Original article

The Protective and Therapeutic Effect of Zamzam Water on Induced Hyperglycemic Wistar Rats

Wesal E. Sabahelkheir[1], Sulafa, H. Mahmoud[2], Murwan, K. Sabahelkhier[1] and Amar M. Ismail[1]

[1]Department of Biochemistry and Molecular Biology; Faculty of Science and Technology, Al-Neelain University, Khartoum, Sudan; [2]Department of Biochemistry, Faculty of Veterinary Medicine, University of Khartoum, Khartoum, Sudan; Department of Chemistry, College of science, Sudan University of Science and Technology.Khartoum, Sudan

ARTICLE INFO

Article history:
Received 2018 October 10th
Reviewed2018November 13th
Accepted 2018 December 3rd

Keywords:
Zamzam Water; Diabetes mellitus; Alloxan

Abstract

The aim of the current study was to investigate the protective and therapeutic effects of Zamzam water on inducing hyperglycemic Wistar rats. Rats classified into DZZ, DTZ, DTT, NTT, and NZZ groups after 2 weeks adaptation. Blood glucose was measured after alloxan induced hyperglycemia using a glucometer. Rats were sacrificed blood was collected, liver and kidney tissue were obtained. Blood glucose, ALT, AST, urea, and creatinine were measured using spectrophotometry method. CBC was estimated using full blood count analyzer. Sections of liver and kidney were stained using hematoxylin stain. The results showed that the treated group with DZZ and DTZ significantly decreased blood glucose levels than control. While a significant increase was observed in urea and creatinine of treaded DZZ group. Results of histopathological section revealed no alteration.

The study concludes that Zamzam water had potential protective and treatment effect on alloxan induced hyperglycemia. Higher pH, calcium, and magnesium might make it is protective and treatment effect, since their role in insulin secretion and carbohydrate metabolism.

[*]**Corresponding author**: amarqqq@yahoo.com

Introduction

Diabetes mellitus (DM) is a general term for heterogeneous disturbances of metabolism for which the main finding is chronic hyperglycemia. The cause is either impaired insulin secretion or impaired insulin action or both (Kerner and Bruckel, 2014). DM reduces the ability of an individual to regulate the level of glucose in the bloodstream resulting in a number of major and some minor complications (Kirti *et al*., 2014). Several pathogenetic processes are involved in the development of diabetes mellitus which include processes which destroy the beta cells of the pancreas with

consequent insulin deficiency and others that result in resistance to insulin action (WHO, 1999). Alloxan is one of the most potent methods to induce experimental diabetes mellitus is chemical induction by Alloxan. It is a well-known diabetogenic agent that is used to induce type I diabetes in experimental animals. Alloxan is a urea derivative which causes selective necrosis of the β-cells of pancreatic islets. In addition, it has been widely used to produce experimental diabetes in animals such as rabbits, rats, mice and dogs with different grades of disease severity by varying the dose of alloxan used (Etuk, 2010). Zamzam water is located inside the Holy Mosque at about 20 meters east of the Ka'ba in Makkah Al-Mukarramah, Saudi Arabia. It is a naturally hard carbonated type of water with unique physical and chemical properties that are different from any other water, this water is different from other water in many ways: first no bacteria can format its source, second it doesn't go moldy nor does it change color, taste or smell, biological growth and vegetation usually take place in most well, this makes water unpalatable owing to the growth of algae leading to changes in taste and odor (Al-Ghamdi, 2012). Furthermore, this water has a higher pH value of 7.9 -8 than the pH value of ordinary water pH 6.5–7 (Huda *et al*., 2016).

Materials and methods

Water Samples

Zamzam water sample was obtained from the well of Zamzam in plastic bottles (9 bottles each of them contains 10 letters of water). Tab water was obtained from the faucet of the local area.

Experimental design

The experiment was conducted on a total of 50 Wistar rats (each weighing between 280 to 400 g) divided into 5 groups each group contains 10 rats.

Induction of diabetes

Diabetes was developed by injecting the animals with alloxan (Sigma, USA) at a dose of 150 mg/kg body weight in 0.1M cold citrate buffer of pH 4.5 intraperitoneal. The onset of diabetes was confirmed in the experimental rats by measuring blood glucose concentration at 48h after injection with alloxan.

Blood Samples collection

In the morning of the termination day rats which had been fasting since the night was slaughtered and blood samples were collected from the rats of all groups and kept in a specific container (plain and EDTA containers). The spot of blood from each rat was taken to measure glucose concentration by glucometer.

