED STATES PHARMACOPEIAL CONVENTION – USP [online], 2005 [viewed 3 August 2022]. Available from: https://www.usp.org/

VIDYASAGAR, D., 2007. Global minute: water and health–walking for water and water wars. *Journal of Perinatology*, vol. 27, no. 1, pp. 56-58. http://dx.doi.org/10.1038/sj.jp.7211629. PMid:17180131.

WORLD HEALTH ORGANIZATION – WHO, 2017. *Guidelines for drinking-water quality*. 4th ed. Geneva: WHO.

WORLD HEALTH ORGANIZATION – WHO. INTERNATIONAL PROGRAMME ON CHEMICAL SAFETY, 1996. *Guidelines for drinking-water quality*. 2nd ed. Geneva: WHO, vol. 2. Health criteria and other supporting information.

YANG, L., LI, D. and CHEN, S., 2016. Hydrogen water reduces NSE, IL-6, and TNF-α levels in hypoxic-ischemic encephalopathy. *Open Medicine*, vol. 11, no. 1, pp. 399-406. http://dx.doi.org/10.1515/med-2016-0072. PMid:28352827.

YUSUF, S., SOENARTO, Y., JUFFRIE, M. and LESTARIANA, W., 2019. The effect of zinc supplementation on pro-inflammatory cytokines (TNF-α, IL-1 and IL-6) in mice with *Escherichia coli* LPS-induced diarrhea. *Iranian Journal of Microbiology*, vol. 11, no. 5, pp. 412-418. http://dx.doi.org/10.18502/ijm.v11i5.1960. PMid:32148672.

ZHU, B. and YANG, X., 2007. The ion chemistry of surface and ground waters in the Taklimakan desert of Tarim Basin, western China. *Chinese Science Bulletin*, vol. 52, no. 15, pp. 2123-2129. http://dx.doi.org/10.1007/s11434-007-0298-6.

ZHU, B., 2016. Natural water quality and its suitability for drinking and irrigation purposes in the Jungar Basin, Central Asia. *Journal of Civil Environmental Engineering*, vol. 6, no. 3, pp. 1-5. http://dx.doi.org/10.4172/2165-784X.1000232.

ZHU, B., YANG, X., RIOUAL, P., QIN, X., LIU, Z., XIONG, H. and YU, J., 2011. Hydrogeochemistry of three watersheds (the Erlqis, Zhungarer and Yili) in northern Xinjiang, NW China. *Applied Geochemistry*, vol. 26, no. 8, pp. 1535-1548. http://dx.doi.org/10.1016/j.apgeochem.2011.06.018.

ZHU, B., YU, J., QIN, X., RIOUAL, P. and XIONG, H., 2012. Climatic and geological factors contributing to the natural water chemistry in an arid environment from watersheds in northern Xinjiang, China. *Geomorphology*, vol. 153-154, pp. 102-114. http://dx.doi.org/10.1016/j.geomorph.2012.02.014.