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Zinc in Alkaline Water (Zamzam) Ameliorates Doxorubicin induced Cardiac Remodeling

Mohammed A. Almujtaba[1], Turky Omar Asar[2], Salma Naqvi[3], Vikas Kumar[4], Fahad A. Al-Abbasi[1], Abdulbasit I. Al-Sieni[1] and Firoz Anwar[1]*

[1]*Department of Biochemistry, Faculty of Sciences, King Abdulaziz University, Jeddah, Kingdom of Saudi Arabia.*
[2]*Department of Biology, College of Science and Arts, University of Jeddah, Jeddah, Kingdom of Saudi Arabia*
[3]*Department of Biomedical Sciences, Gulf Medical University, Ajman, United Arab Emirates*
[4]*Natural Product Discovery Laboratory, Department of Pharmaceutical Sciences, Shalom Institute of Health and Allied Sciences. SHUATS, Naini, Prayagraj, India*

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Zamzam, Doxorubicin, Cardiac remodeling, Alkaline pH, Zinc, Renin

ABSTRACT

The holy Zamzam water is believed to be beneficial in curing several diseases. Studies report that its alkaline nature renders it a therapeutic potential. We aimed to determine the effect of Zamzam water in doxorubicin induced cardiac remodeling in rats. Male Wistar albino rats were divided into 4 groups (n=6) for 28 days of experimental protocol. Group-1 served as normal control (NC), group 2 as doxorubicin control (DC), group 3 as doxorubicin+ Zamzam treatment (DZ) and group 4 as Zamzam control (ZZ). Doxorubicin (1mg/ kg bw) was injected i.p. in DC and DZ groups on first day of study. NC and DC groups were at normal bottled water p.o., while DZ and ZZ groups received Zamzam water p.o. for 28 days. At the end of protocol, the animals were sacrificed, heart preserved, and blood collected for biochemical estimations. Serum levels of cardiac biomarkers, CRP, Fibrinogen, BUN and creatinine increased, while zinc, IL-10 and angiotensin levels decreased significantly in DC as compared to NC. Interestingly, renin level also decreased in DC group. Heart tissues from each group were examined for histological changes. Altered serum levels of the biomarkers were restored on treatment with Zamzam water. Histopathological studies are in agreement with cardioprotective influence of Zamzam in cardiac dysfunction. Research findings endorse the cardioprotective potential of Zamzam in doxorubicin induced cardiac remodeling. We attributed this effect to the presence of zinc in alkaline medium of Zamzam.

INTRODUCTION

Cardiovascular diseases (CVDs) are the most prevalent cause of morbidity and mortality among global population, with an estimate of 19.9 million deaths each year (Kaptoge *et al*., 2019). Among the established predictors of CVD death, hypertension plays an important role (Verdecchia *et al*., 2019). Hypertensive patients are often at the risk of ventricular dilation, infarct thinning and extensive formation of collagen tissues in the damaged zone of heart muscle (Al-Orabi *et al*., 2018). Consequently, the shape of ventricular chamber gets distorted and eccentric hypertrophy is developed, leading to heart failure and cardiac rupture (Matsumura *et al*., 2019). These alterations in cardiac morphology are termed as cardiac remodeling (Cokkinos, 2019). Drugs and adjuvant pharmacological therapies can suppress the early cardiac remodeling and the ventricular infarct expansion (Gelosa *et al*., 2020). However, these drugs are not free from adverse effects and can lose their efficacy in due course of time (Skjødt *et al*., 2019).

* Corresponding author: fanwar1@kau.edu.sa
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Abbreviations
BUN, blood urea nitrogen; bw, body weight; CHF, congestive heart failure; DC, doxorubicin control; Dox, doxorubicin; DZ, Doxorubicin+ Zamzam treatment; ICPMS, Inductively Coupled Plasma Mass Spectrometry; ip, intraperitoneal; LV, left ventricular; NC, Normal control; po, per oral; ROS, Reactive Oxygen Species; Zam, Zamzam; ZZ, Zamzam control.