Biochemical analyses

Glucose was estimated by using a glucometer, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), urea and creatinine were analyzed by Bio System Kits (BioSystem S.A. Costa Brava, 30.80030 Barcelona, Spain). Complete blood count (CBC) was measured by using CBC analyzer.

Statistical analysis

The Statistical Package for Social Sciences (SPSS 16) was used for statistical analysis. Results after two months of water consumption were compared using One-way ANOVA. Statistical significance was set at $p<0.05$.

Histopathological Studies

It has been applied to examine the histopathological changes in the tissues of the kidney and liver for all rats in each group. Rats were slaughtered and dissected, the kidneys and livers were removed and preserved in 10% formaldehyde for fixation of the tissues. Gross from kidney and liver were processed, embedded and stained using the procedure of H and E methods.

Results and Discussion

Figure 1. **Section A**: shows significant increase in means blood glucose level after alloxan injection of DZZ (324.5±98.9), DTT (370.8±83.5) and DTZ (474±123) in comparison to control group NTT (96±16.9) with *P*-value 0.000, 0.000 and 0.000 respectively. In addition, the group received Zamzam water before alloxan injection (324.5±98.9) revealed a significant decrease in mean

glucose levels compared with the other groups that received tap water DTZ (474±123).

Section B: shows no significant difference in means blood glucose levels of DZZ (270.8±124) and DTZ (378±103) in comparison to control group DTT (394±14.9). **Section C**: shows a significant decrease in the mean of DZZ after the treatment (270.8±124) in comparison to before the treatment (324.5±98.9).

Section D: shows a significant decrease in the mean of DTZ after the treatment (378±103) in comparison to before the treatment .(474±123)

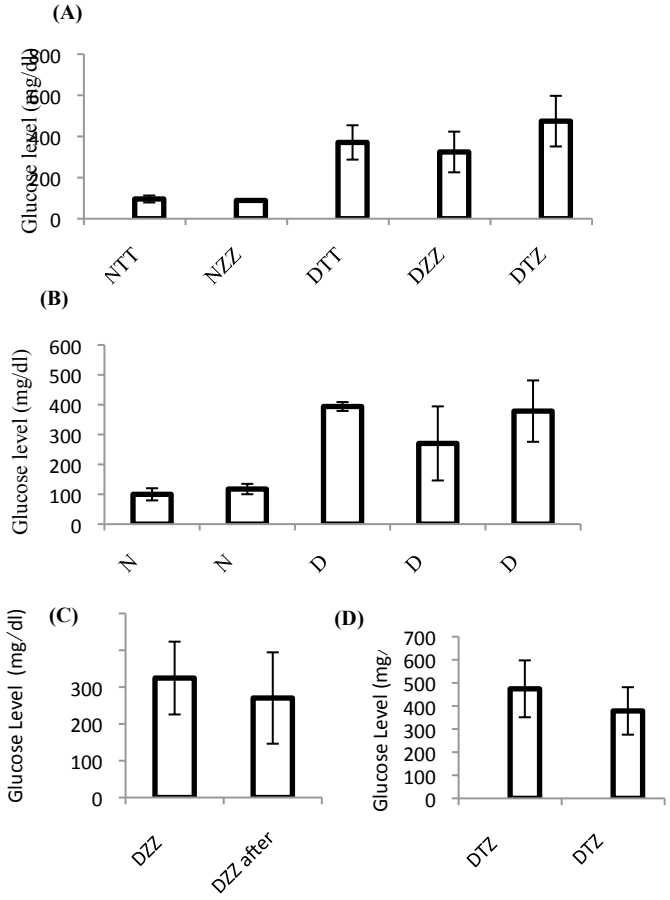


Figure 1. Glucose level in induced hyperglycaemic and Zamzam treated wistar rats.

Figure 1. A, B, C and D represents glucose level after alloxan injection, glucose level after treatment, glucose level of DZZ before and after treatment and glucose level of DTZ before and after treatment respectively.

