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Lipid Profiles and Liver Function Parameters of Postpartum Rats Administered Dry Lake Salt (*Kanwa*)

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Authors' contributions

This work was carried out in collaboration between all authors. Authors YS and LSB designed the study. Authors SMD, AI and HID wrote the protocol and the first draft of the manuscript. Authors YS, SMD and UAU managed the literature searches, carried out analyses of the study and also managed the experimental process. All authors read and approved the final manuscript.

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ABSTRACT

Background: Peripartum cardiomyopathy (PPMC) is a rare but devastating cardiac failure of indeterminate etiology occurring in late pregnancy or early puerperium. Dry lake salt (*Kanwa*) is usually consumed in many parts of Nigeria as laxatives. In the Northern Nigeria, with a high prevalence of PPMC, it is consumed postpartum in large quantities as a traditional practice.
Objectives: This work investigated the effect of *kanwa* on serum lipid profile, some liver function indices, blood pressure and body weight of postpartum rat administered graded doses of *kanwa*.
Place and Duration of Study: Department of biochemistry, Usmanu Danfodiyo University Sokoto, between July 2012 and February 2013.
Methodology: The female rats were grouped into 4 of five animals each postpartum. The rats in the groups were administered 0 mg/kg (control), 100 mg/kg, 200 mg/kg, and 300 mg/kg body

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weight respectively of *Kanwa* orally for four weeks.
Results: The results indicated a significant decrease in HDL-C [group III - 13.20±10.7 mg/dl and group IV - 10.95±9.97 mg/dl] when compared with the controls ($p<0.01$). There is significant increase in TAG especially in group IV (357.30±25.76), while all other lipid profile parameters (T.CHOL, VLDL-C, and LDL-C) assayed were found to have no significant different from the control ($p>0.05$). However, there is a non significant ($p>0.05$) increase in atherogenic index of the treated group as compared with the control. Moreover, blood pressure as well as body weight results are all considered not significantly different from control ($p>0.05$). Lastly, the liver function indices are all found to have no significant changes from the control also.
Conclusions: The result indicated that *kanwa* may play a significant role in the pathogenesis of the PPMC.

Keywords: Kanwa; lipid profile; PPCM; blood pressure.

1. INTRODUCTION

Potash or Natron is generally referred to as *Kanwa* in the northern part of Nigeria and a product of the salt industry in many parts of northern Africa. It occurs as a mixture of different substances with sodium constituting about 30% and other minerals such as potassium, iron, zinc etc in varying proportions depending on locations [1]. The level of potassium is quite below detection in most of the specimen analysed [2]. This indicates that its chemistry is misunderstood by those describing it as "potash". The main mineral constituent identified is trona, $Na_3H(CO_3)_2.2H_2O$, which is basically sodium bicarbonate with water of crystallization. Traces of halite (NaCl) and quartz (SiO_2) were also found to be present [3]. There are different varieties and its composition varies with respect to their locations [1]. Natron has several uses: it is used in cooking as a food tenderizer especially in meat and pulses [4]; [5], to curdle milk, in the tanning industry and in the preparation and enhancement of flavor of local beverages and snuff [6]. It also serves as a salt lick and mineral supplement in ruminants [7] and used in decoctions for the treatment of reproductive ailments such as retained placenta and difficulty in urination. Ethnomedicinal potency has been documented for ailments such as stomach ache, constipation and toothache [6]. Based on these used evidences of *kanwa* we can say that it can be classified among the functional foods. The term is defined by the US Institute of Medicine's Food and Nutrition Board as ''any food or food ingredient that may provide a health benefit beyond the traditional nutrients it contains'' [8]. Functional food: it is similar in appearance to, or may be, a conventional food, consumed as part of a usual diet, and demonstrated to have physiological benefits or reduce the risk of chronic disease beyond basic nutritional functions. However many of the work done on functional foods are from plants perspective [9]. The toxic nature of *kanwa* was noticed in the nursing mothers around Zaria and Malumfashi areas of Northern Nigeria due to the intake of almost 30-40 mg per day [10], this resulted in Peripartum cardiac failure (PPCF) forty days after birth, consequently, such patients suffers from anemia and hypertension [10].

