P-ISSN: 2304-3075; E-ISSN: 2305-4360
International Journal of Veterinary Science
www.ijvets.com; editor@ijvets.com
Research Article
https://doi.org/10.47278/journal.ijvs/2022.173

Correlation between Oxidative Stress Markers and Biochemical Indices of Cardiac Function of Postpartum Albino Rats Administered Natron

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Article History: 22-612 Received: 09-May-22 Revised: 11-Jun-22 Accepted: 25-Jun-22

ABSTRACT

Natron has been implicated in the pathogenesis of peripartum cardiomyopathy, probably due to oxidative stress. The current research investigated the correlation between the antioxidant status of cardiac muscles and serum biochemical indices of cardiac function of postpartum albino rats administered natron. Twenty postpartum rats were divided into four groups, each group having 5 female rats. Group A received distilled water while groups B, C, and D were orally administered 100, 200 and 300mg/kg body weight of natron, respectively. The administration of 300 mg/kg body weight of natron increased malondialdehyde level to 18.27±3.75 from 13.01±2.25nmol/g fresh tissue, significantly ($P<0.05$) decreased activity of superoxide dismutase (SOD) and level of catalase (CAT) to 0.98±0.46 and 4.11±0.14 from 3.50±0.75µmol/min/g and 5.27±1.03ng/g fresh tissue, respectively in the cardiac muscles as compared with the control. Natron administration also increased the serum levels of troponin I and myoglobin and increased the activities of aspartate aminotransferase and lactate dehydrogenase. The cardiac function indices showed significant ($P<0.05$) positive correlations with malondialdehyde and significant ($P<0.05$) negative correlations with antioxidant enzymes. The results, therefore, indicated that the administration of high concentrations of natron led to an increase in markers of oxidative stress in the cardiac muscles, which may be responsible for the observed derangement in the cardiac function indices.

Key words: Peripartum cardiomyopathy, Oxidative stress markers, Biochemical indices, Natron, Albino rats

INTRODUCTION

Natron is a salt deposit of saline lake containing largely hydrated salt of sodium carbonate, traces of other minerals and impurities (Ajiboye et al. 2015; Bankole et al. 2015). It is widely used traditionally in the preparation of many dishes, as a laxative and in the preparations of medicines against different ailments (Omajali and Sanni 2010; Kutshik et al. 2018; Chukwuma 2019). Consumption of natron in large quantities as an additive in the preparation of cereal-based porridge (kunun kanwa) is believed to increase the quality and quantity of maternal milk and the general health of the mother (Saidu et al. 2018a). The consumption of kunun kanwa by nursing mothers in Northern Nigeria has been presumed to be a contributory factor in precipitating the pathogenesis of peripartum cardiomyopathy (PPCM) (Sliwa et al. 2010). PPCM is a dilated form of cardiac injury that develops one month before or up to five months after delivery without identifiable causes or symptoms before late pregnancy (Gupta and Wenger 2018).

Although the precise mechanism of the disease is not clearly understood (Dambazau et al. 2016). It has been speculated to be associated with high sodium concentration in natron and other molecules that result in the generation of reactive oxygen species (ROS) (Saidu et al. 2018b). High sodium intake has been reported to increase blood volume (hypervolumia), preventing cardiac muscles from properly contracting and pumping blood to other body compartments leading to hypoxia (Gupta and Ashraf 2018; Horscroff et al. 2019). Due to high mitochondrial density in cardiac muscles, hypoxia may increase the generation of ROS (Farias et al. 2017). Oxidative stress (OS) manifests when there is the absence or insufficient antioxidant system to counteract the effects of these toxic molecules (Farias et al. 2017). In the cardiac muscles, OS may lead to cardiac injury through the disruption of cellular membranes, resulting in leakage of its components into the serum of affected individuals (Chinedum et al. 2014; Philip et al. 2018).

