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Modulatory Effects of Natron on Biochemical Indices and Cardiac Muscle Gene Expression in Postpartum Albino Rats

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Peripartum cardiomyopathy is a major public health problem that causes significant morbidity, mortality and huge economic burden among peripartum women. The proposal that natron consumption during postpartum period is involved in the development of peripartum cardiomyopathy

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requires further scientific elucidation. The current research investigated the effects of natron administration on antioxidant status of cardiac muscles, serum cardiac troponins among postpartum albino rats. Postpartum rats were group into 4, each containing 5 rats. Colorimetric, sandwich microplate immunoenzymometric assay was used for troponins estimation. Real Time PCR was used for the gene expression studies. Antioxidants status was measured using enzymatic methods. Natron administration reduces oxidative stress at lower dose, while it induces oxidative stress at higher concentration. There was a significant increase (P<0.05) in MDA concentration in groups 3 and 4 and a decrease in group 2 compared with controls. There was a significant decrease (P<0.05) in GPX and SOD activity in groups 3 and 4 and an increase in group 2 compared with controls. There was significant increase (P<0.05) in CAT activity in all groups administered natron compared with controls. There was significant decrease(p<0.05) in troponin I, C and T in groups administered natron compared with controls. Also natron administration up regulates cardiac troponins genes and STAT3 genes. The current study revealed that though natron played a protective role at a low concentration, it may cause oxidative stress in the cardiac muscles at higher doses. Thus, oxidative stress in cardiac muscles and modulation of cardiac muscle genes is likely the mechanism by which high natron intake causes Peripartum cardiomyopathy. Further research is recommended in humans to determine the safety of natron in postpartum period.

Keywords: Peripartum cardiomyopathy; oxidative stress markers; biochemical indices; natron; albino rats.

1. INTRODUCTION

Cardiovascular diseases such as peripartum cardiomyopathy are the most alarming and leading causes of morbidity and mortality among postpartum women. These are diseases caused by additive and interactive effects of inherited and environmental factors. Substantial evidences show that oxidative stress due to the disturbance in the balance between the productions of reactive oxidants (free radical) such as reactive oxygen species (ROS), generated from consumption of exogenous substances such as natron, and antioxidant defense play a vital role in the aetiopathogenesis of the diseases and their complications. The aetiopathogenesis leading to peripartum cardiomyopathy is still unknown. However, studying a number of possible risk factors for developing peripatum cardiomyopathy such natron comsumption, and evaluating oxidative mechanisms thereafter could shade spotlight to understanding of peripartum cardiomyopathy pathology [1,2].

Peripartum cardiomyopathy is a devastating form of cardiac failure affecting women during early postpartum period with high incidence in Northern Nigeria where the consumption of natron postpartum is high. Women in northern Nigeria are involved in unique customary puerperal practices including consumption of natron locally usually as ‘kunun kanwa’ and daily hot water bath for 40 days. These practices are still common in Sokoto and have been proposed to cause volume overload and heart failure [2].

Regulation of cardiac contraction are made by cardiac filament proteins called troponins (Tns).Basically there are three types of troponins; troponin I, troponin C, troponin T. Troponin I (TNNI3) gene encodes for sarcomeric contractile protein troponin I, which is found solely in the cardiac muscle. Cardiac troponin I helps to coordinate contraction of the heart. Troponin T (TNNT2) gene provides instructions for the synthesis of troponin T, which stabilizes the troponin complex during muscular contraction. Troponin T binds Ca^{2+} to form a complex that coordinate cardiac muscle contraction. Tn C transfers the Ca21 signal eliciting contraction to the fiber of cardiac filament [3,4].

Natron consumption may trigger oxidative damage to the genes regulating the synthesis of troponins, this may predispose to postpartum cardiomyopathy. Studies on the effects of natron on antioxidant status and expression of genes encoding for the synthesis of essential cardiac biomolecules in postpartum period are very rare and have not been reported in northern Nigeria to date. It is on this basis the present study was designed to investigate the modulatory effect of natron on biochemical indices and cardiac muscle gene expression in postpartum albino rats.