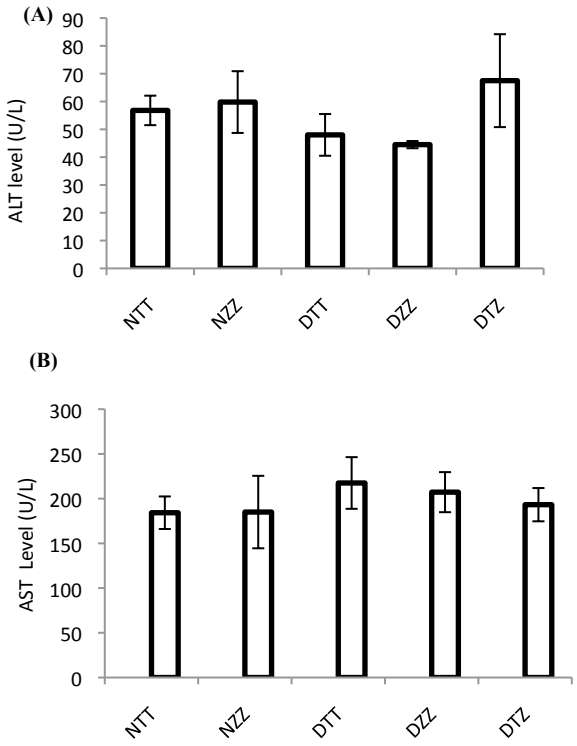
Key: NTT = normal rats receive tab water before and after protection period and during treatment period. NZZ = normal rats receive Zamzam water before and after protection period and during treatment period. DTT = diabetic rats receive tab water before and after protection period and during treatment period. DZZ = diabetic rats receive Zamzam water before and after protection period and during treatment period. DTZ = diabetic rats receive tab water before protection period and Zamzam water after protection period and during treatment period.

Mean activity of ALT, AST and GGT U/L in study groups

Figure2. Section A:shows no significant difference in means blood ALT activity of DZZ (44.5±1.3) and DTZ (67.5±16.7) in comparison to control group DTT (48±7.5).

Section B: shows no significant difference in means blood AST activity of DZZ (207.3± 22.4) and DTZ (193.3±18.7) in comparison to control group DTT (217.5±28.9).

Section C: show no significant difference in means blood GGT activity of DZZ (18.1±4.8) and DTZ (5.6±5.5) in comparison to control group DTT(11.5±9.4) .



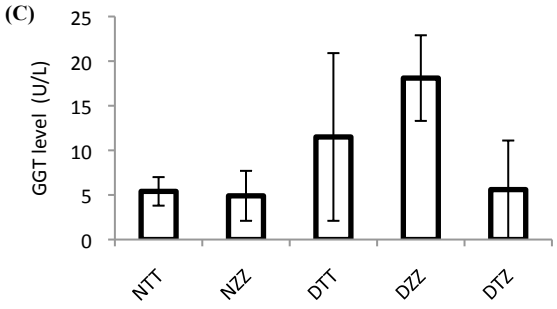


Figure 2. A, B and C represent ALT, AST and GGT levels.

Mean level of urea and creatininemg/dl in study groups

Figure3. Section A: shows significant increase in means blood urea level of DZZ (70±16.6) in comparison to control group DTT (40.3±8.3) with p-value 0.01 and no significant change in the mean of DTZ (64.5±4.7) in comparison to control group DTT (40.3±8.3).

Section B: show significant increase in means blood creatinine level of DZZ (0.45±0.05) in comparison to control group DTT (0.35±0) with p-value 0.03 and no significant difference in mean of DTZ (0.36±0.05) in comparison to control group DTT (0.35±0).

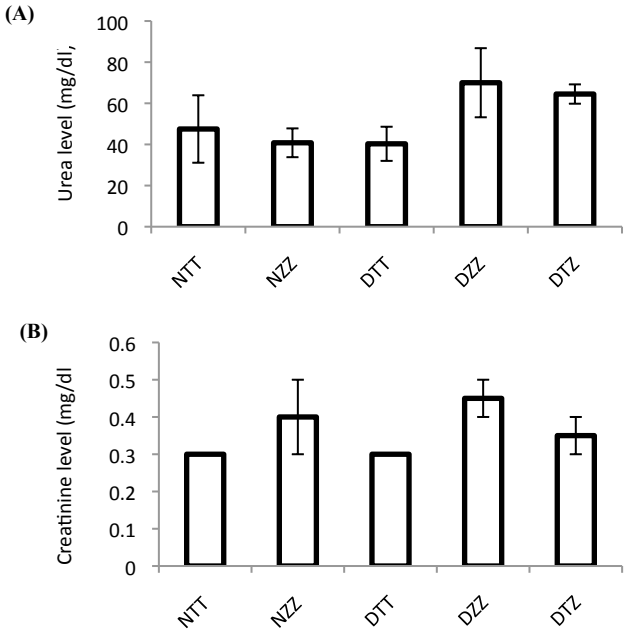


Figure 3. A and B represents urea and creatinine levels.