Peripartum and Postpartum Cardiomyopathy (PPCM) is a form of dilated cardiomyopathy that is defined as deterioration in cardiac function presenting typically between the last month of pregnancy (peripartum) and up to five months after pregnancy (postpartum) [11]. PPCM involves systolic dysfunction of the heart with a decrease of the left ventricular ejection fraction (EF) with associated congestive heart failure and an increased risk of atrial and ventricular arrhythmias, thromboembolism (blockage of a blood vessel by a blood clot), and even sudden cardiac death [11]. PPCM is a diagnosis of exclusion, wherein patients have no prior history of heart disease and there are no other known possible causes of heart failure [12]; [13], but now researchers are investigating cardiotropic viruses, autoimmunity or immune system dysfunction, other toxins that serve as triggers to immune system dysfunction, micronutrient or trace mineral deficiencies, and genetics as possible components that contribute to or cause the development of PPCM [11]. Peripartum cardiomyopathy (PPCM) is a devastating illness afflicting new mothers worldwide. Despite being relatively uncommon in many areas of the world, PPCM is nonetheless an important cause of morbidity and mortality in new mothers. For this reason, it has received welcome attention by investigators in the past decade, with numerous reports and recent general reviews. In Nigeria, it is found to be prevalent in the northern part of

the country which is hugely attributed to the traditional practices in the region in which they take large amount of dried lake salt (*kanwa)* in form of porridge called '*kunun kanwa'*. This is attributed to the large sodium intake in *kanwa* which is one of the predisposing factors of PPCM as revealed by Davidson and co [10]. Therefore this research was design to study the effect of *kanwa* on some biochemical parameters in female albino rat postpartum.

2. MATERIALS AND METHODS

2.1 Chemicals, Reagents and Equipments

All the chemicals, instrument, apparatus and reagents used for this work were analytically graded and purchased from reputable chemical laboratories.

2.2 Experimental Animals

Wistar albino rats of both sexes weighing 135-209 kg body weights were used for this study. The animals were purchased from Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria. The animals were kept in a wire mesh cages at animal house and divided into 4 groups and were allowed to acclimatize for two weeks before the commencement of the experiment. They were fed with pelletized growers' feed (Vital feed, Jos, Nigeria) and were allowed access to water. The rats were treated according to the rules and regulations of animal ethics.

2.3 Collection and Preparation of *'Kanwa'*

The *'kanwa'* used was obtained from fish and vegetable market, Sokoto state. The sample was visually observed to notice its physical properties. It is then carefully dried, ground with mortar and pestle, and then sieved with a small size mesh. The sample were then weighed on a balance and stored in a stoppage container at room temperature until used. This was followed by the oral administration of 100 mg, 200 mg and 300 mg/kg to the various groups respectively of this preparation to the albino rats based on their weight except for the control which was administered with water only.

2.4 Acute Toxicity Studies

The animals were grouped into five groups of 1 animal each. Using 3000 mg/kg body weight, the sample was then administered in single oral dose per kg body weights of the rats at a minimum of 48 hours interval for each animal. The animals were weighed before and after the administration. The animals were observed for toxic symptoms such as weakness or aggressiveness, loss of weight, diarrhea, discharge from eyes and ears, noisy breathing and mortality [14].

2.5 Experimental Design

The animals were allowed to delivered and then randomly divided into four groups of 4 rats each.

Group I Normal untreated (control)
Group II Treated with 100 mg/kg of *kanwa*
Group III Treated with 200 mg/kg of *kanwa*
Group IV Treated with 300 mg/kg of *kanwa*

The appropriate dosages of the 'kanwa' were administered to the animals orally once daily by intubation using intravenous cannula tube for 4 weeks.

2.6 Measurement of Blood Pressure

The baseline blood pressure was measured and subsequent measurements were done every week. This was done by tail-cuff method using non-invasive Ugo Basile, series 58500 blood pressure recorder. The rat was placed in the restrainers and kept in a scanner for 30 minutes to warm the animal prior to obtaining pressure measurement. A cuff was placed on the base of the tail to occlude the blood flow. A transducer was placed close to the cuff which measured the pulse rate. Upon deflation, the non-invasive blood pressure sensor was utilized to monitor the blood pressure and the average of three readings was taken for each rat and the temperature of the rat were monitored throughout the measurement period.

2.7 Blood Sample Collection and Preparation of Serum

The animals were allowed to fast over night before the blood was collected after the last treatment, the animals were anaesthetized with chloroform vapor and blood samples were collected through cardiac puncture into labeled tubes for biochemical analyses. The labeled centrifuge tubes were allowed to stand for 30 minutes to clot and centrifuged at 4000 g for ten minutes and the serum obtained was pipette into labeled tubes.

2.8 Biochemical Analysis

2.8.1 Estimation of serum total cholesterol

Serum total cholesterol (TC) was quantified by enzymatic method using Randox kit [15].

2.8.2 Estimation of serum HDL- C

This was done by enzymatic method using Randox Kit [16].

2.8.3 Estimation of serum triglyceride

This was assayed by the method of Tietz [17] using Randox Kit.