Hence immense interest has developed among the researchers to look for a therapy, free of adverse effects, which can minimize the remodeling of cardiac tissue. Water is considered as a natural healer for many diseases and is used from the time immortal for its therapeutic potential due to high mineral content and alkaline pH (Varner, 2009). Roman bath water, dead sea water are used to heal many skin alignments and deep sea water has found special reference for its hydrotherapy in various body systems (Mooventhan and Nivethitha, 2014). Holy water is believed to possess healing and many spiritual properties since time immortal in many religions of world. The use of holy water for baptism and other spiritual cleansing from Christianity to Hinduism is considered as normal practice (Peterson, 2018; Gottowik, 2016). In Islam Zamzam (Zam) water is considered to be divinely blessed with miraculous properties, that can not only satisfy thirst and hunger, but also have a potential to cure almost all the diseases (Al-Jauziya, 1999). The holy scriptures quote that the Zam well in Makkah was revealed to Hagar, the wife of Ibrahim (Abraham in English) and the mother of Ismail (Crotty, 2012). This well was later rediscovered by Abdul Muttalib, the grandfather of Prophet Mohammad (PBUH). He became the guardian of the well and served its water to Arabs and every pilgrim who visited Makkah for Hajj (Hawting, 1980). Every year, the pilgrims from all over the world take Zam water to their native countries (Ali *et al*., 2009). The demand of this water has drastically risen worldwide in last decade (Sallam, 2015). Government of Saudi Arab, through geological surveys, has set up Zamzam Studies and Research Centre (ZSRC) in order to keep water clean and potable for drinking (Studies, 2005).

Several studies have elicited various therapeutic properties of Zam water. Its healing potential owes to its alkalinity and presence of trace elements like calcium (Ca), magnesium (Mg), sodium (Na), and chloride (Cl) (Shomar, 2012). It has also exhibited anticancer actions by inducing apoptosis in human colon (HCT-116) (Al Doghaither *et al*., 2016) and lung cancer (A594) cell lines (Omar *et al*., 2017). Additionally, it exhibited anti-oxidant properties in type 2 diabetic patients (Bamosa *et al*., 2013). Also, it is antibacterial in nature (Khalid *et al*., 2014), and prevents formation of renal calculi in experimental animals (Al-Ghamdi, 2012). This research is an attempt to determine the effect of Zamzam water in doxorubicin induced cardiac remodeling in rats.

MATERIALS AND METHODS

Water samples

Both the bottled and Zamzam water, for control and test group respectively, were procured randomly from the supermarket of Jeddah city of Saudi Arabia. At the time of purchase, both the waters were packed, sealed and ready to drink, which ensures that both the samples had a quality control.

Instrument, drugs and reagents

The instruments used in the experiment were Ultraviolet-visible spectroscopy (Kyoto, Kyoto, Japan); Centrifuge; ELISA Microplate Reader and mineral ion analyzer Liquiline System. The drug Doxorubicin was purchased from New York, NY, United States, Zam water was procured from Makkah, Saudi Arabia, and Diethyl ether from St. Louis, Missouri, United States. The detection kits used for analysis were purchased as mentioned below:

Detection kits

Zinc quantification kit, Fibrinogen rat in vitro ELISA (Enzyme-Linked Immunosorbent assay) kit, rat high sensitivity C-reactive protein (HS-CRP) ELISA kit, rat Interleukin 10 (IL-10) ELISA kit, rat renin (RENIN) ELISA Kit, were all procured from Abcam, Cambridge, United Kingdom, kits for creatinine and BUN were procured from local chemical supplier. All chemicals used were of analytical grade.

Animals grouping

Twenty-four Wistar male albino rats (170±20 g) were obtained from King Fahd Medical Research Center as approved by institutional ethical committee, and were acclimated for 7 days on a 12-h light/ dark cycle, to the condition of animal research laboratory of Biochemistry Department, Faculty of Science, King Abdulaziz University. The lab temperature was maintained at 22±2°C and relative humidity 50±5%.

The animals were divided randomly into 4 groups of 6 rats each: (i) Normal control group (NC) was fed with normal drinking water for 28 days. (ii) Dox control (DC) group was injected with Dox (1mg/kg bw) i.p. on the first day and was fed with normal drinking water for 28 days. (iii) Dox + Zam (DZ) group were injected Dox (1mg/ kg bw) i.p on the first day, and were given Zam water p.o. for 28 days and (iv) Zam (ZZ) group was fed with Zam water p.o. *ad libitum* for 28 days. Regular laboratory rat food was given to all the four groups throughout the study period. Rat body weight was recorded weekly. Water and food intake were measured daily.

