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Full Length Research Article

Effect of Dried Lake Salt (Kanwa) on Lipid profile and Heart Histology of Female Albino Rats

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ABSTRACT: Peripatum cardiomyopathy is a devastating form of cardiac failure affecting women mainly in their last month of pregnancy or early postpartum with high incidence in Northern Nigeria where the consumption of dried lake salt postpartum is high. The current work was designed to study the effect of dried lake salt on lipid profile and histology of heart in female albino rats. The rats were administered graded doses of the salt for 4 weeks. The group administered 300mg/kg body weight of the dried lake salt has significantly ($P<0.05$) lower high density lipoprotein-cholesterol as compared with the control. There was no significant ($P>0.05$) increase in low density lipoprotein-cholesterol but total cholesterol, triglyceride and very low density lipoprotein- cholesterol levels were lower compared to the control. Atherogenic index of the group administered 300mg/kg body weight was significantly ($P<0.05$) higher compared to the control. The histological examinations of section of the heart reveal chamber dilation, hypertrophy and focal atrophy. The study suggests that consumption of dried lake salt for 4 weeks caused alteration to heart tissue and may cause heart related diseases in rats.
Keywords: Peripartum cardiomyopathy, Dried lake salt, Postpartum, Pregnancy

INRODUCTION

Peripartum cardiomyopathy (PPCM) is a dilated form of cardiomyopathy that causes deterioration in cardiac function presenting typically between the last month of pregnancy and up to five months postpartum (Demakis *et al*., 1971; Reimond and Rutherford, 2001; Sliwa *et al*., 2006). It involves systolic dysfunction of the heart with a decrease in the left ventricular ejection fraction with associated congestive heart failure and for this reason the heart muscle cannot contract forcefully enough to pump adequate amount of blood for the needs of the body's vital organs (Pearson *et al*., 2000; Elkayam *et al*., 2005). It is recognized as a separate entity from idiopathic dilated cardiomyopathy because of its distinct epidemiologic characteristics (Person *et al*., 2000), relatively rapid onset, and association with distinctive autoantigens and autoantibodies (Ansari *et al*., 2002). The global incidence of peripartum cardiomyopathy is not known due to lack of population based data but hospital data suggest that the incidence varies. The reported values are 1 per 15,000 live births in United States (Mielniczuk *et al.,* 2006), 1 per 350 live births in Haiti (Fett *et al*., 2002), 1 per 1,000 live births in South Africa and as high as 1 per 100 live births in Nigeria (Desai *et al*., 1995; Sliwa *et al.,* 2006).

Oxidative stress is known to rise during pregnancy, culminating in the last trimester and this runs parallel to increased total antioxidant capacity postpartum (Toescu *et al*., 2002). Increased production of free radicals and decreased antioxidant capacity occur in congestive heart failure (Ruffolo and Feuerstein, 1998; Sawyer and Colucci, 2000). This pro-oxidant shift in the intracellular redox state may induce cell death by either direct cell membrane damage through lipid peroxidation or apoptosis through activation of transcription factors (Buttke and Sandstrom, 1994). These changes do not only occur in cardiomyocytes but also in cardiac sympathetic nerves, which are very sensitive to oxidative damage (Thompson *et al.,* 1998). Nigeria recorded the highest incidence of peripartum cardiomyopathy with a striking geographical variation in the incidence with a high rate in northern than southern part of the country (Davidson *et al*., 1974; Isezuo and Abubakar, 2007). Women in northern Nigeria are involved in unique customary puerperal practices including consumption of dried lake salt locally usually as 'kunun kanwa' and daily hot water bath for 40 days (Davidson and Parry, 1978). These practices are still common in Sokoto and have been proposed to cause volume overload and heart failure (Isezuo and Abubakar, 2007). It is on this basis that the study was designed to investigate the effect of dried lake salt on lipid profile and heart tissues in female albino rats.

MATERIALS AND METHODS

Chemicals and Reagents

Analytical grade chemicals and reagents were used for this study.