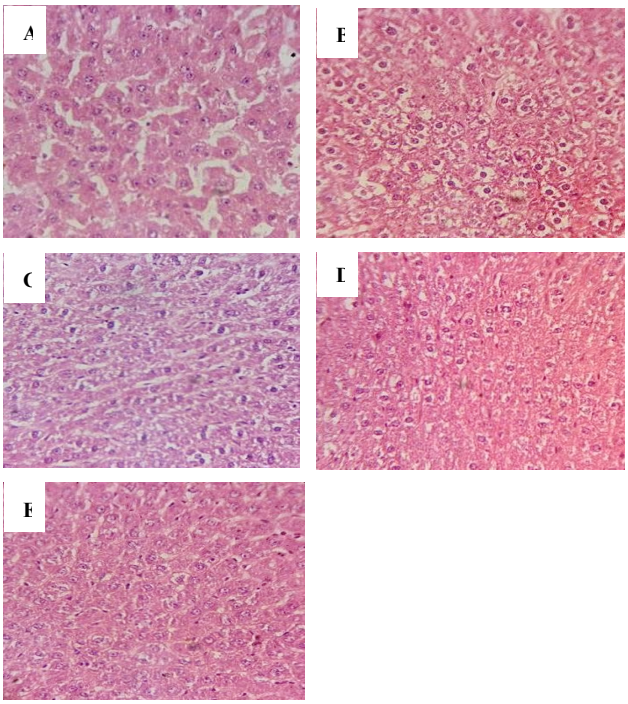


Figure 4. Histological sections of liver organ of studied groups. A= treated by Zamzam Water before and after alloxan; B=treated with tab water before and Zamzam Water after alloxan; C=normally treated by Zamzam Water; D=treated by tab water before and after alloxan; E= normally treated by tab water.

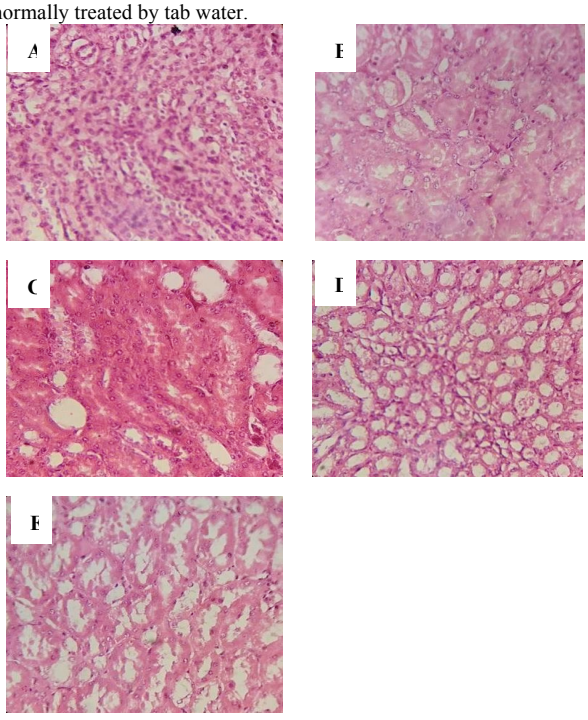


Figure 5. Showed histological sections of kidney organ of study groups. A= group treated by Zamzam water before and after alloxan; B=treated by tab water before and Zamzam water after alloxan;

C=normally treated by Zamzam water. D=treated by tab water before and after alloxan; E= normally treated by tab water.

Discussion

Researchers previously studied the structure of Zamzam and tap water, fortunately, reported interesting findings that, calcium, magnesium and pH are higher in Zamzam water. Previous findings encourage us to hypothesize that Zamzam water could possess protective effect on Alloxan induced hyperglycemia in Wistar Rats.Indeed, the function of calcium and magnesium in insulin action and glucose metabolism.

Concurrent with the previous finding that Alloxan has been used to induce experimental diabetes due to the selective destruction of the insulin-producing pancreatic beta-islets. Alloxan induces a multiphasic blood glucose response when injected into an experimental animal, which is accompanied by corresponding inverse changes in the plasma insulin concentration followed by sequential ultrastructural beta-cell changes ultimately leading to necrotic cell death (Rohilla and Ali,2012).