Sample collection

Rats were monitored for behavioral and physiological changes. On 29th day of the experiment, rats were sedated with 3ml diethyl ether for sampling. Blood was collected in centrifugal tubes from abdominal artery (approximately

3ml/100g body weight), kept for 30 min to clot at lab temperature, clotted samples were then centrifuged at 4500 rpm for 15 min at 4°C. Serum was transferred to 1.5ml tubes and kept at -80°C for further analysis. Heart was harvested and weighed, and a part of it was preserved in 10% phosphate buffered formalin for histological analysis. Remaining part was stored at -80°C for enzyme, DNA and RNA analysis.

Serum analysis

Zinc

Commercially available Zinc quantification kit was used to measure zinc concentration of the serum. According to the manual, serum was deproteinized prior to the assay by adding 1x volume of the 7% TCA solution. Mixture was then centrifuged at 10000 rpm for 5 min. 50 µl serum was placed to the well, 200 µl of zinc reagent mixture of reagent 1 and reagent 2 (4:1) was added and incubated for 10 min at room temperature. OD was recorded at 560 nm. Results were calculated according to the standard curve obtained from standard solution.

Fibrinogen

Fibrinogen rat ELISA kit from Abcam (Cambridge, United Kingdom) was used to measure the fibrinogen content in serum samples. 50 µl of fibrinogen standard and serum samples were added to 96 well plate, wells were covered with a sealing tape and incubated for two hours at room temperature. Then wells were washed five times with 200 µL of wash buffer and 50 µL of biotinylated fibrinogen antibody was added to each well and the plate was incubated again for one hour. Microplate was washed again as mentioned above. 50µL of SP conjugate,50 µL of chromogen substrate and 50µL of stop solution were added step by step and plate was washed after each step. Then OD was measured at 450nm. The concentration was calculated according to the standard curve.

Renin

Renin was measured using a commercially available renin ELISA kit fom Abcam. As per the manual instructions, standards and samples were pipetted in a 96 well plate precoated with antibody specific renin. The immobilized antibody captured renin in the sample. The wells were washed and biotinylated antibody was added. Unbound biotinylated antibodies were washed away and an HRP-conjugated streptavidin was pipetted into the wells. The wells were incubated, rewashed and a TMB substrate solution to the wells was added. Colour developed in proportion to the amount of bound renin in each well. Finally, addition of the stop solution changed the color from blue to yellow. Color intensity was measured at 450 nm.

Interleukin-10, C - reactive protein, angiotensin

Abcam's Rat ELISA kits were used each for the quantitative measurement of IL-10, CRP and angiotensin. Standards and samples were pipetted in a 96-well plate pre-coated with specific antibodies. The wells were washed and biotinylated. Anti- rat specific antibodies were added. Unbound biotinylated antibodies were washed away and HRP-conjugated streptavidin was pipetted into the wells. The wells were incubated, rewashed and a TMB substrate solution to the wells was added. Colour developed in proportion to the amount of bound substance in each well. Finally, addition of the stop solution changed the color from blue to yellow. Color intensity was measured at 450 nm.

Water analysis

The samples of Zam water and the bottled drinking water were outsourced from Saudi Geological Society, Jeddah for analysis of their alkalinity and presence of zinc and inorganic minerals. The samples were analyzed by Inductively Coupled Plasma Mass Spectrometry (ICPMS) technology.

Histology

The animals were sacrificed, heart isolated, 2–3 mm tissues were sliced and fixed in neutral buffered formalin (10% formaldehyde in phosphate buffered saline v/v) over night. After fixation, tissues were dehydrated in ascending gradient of ethanol for one hour in each step (from 70%, 80%, 95%, 100%, 100%, 100%). Dehydrated tissues were then infiltrated by xylene twice for one hour in each step. Finally, tissues were infiltrated by paraffin at 65°C twice, one hour for each step. Fixed tissues were placed in a stainless-steel mold and molten paraffin was poured into mold after 5 second the cassata was placed on top of the mold. The mold with cassata was placed on the freezing table. After one hour, the blocks were solidified. These blocks were sectioned in microtome into 5 micrometer sections. Tissue sections were mounted on the slides and kept in an oven at 60°C for 2 h. Slides containing paraffin sections were placed on a slide holder and deparaffinized with Xylene for 10 min. Xylene was then removed by absolute ethanol. Slides were rehydrated with 100%, 90%, 80% alcohol for 2–3 min each and put into water for 3 min. Excess water was blotted and slides were put into Hematoxylin stain for 5 min. Extra hematoxylin was removed under tap water for 1– 2 min and the slides were further immersed into the Eosin stain for 30 secs. Next, these were dehydrated with 80%, 90%, 100% alcohol and finally with Xylene for 20 min. Coverslip was placed on the slides using one drop of DPX, and slides were dried overnight to make them permanent.