Source of Dried Lake Salt (Kanwa)
The dried lake salt was sourced from the Central Market, Sokoto, Nigeria

Experimental Protocol
Female nursing albino rats weighing between 180-200g were used for the study. The rats were purchased from the Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria. They were allowed access to clean water and food *ad libitum* before and during the experimental period. The animals were randomly divided into 4 groups of 5 rats each and were fed pelletized growers' feed (Vital Feeds, Jos, Nigeria). Group I served as control and was given distilled water, group II, III, and IV were administered 100mg/kg, 200mg/kg, 300mg/kg respectively of dried lake salt solution orally for 4 weeks. The animals were allowed to fast overnight and blood samples were collected from the animals through cardiac puncture into clean labelled test tubes. The blood was allowed to clot and then centrifuged at 4000 rpm for 10 minutes. The serum was used for the analysis of lipid profile. The animals were sacrificed and the heart of each rat was dissected out and stored in containers containing 10% formalin solution for histopathologic study.

Biochemical Analysis
Lipid Profile
Serum total cholesterol was estimated by method of Allain *et al*. (1974). Triglyceride was measured as described by Tietz (1990) while high density lipoprotein-cholesterol was estimated by the method of Burstain *et al*. (1970). Serum low density lipoprotein-cholesterol and very low density lipoprotein-cholesterol were calculated by the formula of friedewald *et al*. (1972) and Atherogenic index was calculated as ratio of LDL-cholesterol to HDL-cholesterol (Abbott *et al.,* 1988)

Tissue Processing for Histopathologic Study
The tissue was chemically fixed and transferred to a cassette, a container designed to allow reagents to freely act on the tissue inside. The cassettes were then immersed in multiple baths of progressively more concentrated ethanol (10%, 40%, 70% and 90% alcohol) so as to dehydrate the tissue. This was then followed by immersion in toluene and later paraffin. The processed tissue was then taken out of the cassette and set in a mould. Additional paraffin was then added to make a 'paraffin block' attached to the outside of the cassette. The paraffin block containing the tissue was then sectioned using a microtome. The paraffin section was then removed by floating on a hot water bath. The tissues were then mounted on glass slides and stained using a combination of hematoxylin and eosin to produce the contrast needed to visualize the tissue with microscope.

Statistical Analysis
The data is expressed as mean ± SEM and ANOVA was used to analyse biochemical parameters followed by Dunnett's multiple comparison test using GraphPad Instat software (Version 3.0, San Diego, USA). A P value of <0.05 was considered significant.

RESULTS
The effect of dried lake salt on serum lipid profile is presented in Table 1. The result indicated the group administered 300mg/kg of the dried lake salt has significantly ($P<0.05$) lower high density lipoprotein – cholesterol as compared with the control. There was no significant ($P>0.05$) decreased in triglyceride, total cholesterol and very low density lipoprotein-cholesterol as compared to the control but low density lipoprotein-cholesterol increased in dose dependent manner, although not significant ($P>0.05$). The group administered 300mg/kg of the dried lake salt has significantly ($P<0.05$) higher atherogenic index as compared with the control.

Table 1: Effect of Dried Lake Salt on Serum Lipid Profile of Albino Rats

Grp	TC (mmol/l)	TG (mmol/l)	HDL-C (mmol/l)	LDL-C (mmol/l)	VLDL-C (mmol/l)	AI
I	2.54±0.10	0.91±0.05	0.63±0.06	1.49±0.07	0.42±0.01	2.36±0.38
II	2.25±0.34	0.84±0.07	0.47±0.05	1.54±0.04	0.38±0.05	3.27±0.67
III	1.73±0.24	0.78±0.08	0.39±0.09	1.57±0.06	0.35±0.02	4.02±0.50
IV	2.23±0.13	0.64±0.08	0.33±0.10*	1.65±0.05	0.34±0.04	5.00±0.73*

Grp I- control, Grp II- administered 100mg/kg of dried lake salt, GrpIII- administered 200mg/kg of dried lake salt, Grp IV- administered 300mg/kg of dried lake salt. TC- total cholesterol, TG- triglyceride, HDL-C- high density lipoprotein-cholesterol, LDL-C- low density lipoprotein-cholesterol, VLDL-C- very density lipoprotein-cholesterol. AI- atherogenic index *P<0.05 when compared with control by Dunette's multiple comparison test.

The result of correlation coefficient of concentration of dried lake salt against atherogenic index, LDL-C and HDL-C is presented in Table 2. There was significantly ($P<0.05$) negative correlation between concentration of dried lake salt and HDL-C while AI and LDL-C show significant ($P<0.05$) positive correlations.