The present study provides evidence that administration of Zamzam water for 60 days significantly decreases blood glucose of the group treated with Zamzam water before Alloxan induced-hyperglycemia in comparison with group tap water before Alloxan received. These findings indicate that, Zamzam water has a protective impact on Alloxan induced-hyperglycemia, in addition to protective effect, Zamzam water also possess therapeutic effect, indicated by significant decrease of blood glucose level in group treated with Zamzam water than tap water. Furthermore, this finding attributed to the role of calcium and magnesium in the release of insulin and carbohydrate metabolism. The finding contradicts the previous study done by Bamosa found that patients receiving Zamzam water had a significant decrease in HbA1c but not in fasting blood sugar (Bamosa*et al*., 2013).

The present study provides evidence that there is a significant increase within a normal range in blood urea and creatinine levels of the group that intake Zamzam water before and after alloxan injection in comparison to group that intake tab water before and after alloxan receive, this indicates that Zamzam water may enhance urea synthetic pathway in the liver. In fact, that after two weeks of treatment Zamzam water, blood analysis of blood urea nitrogen (BUN) and creatinine showed significant differences in positive control group compared to the negative control group, whereas no significant differences were noticed in the level of BUN and creatinine between both the negative control and the test group (Al-Ghamdi, 2012). Independent t-test analysis showed that comparing to tap water Zamzam water revealed insignificant differences in mean level of ALT, AST, GGT and CBC before and after alloxan injection, which indicates that, Zamzam water had neither protective nor therapeutic effect on liver enzymes and CBC count.Concurrent with previous findings that Zamzam water is similarly alkaline, can promote antioxidant mechanisms in normal rats and those stressed with the high dose of gentamicin. In one experiment, the effects of Zamzam water were compared to those of ordinary bottled water in normal rats (Abdullah et al., 2012), which protect tissues damage, histopathological sections of study groups indicate that treatment with Zamzam and tap water for 60 days have no effect on liver and kidney tissues. In fact, that the concentrations of toxic elements such as arsenate, lead and selenium are higher than any other water but still lower than the danger level of human consumption (Huda et al., 2016).

Conclusion

The data of present study concludes that, Zamzam water had potential protective and treatment effect on alloxan induced hyperglycemia. The protective and therapeutic effect observed attributed to the higher pH, calcium and magnesium in Zamzam water, since their role in insulin secretion and carbohydrate metabolism.

References

Abdullah A., Abdelsalam E., Bamosa A. and Kkaled A. (2012). Antioxidant effects of Zamzam water in normal rats and those under induced-oxidative stress. *Journal of Medicinal Plants Research*, 6(42), 5-10.

Al-Ghamdi S. S. (2012). Inhibition of calcium oxalate nephrotoxicity with Zamzam water. *Open Journal of Preventive Medicine*, 2 (1), 67-71.

Bamosa A., Elnour A., Kaatabi H., Al Meheithif A. and Aleissa K. (2013) Zamzam Water Ameliorates Oxidative Stress and Reduces HemoglobinA1c in Type 2 Diabetic Patients. *J Diabetes Metab,* 4 (3), 1-5.

Etuk E. U. (2010). Animals models for studying diabetes mellitus. Agric. *Biol. J. N. Am,* 1(2),131-134.

Huda A. A., Ayat B. A., Sawsan A. R., Shiekhah M. A., Omar A. M. S. and Ulfat M. O. (2016). Evaluation of the Potential Anticancer Activity of Zamzam Water in Human Colon Cancer Cell Line. *Cancer and Oncology Research*, 4 (3), 33-41.

Kerner W. and Bruckel J. (2014). Definition, Classification, and diagnosis of diabetes mellitus, *Exp Clin Endocrinol Diabetes*; page: 384.

Kirti K., Joanna M. T., Shamim I. A., Eva M. K. and Chibber R. (2014). Introduction to Diabetes Mellitus. *Experimental Medicine and Biology Research Gate,* page:1-11.

Rohilla A. and Ali S. (2012). Alloxan-Induced Diabetes: Mechanisms and Effects. International Journal of Research in Pharmaceutical and Biomedical Sciences, 3 (2), 819-823.

WHO, (1999) Department of Non-communicable Disease Surveillance Geneva?