RESULTS

Water analysis

Table I shows the inorganic elements- Cl, SO_4, NO_3, F significantly higher in Zam water in comparison to bottled water. The zinc content in bottled water was markedly higher (5.74) as compared to that in Zam water (0.19).

Total bicarbonate ions (HCO^{3-}) was markedly higher in Zam water (198 mg/l) than that of normal bottled water (32.6 mg/l). Consequently, pH of Zam water was higher (7.4) making it more alkaline than that of bottled water (6.3). T.D.S was measured to be abundantly high in Zam water (429.17 mg/l), while it was just 81.47 mg/l in bottled water. The chemical compositions indicate strong alkaline nature of Zam. However, no significant difference was seen in the hardness of between the two water samples.

Blood parameters

Figure 1 shows the level of Zn, CRP, Il-10, fibrinogen, angiotensin, creatinine, BUN and renin in rat serum. The results showed that the zinc level in Dox induced (DC) group was 0.55 ± 0.12 mcg/ml, which was significantly lower than that of normal control (NC) group. However, despite low zinc content in alkaline Zam water, the treatment group exhibited a significant attenuation in serum zinc level (0.96 ± 0.07 mcg/ml, $p<0.05$) as compared to DZ control group (Fig. 1A).

The DC group exhibited plasma concentration of CRP as 13.40 ± 2.66 mg/dl, which was significantly higher ($p<0.05$) than that of NC (7.70 ± 0.44 mg/dl). However, treatment with Zam water showed a significant reduction ($p<0.05$) in this level (7.97 ± 1.29 mg/dl) as compared to DC group (Fig. 1B).

IL-10 in DC group (3.73 ± 0.50 pg/ml) was about half the level as that of NC (8.07 ± 0.34 pg/ml), $p<0.05$. The concentration was found to be significantly increased (7.13 ± 1.07 pg/ml) in DZ group when compared to DC group ($p<0.05$) (Fig. 1C).

The DC group exhibited a significant increase in plasma fibrinogen (4.37 ± 0.74 g/L) as compared to NC group (3.0 ± 0.51 g/L, $p<0.01$). However, treatment with Zam water significantly decreased the plasma fibrinogen to 3.62 ± 0.22 g/L as compared to untreated Dox induced cardiac remodeling group ($p<0.05$) (Fig. 1D).

The angiotensin level was significantly ($p<0.05$) decreased in DC group rats (16.0±2.26 nmol/ml/min) as compared to NC group (27±2.52 nmol/ml/min). Angiotensin in DZ group (31.0±6.67.nmol/ml/min) was significantly higher than that of DC group ($p<0.05$) (Fig. 1E).

In DC animals, the level of creatinine significantly increased to 1.60 ± 0.06 mg/dl in comparison to normal control NC animals (0.73 ± 0.13 mg/dl, $p<0.001$). However, creatinine level of treated DZ group was 1.04 ± 0.09 mg/dl, which was significantly lower as compared to DC group ($p<0.01$) (Fig. 1F).