Peripartum cardiomyopathy causes serious morbidity and mortality among women in Northern Nigeria. Studies on the effects of natron on antioxidant status and lipid peroxidation in postpartum period are very rare. Also there is

gross paucity of literature about natron influence on expression of genes coordinating the synthesis of essential cardiac biomolecules such as troponins . There are currently needs to discover better understanding of etiopathology and risk factors of peripartum cardiomyopathy. The present study could generate and provide evidenced based information on effects of natron consumption on antioxidant status of cardiac muscle and gene expression of vital biomolecules of the heart. This could potentially improve the postnatal care offered to nursing mothers .

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

Analytical grade chemicals and reagents was used for this study.

2.2 Source of Natron (Kanwa)

The natron was purchased from the Central Market, Sokoto, Nigeria.

2.3 Natron (Kanwa): Purchase And Preparation

The natron was made into powder form using mortar and pestle, weighed and stored at room temperature before used.

Natron solutions were freshly prepared, each corresponding to the respective dosage for each group of rats using 1mL of distilled water.

2.4 Experimental Animals

Were purchased and kept in the animal house of the Faculty of Pharmaceutical Science, Usmanu Danfodiyo University Sokoto. A total of 28 adult rats, 20 females and 8 males, averagely 11 weeks old and 160g weight was used for the study. They were allowed access to clean water and food *ad libitum* . Thereafter were allowed to acclimatised for a week before the commencement of the experiment.

2.5 Breeding

The rats were grouped into 4, each containing 5 females and 2 males in a cage. They were allowed to breed for the period of 6 weeks. After conception, the females were isolated till they deliver.

2.6 Natron Administration

The postpartum rats were randomly regrouped into 4, each containing 5 rats respectively. Natron preparations was administered orally daily for 28 days as shown below:

- **Group 1:** 1.0 mL of distilled water (control)
- **Group 2:** 100 mg/kg body weight of natron.
- **Group 3:** 200 mg/kg body weight of natron.
- **Group 4:** 300 mg/kg body weight of natron.

2.7 Sample Collection And Preparation

2.7.1 Blood

The animals were allowed to fast overnight and blood samples was collected from the animals through cardiac puncture into clean labelled test tubes after being anaesthetized with chloroform. The blood was allowed to clot and then centrifuged at 4000 rpm for 10 minutes. The serum was used for the analysis of some cardiac biomarkers.

2.7.2 Cardiac muscle samples

The animals were dissected, and the respective cardiac tissues were removed instantly using a scalpel. 20mg of tissue was homogenized in 0.3mL binding buffer 4(BB4) for gene expression analysis.50mg of tissues was homogenized in 1mL of extraction solution for antioxidant analysis. The homogenate was centrifuged at 10,000 × g for 15minutes at 4°C.

The supernatant was aspirated into clean sample tubes and stored at -20°C until analysis.

2.7.3 Analyses of Cardiac Muscle Antioxidant Status

Malondialdehyde level was estimated in the cardiac muscle homogenate via the method of Shah and Walker's [5]. The activities of superoxide dismutase (SOD) was assayed using the method of Malstrom *et al.* (1975). Glutathione peroxidase (GPx) activity was measured using enzymatic method described by Ursini *et al.* (1985). Catalase activity was measured using the method of Johansson *et al.* (1988).

2.7.4 Estimation of the Serum Levels of Cardiac Function

The levels of cardiac function proteins: Troponins I, C and T were estimated using Pars Biochem ELISA kits following the method of Apple *et al*. [6].

2.7.5 Gene Expression Studies Using Real Time PCR

- Total RNA was isolated from the cardiac muscles of postpartum Albino rats using transgen® isolation kit (Shanghai ZJ Bio-Tech Co., Ltd., Shanghai, China) following the manufacturer's guidelines.
- The RNA was quantified using Nanodrop spectrophotometer (Bioevopeak Nucleic acid analyzer, SP-MUV200F, Shandong, China).
- cDNA synthesis and Real time PCR was performed according to TransScript® Green one-step qRT-PCR Supermix kit protocol (AQ211-01, TransGen Biotech, China) on a Rotor-Gene Q 5plex HRM qPCR machine (QIAGEN, Germany).
- Ct values was normalized to house-keeping gene using the ΔΔCt method.