Table 2: Correlation Coefficient (r) of Concentration of Dried Lake Salt against AI, LDL-C and HDL-C

Parameter	Correlation Coefficient (r)	P Value
AI	0.998	0.0012
LDL-C	0.982	0.0176
HDL-C	-0.732	0.0268

AI- atherogenic index, HDL-C- high density lipoprotein-cholesterol, LDL-C- low density lipoprotein-cholesterol

Photomicrograph (Histopathology) of the Heart

The section of the heart of normal rat is presented in Figure 1a and 1b showing endocardium, myocardium and pericardium. The myocardium is composed of round to spindle shape with regular nuclei and abundant cytoplasm. The section of the heart of rats administered 100mg/kg of dried lake salt is presented in Figure 2a and 2b showing chamber dilatation and ventricular atrophy. The section of the heart of rats administered 200mg/kg of dried lake salt is presented in Figure 3a and 3b showing chamber dilatation and atrophy. The section of the heart of rats administered 300mg/kg of dried lake salt is presented in Figure 4a and 4b showing chamber dilatation and hypertrophy

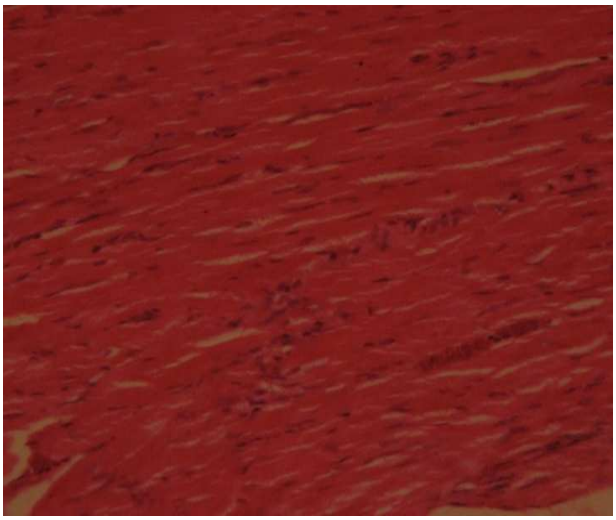

Figure 1a: Photomicrograph of Normal Heart Cardiac Myocyte Stained with H and E x200

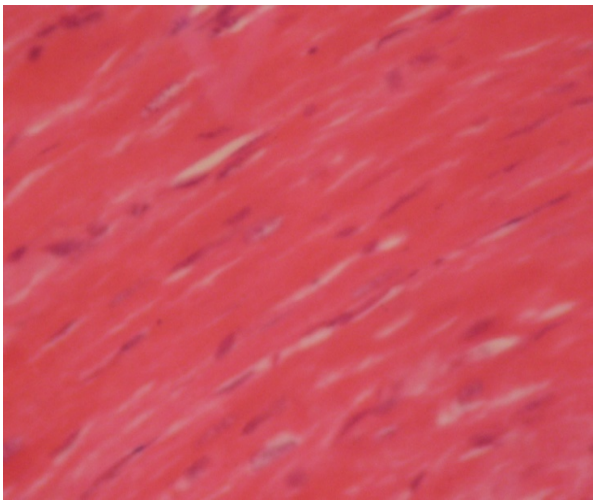

Figure 1b: Photomicrograph of Normal Heart Cardiac Myocyte Stained with H and E ×400

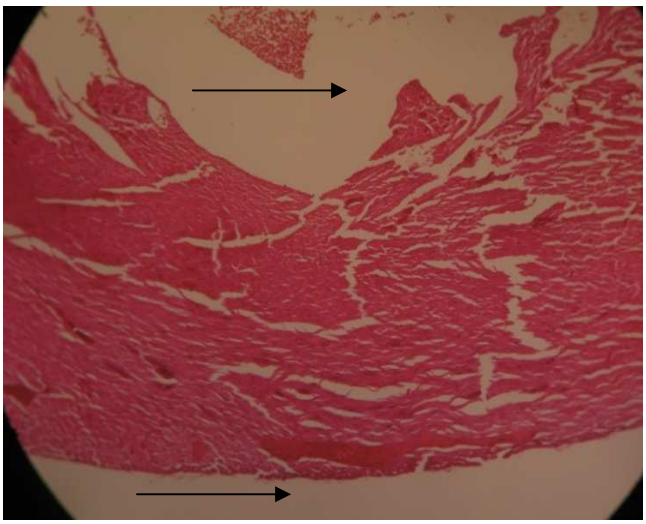

Figure 2a: Photomicograph of Heart showing Chamber Dilatation Stained with H and E x40