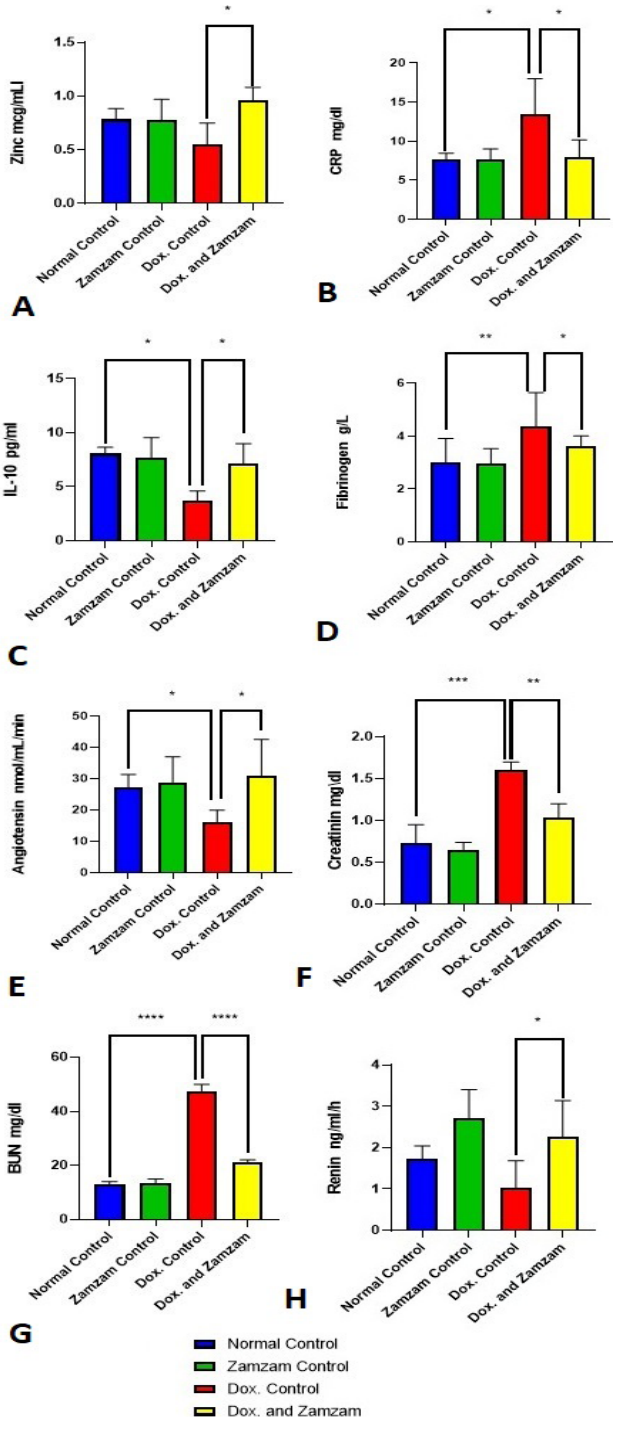

Fig. 1. Effect of Zam water on plasma concentration of various parameters in Dox - induced cardiac remodeling. Values are given as mean ± SD, n= 6; * represents significant difference from Dox control group (*$p<0.05$; **$p<0.01$; ***$p<0.001$).

Table I. Concentration of some elements, ions, salts and pH of bottled and Zam water.

Parameters	Bottled water	Zam water
Cl	28.9	61.4
HCO_3	32.6	198
NO_3	0.05	23.2
SO_4	7.3	56.2
F	0.12	0.57
Zn	5.74	0.19
T.D.S	81.47	429.17
pH	6.3	7.4
Total Hardness	31.86	129.95

BUN was assessed as a marker to detect the renal damage. The results showed that the BUN content in the DC animals was significantly higher (47.50 ± 1.44 mg/dl, $p<0.0001$) as compared to the animals of normal group, while that of the animals treated with Zam water was significantly decreased (21.0 ± 0.58 mg/dl, $p<0.0001$). This indicated that Zam, which is rich with zinc content and alkalinity, showed renal protection (Fig. 1G).

In DC group animals the level of plasma renin was lower (1.02 ± 0.38 ng/ml/h) than that in normal control (1.72 ± 0.19 ng/ml/h). Lower protection of renin indicated the destruction of juxtaglomerular cells in DC group. However, the group treated with Zam water, which is more alkaline than bottled water, and contains higher zinc content, exhibited significant increase ($p<0.05$) in the renin level (2.27 ± 0.50 ng/ml/h). It indicates that the presence of zinc and alkaline combination in Zam water exhibited renal protection, and thus attenuated the functions of juxta glomerulus to produce renin (Fig. 1H).

Histopathology

The histological study of the hearts of doxorubin-induced cardiac remodeling in the control (DC) group demonstrated that the myocytes loose muscle diameters, fat deposits, fibrinogen thickness, highly irregular distribution of eosinophils, ventricular cell arrangement and intracellular gaps were all markedly increased ($P<0.01$, Fig. 2iii) as compared to those in both normal (NC) and Zam control (ZZ) groups (Fig. 2i and ii). Administration of Zam water for 28 days reversed these pathological changes in DZ group. The treated group (Fig. 2iv) demonstrated normal cardiomyocyte architecture with lesser intracellular spaces, less eosinophilic filtration and milder fat deposits as compared to those of DC group ($P<0.05$, Fig. 2).