2.8.4 Estimation of serum LDL- C

This was calculated using Friedewald formula [18].

$$\text{LDL –C (mg/dl)} = \text{TC} - (\text{HDL- C}) - \left(\frac{TG}{5}\right)$$

2.8.5 Estimation of serum VLDL- C

This was calculated using Friedewald formula [18].

$$\text{VLDL-C (mg/dl)} = \frac{TG}{5}$$

2.8.6 Estimation of atherogenic index

This was calculated as the ratio of LDL-cholesterol to HDL-cholesterol [19].

2.8.7 Estimation of serum total protein

This was assayed by Biurette method using Randox kit.

2.8.8 Estimation of serum albumin

Albumin was estimated by Bromocresol green (BCG) method using Randox kit.

2.8.9 Estimation of serum globulin

Serum globulin = total protein – albumin

2.8.10 Estimation of serum aminotransferases and alkaline phosphatase

These are all assayed by calorimetric end point method.

2.9 Statistical Analysis

The data is expressed in form of mean **±** standard deviation with $p>0.05$ all considered not significantly different from the control, with ANOVA using instat3 software.

3. RESULTS AND DISCUSSION

3.1 Acute Toxicity Studies

All the animals were observed for 2 days each (48 hours) and neither mortality nor toxic manifestation was recorded. Therefore LD_{50} was greater than 3000 mg/kg body weight of the animal (LD_{50}>3000 mg/kg).

3.2 Blood Pressure

The blood pressure of the female albino rat treated with *kanwa* is presented in Table 1. The result indicated that there is no significant ($p>0.05$) change in the treated animals when compared with the control.

3.3 Body Weight

The body weight of the female albino rat treated with *kanwa* and control is presented in Table 2. The result indicated that there is no significant ($p>0.05$) change in the treated animals when compared with the control.

3.4 Lipid Profile Parameters

The lipid profile parameters of the female albino rat treated with *kanwa* and control is presented in Table 3. Among all the lipid profile parameters of treated animal, HDL-C was found to have a significant decrease ($p<0.01$) when compared with the control in a dose dependent manner. Also TAG levels increases significantly especially in group IV, while LDL-C, and VLDL-C are considered non significant ($p>0.05$). However, AI is found to have non-significant increase in a dose dependent manner at $p>0.05$.

3.5 Liver Function Parameters

This was presented in Table 4. All the liver function parameters investigated in this study was found to be non-significantly different from the control group ($p>0.05$).

Table 1. Blood pressure of female albino rats treated with *kanwa*

Weeks		Group I	Group II	Group III	Group IV
Before	SP	119.83±2.12	117.19±2.75	125.57±8.31	143.00±3.61
	DP	79.17±0.71	79.25±0.35	78.96±1.77	79.06±0.59
	PR	164.84±1.18	283.63±19.2	241.93±40.65	180.83±21.36
First week	SP	119.38±0.53	120.5±0.00	119.83±0.46	119.87±2.72
	DP	79.75±0.35	78.56±0.68	78.98±0.68	79.36±0.26
	PR	281.88±36.59	270.46±16.87	270.46±16.87	252.38±39.16
Second week	SP	118.84±2.60	120.46±0.88	120.19±0.38	120.84±1.00
	DP	80.33±1.42	77.75±1.19	79.56±0.34	80.42±1.14
	PR	255.50±14.38	276.44±26.45	261.27±4.23	276.58±33.15
Third week	SP	117.34±6.13	120.09±0.65	120.65±1.04	120.86±0.75
	DP	82.67±4.72	79.25±0.96	80.19±1.28	79.61±0.67
	PR	239.38±74.07	257.77±85.78	306.15±56.73	274.53±86.01
Forth week	SP	123.37±0.53	123.42±0.56	123.13±0.83	122.64±1.85
	DP	75.88±0.53	77.71±2.31	77.62±1.53	77.64±2.19
	PR	176.63±6.19	222.27±36.29	265.15±95.57	240.00±22.96

Sp- Systolic pressure, Dp- Diastolic pressure and Pr- Pulse rate

Table 2. Body weight of female albino rats treated with *kanwa*

GP	Before	1st week	2nd week	3rd week	4th week
I	**ND**	**ND**	135.62±11.63	138.67±12.42	146±16.97
II	209±45.38	200.25±18.75	200±18.66	195.25±20.65	203±2 4.81
III	201.5±18.30	201.5±18.41	202.25±19.67	205.5±21.70	208.75±17.37
IV	205.25±69.06	195.2±46.25	180.0±39.03	189±51.06	193.33±54.72