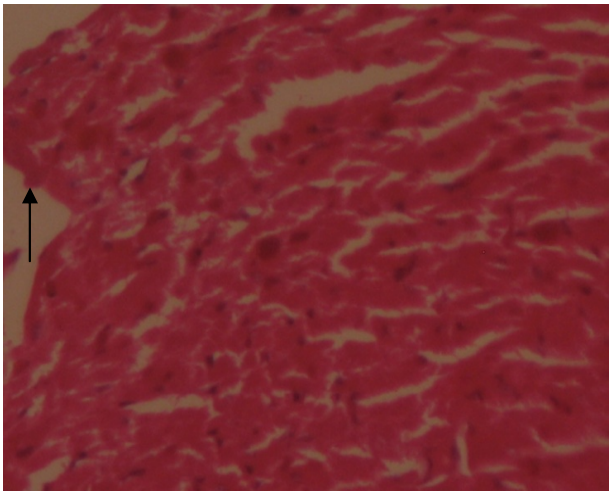

Figure 2b: Photomicrograph of Heart showing Ventricular Atrophy Stained with H and E x200

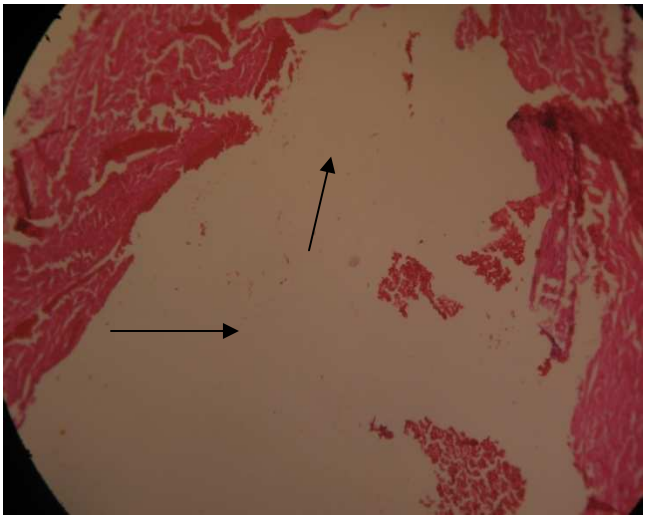

Figure 3a: Photomicrograph of heart showing Dilatation of chamber Stained with H and E x100

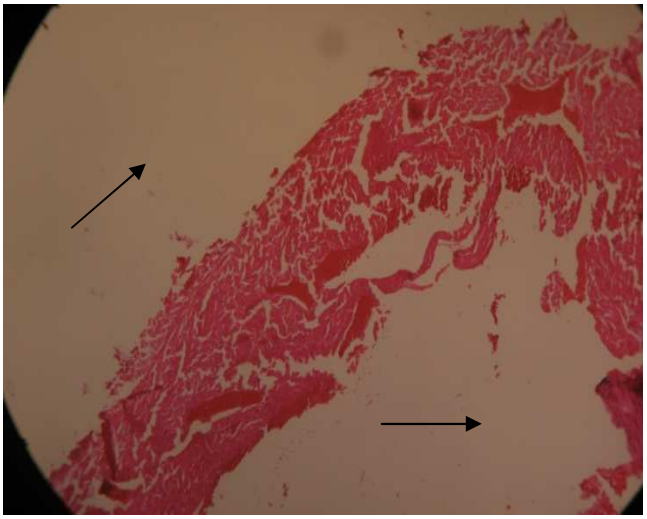

Figure 3b: Photomicrograph of Heart showing Chamber atrophy Stained with H and E x100

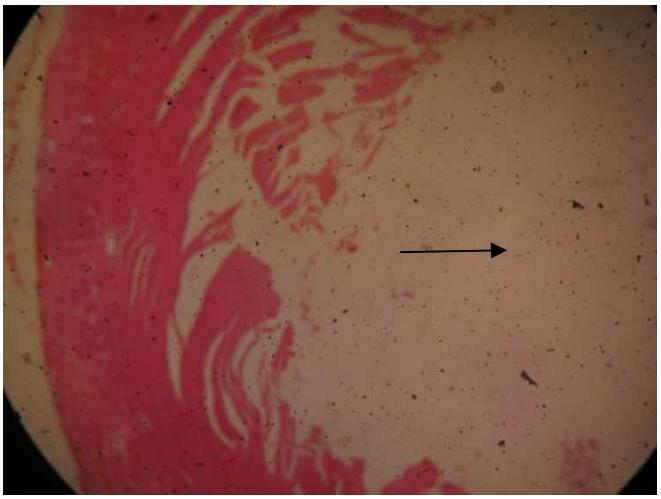

Figure 4a: Photomicrograph of Heart showing Chamber dilatation Stained with H and E x100