2.8 Data Analysis

The experimental data were statistically analyzed using SPSS version 25.0 (Inc., Chicago, IL, USA) for windows and expressed as mean ± standard error of mean. A one-way analysis of variance (ANOVA) was used to analyze the data followed by a Tukey's *post hoc* test to compare the mean differences among the various groups. The level of significance was set at p≤0.05.

The Delta-Delta Ct algorithm(Livak method) was used to calculate the log-fold change between Ct values of target genes and reference gene (GAPDH) expressed.

3. RESULTS

Table 1 Shows cardiac malondialdehyde (MDA) concentration and antioxidant enzyme activity in postpartum albino rats administered natron. There was significant increase (P<0.05) in MDA concentration in group 3 and 4 and decrease in group 2 compared to controls. There was significant decrease (P<0.05) in GPX and SOD activity in group 3 and 4 and increase in group 2 compared with controls. There was significant increase (P<0.05) in CAT activity in all groups administered natron compared to controls. Table 2 Shows serum troponin I,C and T concentration in postpartum albino rats administered natron. There was significant decrease in troponin I,C and T in groups administered natron compared to controls. Fig. 1. Shows the effect of Natron Administration on expression of the TNNT2 gene of postpartum Albino Rats. There was increase expression of TNNT2 gene with increase natron concentration. Fig. 2. Shows the effect of Natron Administration on expression of the TNNI3 gene of postpartum Albino Rats. There was increase expression of TNNI3 gene with increase natron concentration. Fig. 3. Shows the effect of Natron Administration on expression of the STAT3 gene of postpartum Albino Rats. There was increase expression of STAT3 gene with increase natron concentration. Fig. 4. Shows the effect of Natron Administration on expression of the TNF-α gene of postpartum Albino Rats. There was decrease expression of TNF-α gene in group 3 and 4 compared to controls.

Table 1 Shows cardiac malondialdehyde (MDA) concentration and antioxidant enzyme activity in postpartum albino rats administered natron. There was a significant increase (P<0.05) in MDA concentration in groups 3 and 4 and a decrease in group 2 compared with controls. There was a significant decrease (P<0.05) in GPX and SOD activity in groups 3 and 4 and an increase in group 2 compared with controls. There was significant increase (P<0.05) in CAT activity in all groups administered natron compared with controls.

Table 1. Cardiac Malondialdehyde (MDA) Concentration And Antioxidant Enzyme Activity In Postpartum Rats Administered Natron

Parameter	Group 1(n=5)	Group 2(n=5)	Group 3(n=5)	Group 4(n=5)
MDA(nmol/mL)	0.93±0.05[a]	0.53±0.04 [c]	0.99±0.07 [b]	1.20±0.08 [b]
GPX(U/mL)	13.61±0.28 [a]	15.40±0.29 [c]	6.81±0.59 [b]	4.59±0.39 [b]
CAT(U/mL)	137.68±12.3 [a]	256.04±25.28 [b]	175.80±16. 34 [b]	169.38±17. 22 [b]
SOD(U/mL)	0.62±0.05 [a]	0.49±0.06 [b]	0.35±0.04 [b]	0.32±0.04 [b]

Values are mean ± SEM. Value with different superscripts on the same row differ significantly(p<0.05). Group 1: postpartum + 1.0 mL DH2O (control); Group 2: postpartum + administered 100mg/kg body weight of natron; Group 3: postpartum + administered 200mg/kg body weight of natron; Group 3: postpartum + administered 300mg/kg body weight of natron; MDA: malondialdehyde; GPX: Glutathione peroxidase; CAT: Catalase; SOD: Superoxide dismutase. n : number of participant

Table 2. Serum Troponin I, C And T Concentration In Postpartum Albino Rats Administered Natron

Parameter	Group 1(n=5)	Group 2(n=5)	Group 3(n=5)	Group 4(n=5)
Troponin I(µg/L)	3.86±0.09 [a]	3.75±0.08[b]	3.60±0.12[c]	3.76±0.15 [b]
TroponinC(nm/L)	3.79±0.20 [a]	3.48±0.19 [b]	3.91±0.11 [c]	3.47±0.17 [b]
Troponin T(µg/L)	3.53±0.11 [a]	3.46±0.07 [b]	3.26±0.14 [b]	3.60±0.07 [c]

Values are mean ± SEM. Values bearing different superscripts on the same row differ significantly($p<0.05$). Group 1: postpartum + 1.0 mL DH_2O (control); Group 2: postpartum + administered 100mg/kg body weight of natron; Group 3: postpartum + administered 200mg/kg body weight of natron; Group 3: postpartum + administered 300mg/kg body weight of natron; n : number of participant

Table 2 Shows serum troponin I,C and T concentration in postpartum albino rats administered natron. There was significant decrease in troponin I,C and T in groups administered natron compared to controls.