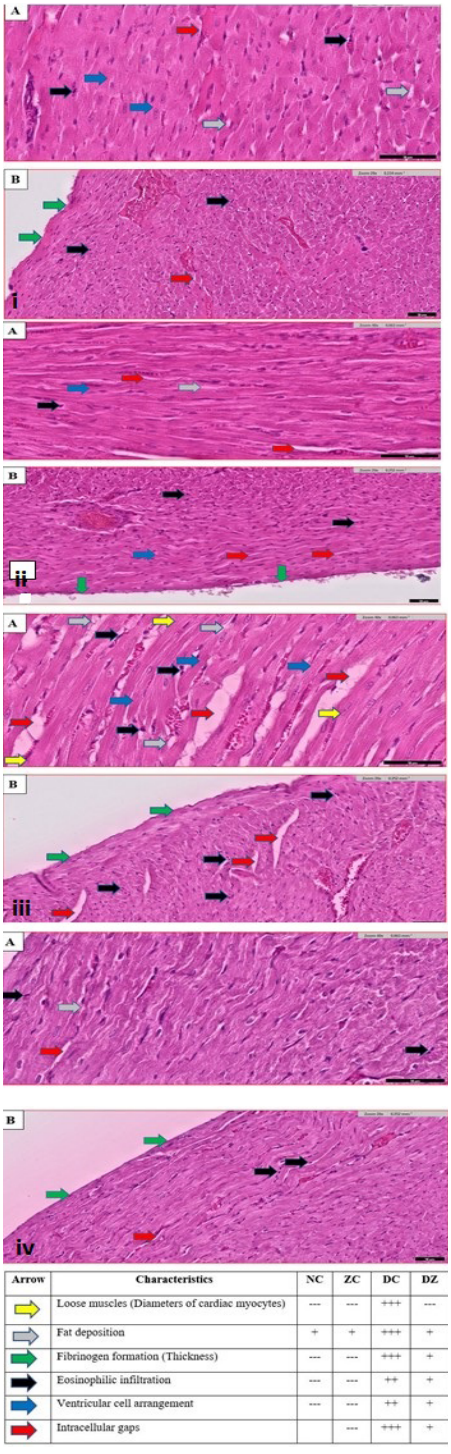

Fig. 2. Heart histology image of random sample from (i) Normal control (NC) (ii) Zamzam Control (ZC) (iii) Dox-induced cardiac remodeling control (DC) group and (iv) Dox-induced cardiac remodeling control + Zamzam (DZ) groups. (A-H and E stained; 40 X, B- H and E stained; 20 X, respectively).

DISCUSSION

In the field of medical therapeutics, vehicles used in drug formulation for various dosage forms can influence activity of main pharmaceutical ingredient (Pottel *et al*., 2020). Although, water is the most commonly used vehicle, but physiological functions of drinking water on human under disease state are still not much explored (Rahman *et al*., 2016). Human body is composed of approximately 60 to 80% of water, and it can influence cellular functions in organ development and their physiological actions (Pottel *et al*., 2020). According to Functional Water Association of Japan, both activated and natural water, can have specific roles in attenuating the altered or diseased state of the body (Bari *et al*., 2003). Electrochemically reduced water with alkaline pH has potential of scavenging ROS, and is popular for its beneficial health effects in many countries of the world including Japan (Shirahata *et al*., 2007). Zam water is more alkaline than the normal bottled drinking water. Prior studies conducted on Zam revealed that it possesses anti-diabetic potential, which is attributed to its alkaline nature (Bamosa *et al*., 2013). Similarly other studies reported therapeutic actions of Zam in conditions like cancer, osteoporosis, cardiovascular diseases, teratogenicity, lipid profile and oxidative stress (Al-Juwaie *et al*., 2020). In the present study, we explored the potential of Zam water in Dox induced cardiac modelling.