***ND**- not detected, GP- group*

Table 3. Lipid profile parameters of female albino rat treated with *kanwa*

	T.CHOL (mg/dl)	TAG (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	VLDL-C (mg/dl)	AI (mg/dl)
I	197.00±0.28	281.30±50.35	48.82±4.31	91.92±14.67	56.26±10.07	1.904±0.47
II	174.3±56.82	275.20±109.98	24.63±7.24	94.64±41.68	55.04±22.00	4.43±2.86
III	134.05±40.81	239.40±120.12	13.20±10.7**	58.53±36.05	47.88±24.03	7.48±5.81
IV	172.67±48.81	357.30±25.76	10.95±9.97**	82.53±49.63	71.46±5.15	9.35±3.98

T.CHOL - total cholesterol, TAG- triacylglycerol, HDL-C- high density lipoproteins cholesterol, LDL-C- low density lipoproteins cholesterol, VLDL-C - very low density lipoproteins cholesterol, AI- atherogenic index

Table 4. Liver function parameters of female albino rat treated with *kanwa*

Parameters	Group I	Group II	Group III	Group IV
Albumin (g/dl)	0.84±0.007	2.14±0.70	1.29±0.58	0.79±0.58
Total protein (g/dl)	4.50±0.13	5.84±0.97	5.38±1.37	4.40±0.40
Globulin (g/dl)	3.65±0.12	3.69±1.43	4.08±0.82	3.60±0.26
ALP (U/L)	8.23±2.59	2.44±1.85	3.96±4.19	4.57±3.00
ALT(U/L)	36.23±0.49	24.46±1.28	29.11±7.65	35.31±3.76
AST (U/L)	19.70±1.86	26.19±5.16	15.28±4.05	20.36±3.27

ALP- alkaline Phosphatase, ALT- Alanine transaminase, AST- Aspartate transaminases

3.6 Discussion

Peripartum cardiomyopathy is a rare but devastating cardiac failure of indeterminate etiology occurring in late pregnancy or early puerperium [20]. From Table 1 above, the values of Blood pressure, as well as that of pulse rate of treated animal showed no significant (p>0.05) different from that of the control. This showed that *kanwa* may have no effect on the blood pressure. These contradicted findings of Davidson et al. [10] as they reported exhibition of

hypertension in nursing mothers taking *kanwa*, which may well, as they reported, increase extracellular fluid and plasma volumes and lead to increased cardiac work. The contradiction might be due to the difference in the amount of dosage as well as duration of administration or may be anonymous reason. However, Carson and Ooi [21] reported that the blood pressure may be normal in PPCM patients. From Table 2, administration of *Kanwa* has no significant impact on the body weight of the treated animals; as such the body weights are not affected at the given dosage. However, the large variability of body weight that exists between the treated animals and the control group make it difficult to directly draw conclusions. From Table 3, HDL-C shows a significant decrease in a dose dependent manner, especially for group 2 and 3 when compared with untreated group (p<0.01). There is significant increase in TAG (357.30±25.76) and atherogenic index (9.35±3.98) coupled with decrease in HDL-C (10.95±9.97) in group IV when compared to non-significant difference between other groups (II and III) and control group, suggests potential risk of developing cardiomyopathy and other possible cardiac problems. This is consistent with Muhammad et al. [22], as they reported dose dependent decrease of HDL-C. These results show characteristic dyslipidemia in the treated animals of this study. Dyslipidemia are lipid disorders that can accelerate the atherosclerosis process and its consequences, such as heart failure and coronary atherosclerosis [23,24]. A meta-analysis has confirmed that higher salt intakes are associated with a greater incidence of strokes and cardiovascular events [25]. In this study, although we used a variant of normal salt, but *kanwa* was also found to have a significant similarity with normal salt [1]. It was also reported that, metabolic abnormalities in the postprandial state are known to contribute to endothelial dysfunction and atherosclerosis progression in healthy people [26]. Meanwhile, Ross, [27] considered endothelial dysfunction, to be an initial step in the development of atherosclerosis, and it has been shown with higher salt intakes by Bragulat et al. [28] and Tzemos et al. [29]. It was also described by Ceriello et al. [30] that; increases in metabolic factors, such as glucose and triglycerides, have been shown to induce endothelial dysfunction postprandially by increasing oxidative stress and decreasing the bioavailability of nitric oxide.

Total cholesterol, LDL-C, and VLDL-C all shows a non significant difference (p>0.05), while Atherogenic index shows a non-significant increase in treated animals when compared with the control in a dose dependent manner. All the liver function parameters investigated in this study was found to be non-significantly different from the control group (p>0.05).

4. CONCLUSION

In all the treated animals, derangements in lipid metabolism are presented which can accelerate the atherosclerotic process and its consequenses. This can be a possible mechanism of developing peripartum cardiomyopathy in individual taking dried lake salt. The limitation of the study is; relatively small numbers of experimental animals were used.

5. RECOMMENDATIONS

Further studies should also focus on the compositional analysis (both qualitative and quantitative) of the main constituents of *Kanwa.*

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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