Fig. 1 shows the effect of Natron Administration on expression of the TNNT2 gene of postpartum Albino Rats. There was increase expression of TNNT2 gene with increase natron concentration.

Fig. 2 shows the effect of Natron Administration on expression of the TNNI3 gene of postpartum Albino Rats. There was increase expression of TNNI3 gene with increase natron concentration.

Fig. 3 shows the effect of Natron Administration on expression of the STAT3 gene of postpartum Albino Rats. There was increase expression of STAT3 gene with increase natron concentration.

Fig. 4 shows the effect of Natron Administration on expression of the TNF-α gene of postpartum Albino Rats. There was decrease expression of TNF-α gene in group 3 and 4 compared to controls.

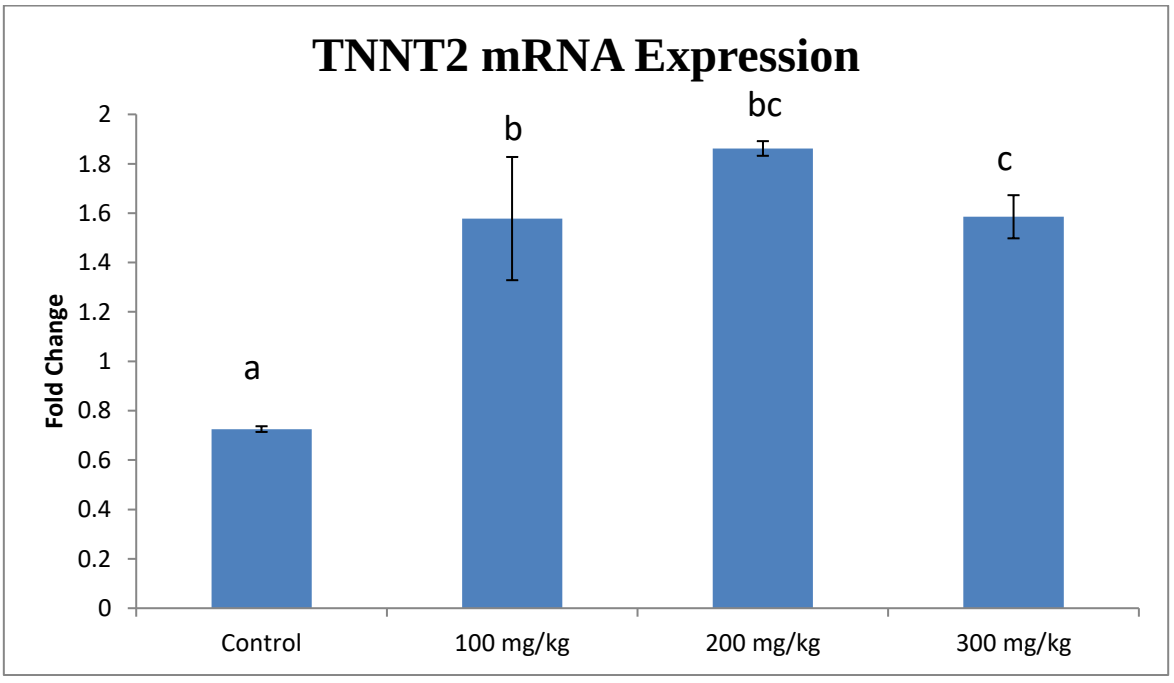

Fig. 1. Effect of natron administration on the expression of TNNT2 gene of postpartum albino rats. Postpartum rats were administered with natron at 100, 200 and 300 mg/kg body weight for 28 days. The Delta-Delta Ct algorithm(Livak method) was used to calculate the log-fold change between Ct values of target genes and reference gene (GAPDH) expressed.