Dox is one of the most potent anticancer drug from the family of anthracycline obtained from Streptomycous species used in the treatment of many cancers (Khasraw *et al*., 2012). It exerts its action by various interactive mechanisms towards numerous tumors including topoisomerase inhibition, DNA intercalation and free radical generation of ROS (Upadhayay *et al*., 2020). It exerts cytotoxic actions on tumor cells, however, apoptosis and mitochondrial dysfunction caused by ROS also affect other normal organs including heart, brain and kidneys (Xu *et al*., 2020). Dox – induced cardiotoxicity is irreversible, and is responsible for CHF, myopathy and left ventricular (LV) remodeling (Sun *et al*., 2020). Further, cardiac remodeling, cell death and chamber dysfunction are directly associated with alteration in renin and angiotensin levels (Tocchetti *et al*., 2014).

Cardiovascular disorders and mortality are often associated with deficient zinc level in the serum (Yu *et al*., 2018). Zinc is an essential micro nutrient that acts as a vital cofactor for energy transfer and physiological heart function, possesses antioxidant potential and is involved in multiple signaling pathways (Alexanian *et al*., 2014). Zinc content in Zam water is significantly lesser than in bottled water. Despite this, the serum zinc concentration in Zam-treated animals was significantly higher, and hence showed a significant protection in cardiac morphology in dox-induced animals. However, high zinc content of bottled water wasn't able to attenuate cardiac remodeling. We proposed that zinc, under an alkaline environment of Zam, influenced multiple factors and attenuated cardiac toxicity/ remodeling.

The elevating inflammatory mediators in acute phase of cardiac remodeling include high sensitivity- C Reactive Protein (hs-CRP), cytokines and fibrinogen. CRP increases in serum with infection, inflammation surgery, heart attack and/ or other heart diseases (Kamath *et al*., 2015). Elevated level of CRP is an early indicator of Dox induced cardiac toxicity (Cao *et al*., 2017). Likewise, in our study, CRP level significantly raised in DC animals. Numerous studies indicate that inflammation occurs at acidic pH (Haka *et al*., 2009). CRPs bind to the ligand proteins in the acidic environment. At low pH, protein misfolding takes place, which implicates for toxic conditions. However, treatment with Zam reduced this level to normal. This is indicative of anti-inflammatory potential of Zam water owing to its alkaline nature. Our results are comparable with other previous study obtained in postmenopausal women (Schoppen *et al*., 2004) treated with sodium rich carbonated mineral water that can reduce the risk of cardiovascular risk in females. Further similar results were obtained in reduction of cardiovascular risk by sodium-bicarbonate mineral water in moderately hypercholesterolemic young adults (Pérez-Granados *et al*., 2010). Extensive studies and research established zinc as highly antioxidant element (Prasad, 2014), and has a strong tendency to decrease CRP (Bao *et al*., 2010). Thus, it can be strongly stipulated that presence of zinc at alkaline pH of Zam water renders it a potent anti-inflammatory and anti-oxidant activity.

Serum level of IL-10, an anti- inflammatory cytokine, is decreased in cardiac toxicity (Sikka *et al*., 2013). In a study by (Jung *et al*., 2017) administration of IL-10 to mice post-MI Left ventricular remodeling reduced the inflammation and improved the LV physiology (Jung *et al*., 2017). In the present study, administration of Dox significantly reduced the anti-inflammatory cytokine, IL-10 in the control group. However, IL-10 level significantly restored to its normal on treatment with Zam. We postulated that alkalinity and presence of zinc in water provided it anti- inflammatory potential.

Our findings also showed a significant increase in plasma fibrinogen level in Dox induced rats. Fibrinogen, has a marked role in formation of thrombus, and is involved in endothelial cell injury and platelet aggregation (Mnafgui *et al*., 2016). Increased fibrinogen level in this study, thus, indicated increased cross-linking of platelets which may have resulted in increased myocardium infarction size. However, treatment with Zam water significantly

decreased the plasma fibrinogen level and hence prevented the myocardial structure.

The histopathological results support the attenuation in remodeled tissue by Zam administration. Cardiac tissues revealed significant decrease in eosinophilic infiltration, fibrin formation, fat deposition, ventricular cell arrangement and intracellular gaps in Zam treated DZ animals as compared to Dox-induced untreated rats.