Cite This Article as: Bello HA, Saidu Y, Abubakar MK, Wali U, Abbas AY and Muhammad SA, 2022. Correlation between oxidative stress markers and biochemical indices of cardiac function of postpartum albino rats administered natron. International Journal of Veterinary Science x(x): xxxx. https://doi.org/10.47278/journal.ijvs/2022.173

PPCM has been described to be most prevalent in the Northern part of Nigeria, accounting for one case in every 100 live birth, probably due to the high intake of kunun kanwa by nursing mothers in the region (Sliwa et al. 2010; Saidu et al. 2018a). Saidu et al. (2018b) reported that the intake of natron in high doses caused a significant increase in levels of cardiac function indices in the serum of albino rats, probably due to OS. Previous studies have indicated significant OS in the kidney, and liver of albino rats following administration of graded doses of natron (Ajiboye et al. 2015; Kutshik et al. 2018). Similarly, our previous study revealed that natron induced OS and caused dyslipidemia in rats (Saidu et al. 2020). However, there is a shortage of information on the OS status of cardiac muscles of patients with PPCM. The current work investigated the correlation between the antioxidant status of cardiac muscles and serum biochemical indices of cardiac function of postpartum rats administered graded doses of natron as an index to predict the possible relationship between PPCM and OS.

MATERIALS AND METHODS

Chemicals and Reagents

Analytically graded chemicals and reagents were used for the study.

Natron Purchase and Preparation

Natron was bought from Fish and Vegetable Market, Sokoto, Sokoto State, Nigeria. It was identified at the Department of Chemistry, Usmanu Danfodiyo University, Sokoto. The sample was ground using mortar and pestle into a fine powder, weighed and stored in a desiccator at room temperature before use. Three stock solutions were freshly prepared weekly, each corresponding to the respective dosage for each group of rats using 100 mL of distilled water and kept in a refrigerator.

Experimental Animals

Twenty-eight rats (20 females and 8 males) weighing 160-180g were purchased from the Department of Biological Science, Usmanu Danfodiyo University, Sokoto (UDUS). They were kept in the Animal House of the Department of Biochemistry of the university freely permissible to air, water and feed before and throughout the experimental time. The rats were allowed to acclimatize for a week before the commencement of the experiment. All procedures were approved by the Institutional Animal Ethics Committee of UDUS.

Preparation of Postpartum Rats

A total of 28 rats was utilized for this experiment and divided into 4 groups, each group having 5 females and 2 male rats and allowed to copulate for 6 weeks. During the period, the females were observed for conception and isolated until they deliver their pups.

Natron Administration

Following delivery, the postpartum rats were randomly regrouped into 4, each group having 5 rats. Natron was administered orally in a 1.2mL dosage form using a clean cannula daily for 28 days as follows:

Group A: Postpartum albino rats received 1.2mL of distilled water
Group B: Postpartum albino rats received 100mg/kg body weight of natron
Group C: Postpartum albino rats received 200mg/kg body weight of natron
Group D: Postpartum albino rats received 300mg/kg body weight of natron

Sample Collection and Preparation

Following the last day of natron administration, the animals were allowed to fast overnight, then humanly sacrificed by placing each in a jar with cotton wool sucked in chloroform and blood samples were collected into clean centrifuge tubes. The blood was allowed to clot for about 15 minutes and then centrifuged at 4000rpm for 10min using a benchtop centrifuge. The serum was then aspirated and transferred to another set of clean labelled sample tubes and stored at -4°C until required (Parasuraman et al. 2010). Using a scalpel, the animals were dissected, and the respective cardiac tissues were removed instantly, rinsed with a 50mM phosphate-buffered saline (pH 7.4) to remove the blood and then homogenized in 10mL cold 50mM phosphate, pH 7.0 containing 1 mM EDTA per gram tissue. The homogenate was then centrifuged at 10,000 × g for 15 minutes at 4°C. The supernatant was aspirated into clean sample tubes and stored at -20°C until analysis.

Analyses of Cardiac Muscle Antioxidant Status

Malondialdehyde level was estimated in the cardiac muscle homogenate via the method of Buege and Aust (1978) while the activities of superoxide dismutase (SOD) and glutathione peroxidase (GPx) were assayed using Cayman's Assay kits following the methods of Sun et al. (1998) and Paglia and Valentine (1967), respectively. The level of catalase (CAT) was estimated in the homogenate using Pars Biochem ELISA kit using the method of Apple et al. (1999).