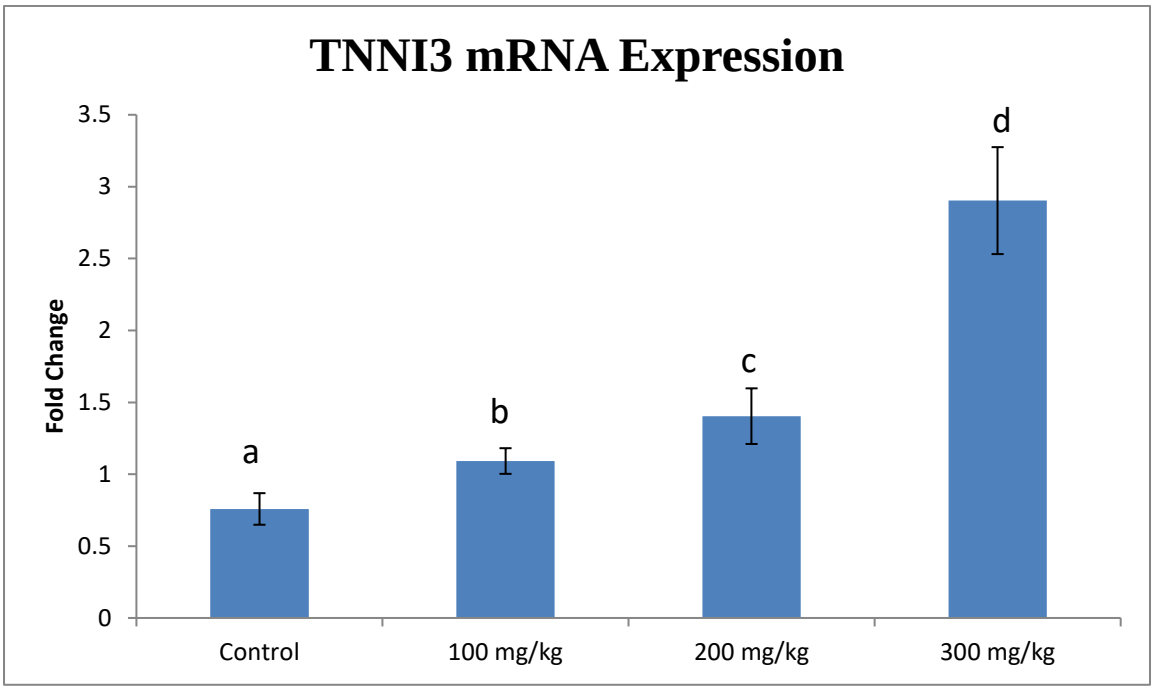

Fig. 2. Effect of natron administration on the expression of TNNI3 gene of postpartum albino rats. Postpartum rats were administered with natron at 100, 200 and 300 mg/kg body weight for 28 days. The Delta-Delta Ct algorithm(Livak method) was used to calculate the log-fold change between Ct values of target genes and reference gene (GAPDH) expressed.