Apart from its effect on cardiac system, Dox has also been reported for hepatic and renal toxicities (Su *et al*., 2015). ROS generated by Dox can damage the DNA by lipid peroxidation and other oxidative modifications (Ghibu *et al*., 2012). Additionally, these free radicals have deleterious actions on kidney as they affect glomerular hyper permeability and cause degeneration of renal tubules (Afsar *et al*., 2020). Induction of Dox in this study resulted in the elevated levels of renal markers- creatinine and BUN. Similar outcomes have been reported in other studies, which reveal that increased levels of renal function biomarkers in serum are indicative of tubular blockade and compromised kidney functions (Rizvi and Kashani, 2017). This nephrotoxicity is attributed to lipid peroxidation and free radical generation in glomerular cells and prevention of protein synthesis in renal tubules (Roomi *et al*., 2014). On the contrary, Zam water exhibited reno-protection, as treatment in DZ group significantly reduced both creatinine and BUN levels as compared to DC group. Our results are comparable to those found in the previous studies conducted on different animal models, where preconditioning with zinc significantly reduced the serum creatinine and urea concentration in the study animals induced with renal ischemia (O'Kane *et al*., 2018).

It is well established that renal hemostasis is regulated by RAS (Navar, 2014). As per the RAS physiology, the juxtaglomerular cells of kidney secrete renin into the circulation, which bind to a liver produced substrate, Angiotensinogen, and form angiotensin-I (AT-I). Thereafter, ACE hydrolyzed AT-I to AT-II (Kobori *et al*., 2007). In this study, both renin as well as angiotensin levels were found to be significantly lower in DC group. These observations are completely contradictory to most of the studies which report elevation of these components (Sobczuk *et al*., 2020) Our findings are strongly supported by renal damage markers. Increased levels of creatinine and BUN clearly indicate the damage to kidney (Cheng *et al*., 2020). Dox at the dose of 1 mg/kg bw caused degenerative changes in the juxtaglomerular cells. Damaged juxtaglomerular cells are not efficiently able to perform their functions, i.e., production of renin. This explains the low renin level in DC group of this study. Also, renin converts angiotensinogen to angiotensin. Inadequate release of renin will consecutively result in lesser conversion. This is in accordance to the significantly lower level of angiotensin in DC group. However, administration of Zam water for 28 days was able to replenish the damage caused to juxtaglomerular cells. It showed the better preserved cellular and tubular structure with regeneration of the juxtaglomerular cells and restored their activity. As a result, renin and angiotensin level increased significantly in DZ group as compared to DC group. Interestingly, the juxtaglomerular protection, and hence, renin production was found to be higher (2.27 ± 0.50) in dox-induced treated (DZ) group than that of normal control (1.72 ± 0.19) group. This can be attributed to alkaline pH and high zinc content in Zam water (Table I). Oral alkaline agents have shown reno- protection in patients with chronic renal diseases (Abe *et al*., 2020) Also, Zinc has a potential outcome against cardiac and ischemic injuries (Xu and Zhou, 2013). Rao *et al* reported in their study that administration of zinc in ischemic rats could reduce the necrosis and tubular damage (Rao *et al*., 2017). In view of these reports, it is possible that Zam water, rich with both, alkaline pH and zinc content, has shown synergistic protection in DZ group of our study. As a result, juxtaglomerular cells retained their potential to produce renin, which in turn, converted more of the angiotensinogen to angiotensin. Hence their level increased in the treated (DZ) group. Another possible mechanism supporting elevated angiotensin is that the zinc affects/controls the homeostasis of ACE-2 expression which is regulated by Sirt-1 (Patel *et al*., 2016), and sirt-1 is further regulated by Zinc (Rosenkranz *et al*., 2016). Zinc dependent expression is already established for zinc metalloenzyme such as metallothionin and matrix metalloproteinases (Wessels *et al*., 2020).

CONCLUSION

Our findings in this study reported that doxorubicin induced cardiac remodeling is associated with the reduced zinc level, increased inflammation and damaged renal function. Zamzam water, which is highly alkaline in nature, showed cardio-renal protection by reducing free radical generation and inflammation. A novel finding in this study was that despite renal damage, renin level decreased in doxorubicin control group, which was attenuated by treatment with Zam. We postulated that the presence of zinc in alkaline pH of Zamzam water resulted in prevention of cardiac-remodeling through its anti-inflammatory actions.

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Statement of conflict of interest

The authors have declared no conflict of interest.

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