Estimation of the Serum Levels and Enzyme Activities of Cardiac Function

The levels of cardiac function proteins: myoglobin, troponins I and T were estimated using Pars Biochem ELISA kits following the method of Apple et al. (1999). Whereas the activities of cardiac function enzymes: aspartate transaminase (AST), lactate dehydrogenase (LDH) and creatine kinase (CK) were assayed using Agappe's kits following the method of International Federation of Clinical Chemistry (1989).

Data Analysis

Data were expressed as mean±standard error of the mean (SEM). All the parameters were evaluated using a one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison tests. Correlations between the variables were carried out using Pearsons's correlation coefficients with GraphPad InStat software (version 3.05). The limit of statistical significance was set as $P<0.05$.

RESULTS

The result of the effect of natron on the level of malondialdehyde of cardiac muscle is shown in Fig. 1.

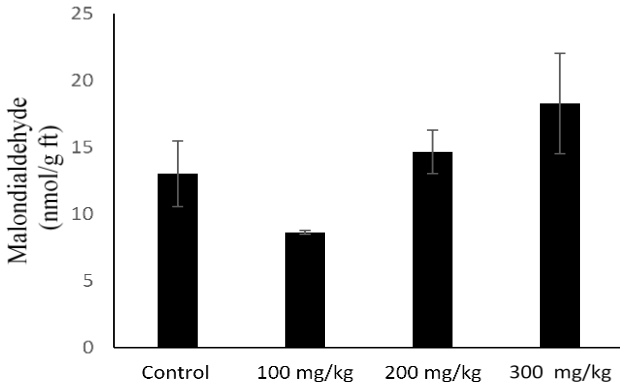

Fig. 1: Malondialdehyde level in the cardiac muscles of postpartum rats administered graded doses of natron: Values are expressed as mean±SEM, g ft: gram fresh tissue.

The result indicated that administration of 100mg/kg body weight natron decreased MDA level compared to control. However, the levels were observed to increase in a dose-dependent manner with increased natron concentration. The effects of natron administration on the activities of SOD and GPx and the level of CAT in cardiac muscles are shown in Fig. 2. The result indicated that SOD activity significantly decreased at a concentration of 200 and 300mg/kg of natron when compared with control. Administration of 100 mg/kg body weight of natron increased activity of GPx and level of CAT in cardiac muscles. However, administration of 200 and 300mg/kg body weight of natron decreased the levels in a dose-dependent manner.

The effects of natron on biochemical indices of cardiac function enzymes and proteins are presented in Table 1. The result showed that administration of natron did not significantly change the levels and activities of the cardiac indices, but the values increased in a dose-dependent manner except for troponin T. Table 2 presents the correlation coefficients (R^2) between oxidative stress markers (MDA, SOD, GPx, CAT) of cardiac muscles and serum biochemical indices of cardiac function. The results indicated significant positive correlations between cardiac markers and MDA. However, significant negative correlations were observed between SOD, GPx and CAT.

DISCUSSION

The high sodium present in natron may be associated with an increased generation of ROS in the cardiac muscles (Shazia et al. 2012; Imafidion et al. 2016; Kutshik et al. 2018). 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 may lead to lipid peroxidation of the cardiomyocytes membranes because MDA is an index of lipid peroxidation (Farias et al. 2017). Ajiboye et al. (2015) and Imafidion et al. (2016) have earlier reported increased levels of MDA in albino rats administered with natron.

The observed significant decrease in SOD activity might be due to the overwhelming effect of ROS 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 (Budueli et al. 2006). 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 (Ajiboye et al. 2015).

Similarly, CAT follows the same pattern as GPx. The observed increase at 100 mg/kg body weight suggests the protective role of natron, which stimulates the synthesis of the enzyme. On the other hand, the observed decrease in CAT with an increase in natron concentration might be due to an increase in free radicals that overwhelmed the enzyme activity or disrupt the enzyme architectural structure (Budueli et al. 2006). Another plausible explanation may be due to the direct effects of natron on the genes of the enzyme (Rando 2003). Overall, the decrease in antioxidant enzyme activities in animals at a higher dose of natron in this study is consistent with our earlier study, which showed a reduction in antioxidant status after natron administration (Saidu et al. 2020).