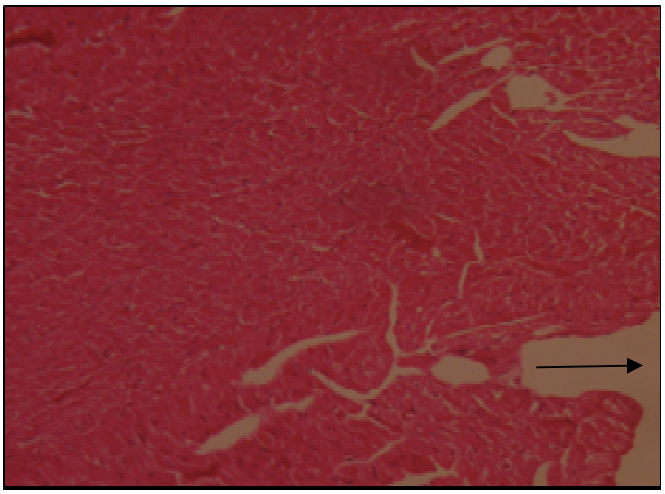

Figure 4b: Photomicrograph of heart showing Hypertrophy Stained with H and E x100

DISCUSSION

Peripartum cardiomyopathy is a devastating illness afflicting new mothers worldwide. Despite being relatively rare in many areas of the world, peripartum cardiomyopathy is nonetheless an important cause of morbidity and mortality in women of child-bearing age. For this reason, it has received keen attention by many researchers and investigators (Ramaraj and Sorrell, 2009; Selle *et al*., 2009; Ntusi and Mayosi, 2009; Tibazarwa and Sliwa, 2010). The lower levels of total cholesterol in the groups that were administered graded doses of dried lake salt, although not significant could be one of the factors that led to the changes observed in the heart of the rats. Studies have proposed that high cholesterol levels beneficially modulate inflammatory activation by neutralization of endotoxin (Rauchhaus *et al*., 2000). This assertion confirms the study that suggests low cholesterol levels are rather indicators of severe heart failure (Rauchhaus *et al*., 2003). LDL- C increased in dosage dependent fashion which suggests that high dried lake salt consumption may be linked to complications of peripartum cardiomyopathy as evidenced by the result of the histology of the heart. This could further buttress the result of atherogenic index which was significantly higher at 300mg/kg of the dried lake salt. High LDL-C

may undergo oxidation which can cause oxidative damage to the cardiomyocytes because of the sensitivity of these cells to lipid peroxidation and this could probably be responsible for the chamber dilation and hypertrophy observed in our study. Study by Geoge *et al.* (2006) indicates that oxidized LDL, a marker of oxidative stress was elevated in PPCM.

The histologic analysis of the heart indicate cardiac chamber dilatation, hypertrophy and focal atrophy all of which are cardinal features of PPCM in the groups administered different doses of dried lake salt.

The dilation of the heart chamber and ventricular hypertrophy might result from heart muscle weakening and reduced ventricular compliance. Hypertrophy and dilation are results of cardiac remodelling which may be deleterious because of high wall stress and increase in oxygen demand of the heart. The result of this study further confirm the report by Isezuo and Abubakar, (2007) that the practice of consumption of dried lake salt couple with hot water bath may be responsible for volume overload and heart failure observed in PPCM subjects in their study in Sokoto, northern Nigeria. The significantly positive correlations between AI and LDL-C against concentration of dried lake salt administered indicate that dried lake salt may precipitate atherosclerosis. The inverse relationship between concentration of dried lake salt and HDL-C may also interfere with anti atherogenic effect of HDL-C. Thus, alteration in the heart tissue may also be attributed to these changes in our study

To the best of our knowledge, this research is first of its kind to use dried lake salt to simulate PPCM in experimental animal model. The findings in our study are striking as indicated by the result of histology. We conclude that the practice of consumption of 'Kunum kanwa' as a puerperal practice may be the major risk factor for PPCM. Further researches are required in a large sample study to ascertain whether the consumption of dried lake salt is one of the major contributory factors in the pathogenesis of PPCM in northern Nigeria.

CONCLUSION

The findings of the study indicate that consumption of dried lake salt result in severe heart damage and play a role in the pathogenesis of PPCM as revealed in our experimental animal model.

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