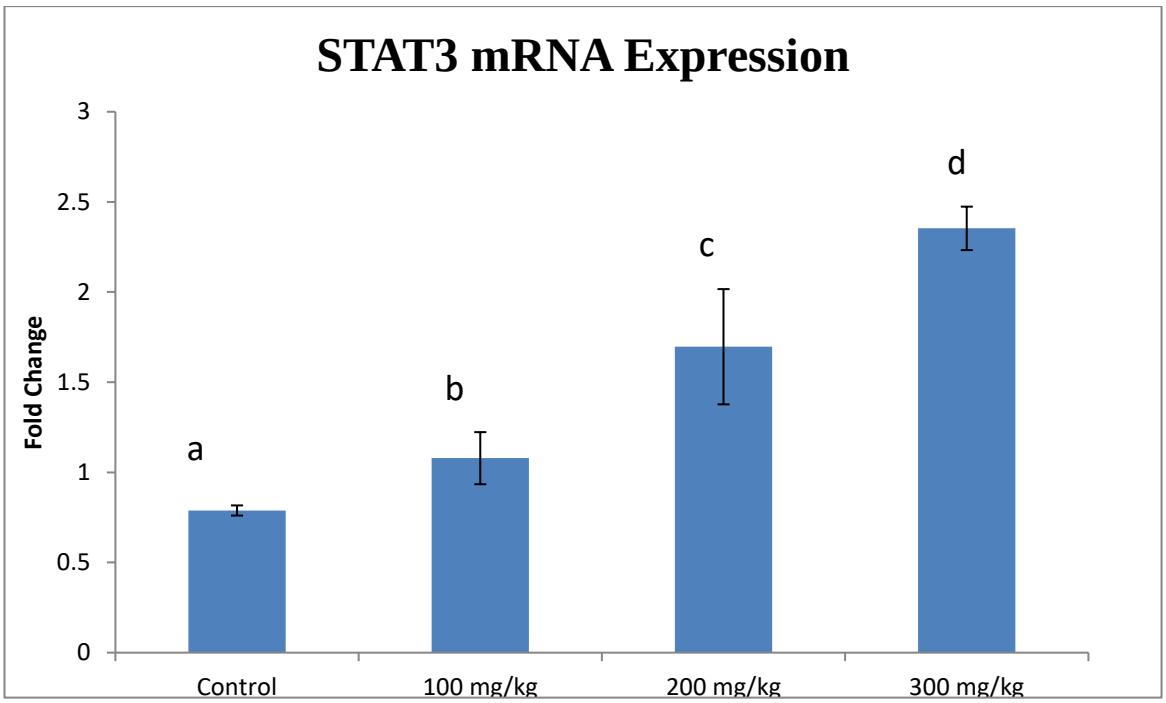

Fig. 3. Effect of natron administration on the expression of STAT3 gene of postpartum albino rats. Postpartum rats were administered with natron at 100, 200 and 300 mg/kg body weight for 28 days. The Delta-Delta Ct algorithm(Livak method) was used to calculate the log-fold change between Ct values of target genes and reference gene (GAPDH) expressed.