Histological examinations from previous studies revealed that natron displayed all the cardinal features of PPCM in rat models (Muhammad et al. 2014; Saidu et al. 2018b). The observed decrease in the values of some of the cardiac function indices at 100 mg might be associated with the health benefits of natron, which may serve therapeutic purposes at a lower concentration. However, the observed increase in the levels of the cardiac proteins (TnI and MB) and activities of AST and LDH in a dose-dependent manner suggest that natron at a higher dose could lead to sodium overload, resulting in cardiac chamber dilation and subsequent disruption of cardiac membranes. Furthermore,

Table 1: Serum cardiac proteins and enzymes of postpartum rats administered graded doses of natron as an index of cardiac function.

Group	TnI (µg/L)	TnT (µg/L)	MB (µg/L)	AST (U/L)	CK (U/L)	LDH (U/L)
Control	1.32±0.05	1.68±0.33	0.14±0.01	130±20	170±20	430±110
100 mg/kg bw	1.27±0.13	1.74±0.22	0.14±0.02	110±30	150±20	470±170
200 mg/kg bw	1.37±0.22	2.74±0.42	0.15±0.02	140±10	160±20	530±40
300 mg/kg bw	1.68±0.19	1.61±0.15	0.18±0.02	180±40	180±40	800±110

Values are expressed as mean±SEM, MB: Myoglobin, TnT: Troponin T, TnI: Troponin I, CK: creatine kinase, LDH: Lactate dehydrogenase, AST: Aspartate aminotransferase.

Table 2: Correlations between serum levels of cardiac function indices and antioxidant status of cardiac muscles of postpartum albino rats administered graded of natron.

	MDA	SOD	GPx	CAT
Cardiac indices		(Correlation coefficient)		
AST	0.7455*	-0.7741*	-0.3501	-0.6377*
CK	0.6438*	-0.7127*	-0.7548*	-0.4463
LDH	0.7117*	-0.7407*	-0.7914*	-0.7014*
MB	0.5879*	-0.8031*	-0.8692*	-0.6931*
TnI	0.7420*	-0.7406*	-0.7384*	-0.5637*
TnT	0.2244	-0.5071	-0.5485*	-0.4627

Correlation coefficients with * are considered to be significant at $P<0.05$, MB: Myoglobin, TnT: Troponin T, TnI: Troponin I, CK: creatine kinase, LDH: Lactate dehydrogenase. AST: Aspartate aminotransferase, SOD: Superoxide dismutase, GPx: Glutathione peroxidase, CAT: Catalase, MDA: Malondialdehyde

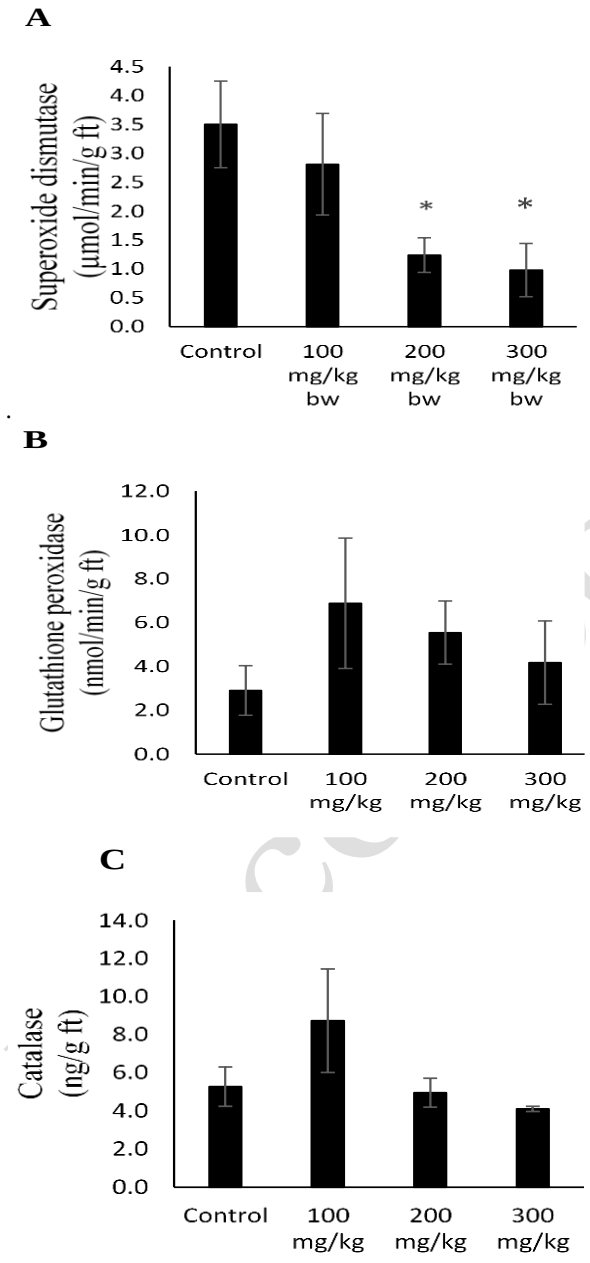

Fig. 2: Antioxidant status of cardiac muscles of postpartum rats administered graded doses of natron: Values are expressed as mean±SEM. (A) superoxide dismutase (B) glutathione peroxidase (C) catalase. Bars with an asterisk are considered significantly different from the control. at $P<0.05$. g ft: gram fresh tissue, bw: body weight.

these changes may result in the leakage of the cardiac proteins or enzymes into the serum (Muhammad et al. 2014; Saidu et al. 2018b). The CK activity of the treated rats was similar to the control, however, the activity increases with an increase in the concentration of natron. The observed sudden decrease in TnT level at 300mg/kg body weight might either be due to a random or systematic error, or other causes that are not well understood.

The observed significant positive correlations between serum biochemical indices of cardiac function indices and cardiac muscle MDA, and significant negative correlations with antioxidant enzymes indicated that OS in the cardiac muscles may be responsible for the increased levels of specific cardiac proteins and increased activities of cardiac function enzymes in the serum. Additionally, disruption of the cellular membranes may lead to leakage of the markers of cardiac markers into the serum. In other words, the mechanism by which these markers are released as a result of OS includes the disruption of cellular membranes by free radicals, leading to the release of specific cardiac proteins into circulation following myocardial injury (Palanisamy et al. 2010; Philip et al. 2018).

Hypoxia, which can be occasioned by high Na^+ intake from natron consumption can inhibit the complete oxidation of pyruvate to CO_2 and H_2O, and by simple equilibrium, it has to be metabolized anaerobically to lactate through lactate dehydrogenase. Following OS and cardiac injury, there is an increased possibility of release of lactate into circulation and therefore increased LDH activity (Rodwell et al. 2015; Kong et al. 2016; Farias et al. 2017). Similarly, hypoxic cells need energy for better performance and therefore increase the activity of CK to augment the ATP levels (Brewster 2018) and the requirement of energy by the cardiomyocytes during injury stimulates aspartate aminotransferase to function tirelessly to provide oxaloacetate that would serve as a precursor for glucose synthesis through gluconeogenesis (Giannini 2003; Nelson and Cox 2013). Taken together, the data suggest a direct relationship between increased oxidative stress and increased biomarkers of cardiac injury

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. The observed significant correlation coefficients indicated that oxidative stress might be accountable for the increase in serum levels of cardiac function indices. Thus, oxidative stress in cardiac muscles is likely the mechanism by which high natron intake causes PPCM.

Conflict of Interests

The authors declare no potential conflicts of interest.

Acknowledgements

This work was supported by a grant from the Tertiary Education Trust Fund, Nigeria with grant number: TET/DR&D/CE/UNI/SOKOTO/IBR/2016/VOL1.

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