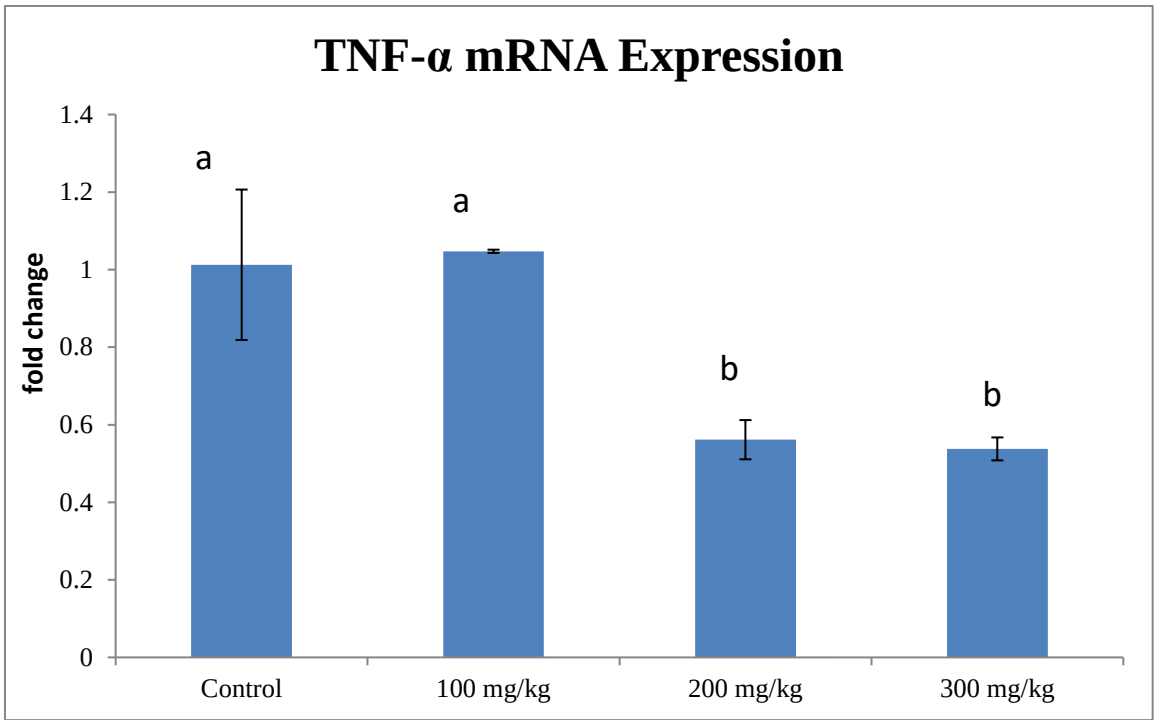

Fig. 4. Effect of natron administration on the expression of TNF-α gene of postpartum albino rats. Postpartum rats were administered with natron at 100, 200 and 300 mg/kg body weight for 28 days. The Delta-Delta Ct algorithm(Livak method) was used to calculate the log-fold change between Ct values of target genes and reference gene (GAPDH) expressed.

4. DISCUSSION

The observed decrease in MDA level at 100 mg/kg body weight of natron compared to control is an indication that ROS generated during post-delivery is counteracted by natron, probably due to the presence of some antioxidant minerals. This suggests that natron at a lower dose could have therapeutic benefits. The increased MDA level at higher doses suggests that the consumption of natron at these doses may be detrimental to the health. Natron at a higher dose is known to induced oxidative stress, which may lead to lipid peroxidation of the cardiomyocytes membranes because MDA is an index of lipid peroxidation. This may be responsible for high level of MDA found in this study at higher dose of natron administration. Ajiboye *et al*. [7]; Imafidion *et al*. [8] and Farias *et al*. [9] have earlier reported increased levels of MDA in albino rats administered with natron. Also studies have shown that high sodium present in natron may be associated with an increased generation of ROS in the cardiac muscles [8,10,11,2].

The observed significant decrease in GPX and SOD activity might be due to the overwhelming effect of their activities to mitigate oxidative stress associated with ROS generated from natron and post-delivery OS. It might also be due to the presence of molecules in natron that inhibits its activity or due to disruption of the enzyme's structure by ROS. The increased activity of GPx following administration of 100 mg/kg body weight of natron is an indication of the protective role of natron to counteract the post-delivery OS probably due to the presence of selenium (GPx cofactor) in natron. This may also be responsible for the observed decrease in MDA level at the same concentration. However, the observed increase in MDA level at higher doses of 200 and 300 mg/kg body weight of natron may be due to an increase in ROS generation by natron, which overwhelms the antioxidant enzymes' capacity to neutralize ROS. Overall, the decrease in antioxidant enzyme activities in the animals at a higher dose of natron in this study is consistent with earlier study, which showed a reduction in antioxidant status after natron administration [1,2]. The observed increase in CAT with an increase in natron concentration might be due to the body compensatory response to mop up an increase in free radicals induced by natron administration.

Another possible explanation may be due to the direct effects of natron on the genes of the enzyme. This may suggests the protective role of natron, which stimulates the synthesis of the enzyme. Similar findings were reported by [1,2].

The observe decrease levels in troponin I,C and T in groups administered natron compared to controls may be due to increase turnover of the troponin molecules in cardiac muscle as a result of oxidative stress induced by natron. TNNT2, TNNI3 and STAT3 gene expression studies show increase expression, TNF-α shows decrease expression. This indicate that natron administration affect the gene expression of cardiac troponins in cardiac muscle. The study also indicate that natron consumption down regulate expression of the imflammatory cytokine TNF-α. These findings may suggest that the observe increase in the expression of TNNT2, TNNI3 and STAT3 genes could be a compensatory response to cardiac demand of cardiac proteins (troponins).

5. CONCLUSION

The current study revealed that natron played a protective role at a low concentration. However, it can cause oxidative stress in the cardiac muscles at higher doses. Thus, oxidative stress in cardiac muscles and modulation of cardiac muscle genes is likely the mechanism by which high natron intake causes PPCM.

6. RECOMMENDATIONS

1. Further research is recommended in higher animals such as humans.
2. High doses of sodium should be avoided during the postpartum period.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

ETHICAL APPROVAL

The rats were handled according to rules and regulations of animals ethics (Ethical approval no:PTAC/N/(AC)/OT/78 – 24).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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