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This work was carried out in collaboration between all authors. Author RAD designed the study, performed statistical analysis, and wrote both protocol and first draft of the manuscript. Authors ML and RSUW supervised collection and arrangement of the data for the study. IMS help with some literature, SU immensely contributed also in the literature review while TSS further proofread and arranged the paper for publication. All authors managed literature searches, arrange tables and graphs. All authors read and approved the final manuscript.

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Comparison of Lipid Parameters in Rats Exposed to Hypertensive Condition and Given Separate Interventions of Camel Milk and Urine

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Abstract:

Lipid parameters; mainly Triglycerides (TRIG), Total Cholesterol Content (TC), cholesterol for lipoproteins of High Density, (HDL) and cholesterol for lipoproteins of Low Density (LDL) were measured from different rats recruited for both test and control study in rats exposed to hypertensive state for 28 days. This research was aimed to evaluate and identify if these mentioned key lipid values were adversely affected consequent from camel milk and urine separate intervention within hypertensive study setting. The controls have hypertensive positive rats; PC in addition to their negative; HNC and normal; NC peers. There are 3 graded Camel Milk (CM) and Camel Urine (CU) tests components each. From the blood sera of the killed rats; the aforementioned lipid parameters were determined. The obtained values were compared between all the 5 research groups. TRIG, HDL and TC values from both graded milk and urine tests align much with their NC and PC peers in the dual study. The HNC however have differed with some significance from both the CM/CU tests and the other instituted controls; which is the (NC/PC) too. In conclusion, the key lipid parameters studied were not adversely affected by their interaction with CM and CU among the separate hypertensive-treated, hypertensive positive and normal controls set ups.

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INTRODUCTION

Measurable lipids found in the blood serum which features some of the test routinely done to evaluate cardiovascular health may be modified (Amit *et al*., 2011, Ankur *et al*., 2012). Hyperlipidemia defined or indicated by excessive presence of lipids and or substances containing fat in the serum has become etiological parameters crucial to the occurrence of atherosclerosis including ischemic cerebrovascular illness and coronary heart diseases (Grundy and Vega, 1988, Onwe *et al*., 2015). It may be caused due to either defective traits in the genetic composition or by some marked metabolic imbalance with natural etiology such as hypothyroidism, primary biliary cirrhosis, diabetes mellitus and excessive alcohol intake, which this last one being habit associated life style (Begun and Aslama, 2012). Still other pathological conditions including pancreatitis, myocardial infarction and heart attack may all be linked to the direct dyslipidemic levels present in the blood (Balamurugan and Shantha, 2010).

Yet still higher blood levels of cholesterol lipoprotein content that doesn't belongs to High Density and Low Density might be as a result of adverse consequences of obesity, diet, or when illnesses associated with hereditary root and cause (such as defective receptor-protein alterations to its genetic composition for LDL in classical hypercholesterolemia involving the family) as pointed earlier or due to type 2 diabetes and other diseases that may be present (Durrington, 2003).

For cardiometabolic risk assessment, blood monitoring of lipid levels and its evaluation have become vital and integral part in establishing the true cause of what went wrong and diagnosing the CVDs (Kasia *et al*., 2020, Zalts *et al*., 2024). Due to its serving as major therapeutic target in prevention of both the primary and secondary atherosclerotic cardiovascular diseases (ASCVD) as well as in recognition of critical role it usually played in atherogenesis; the LDL happened to be the most important component that is measured; albeit not being estimated directly (Mach *et al*., 2019). The stability of lipid parameters samples have become crucial in the subsequent analysis of cholesterol contents belonging to either lipoproteins of high-density (HDL-c), or for low density (LDL-c) categories, as well as cholesterol content in totality (TC), and triple glyceride fatty acids (TGs) (Kasia *et al*., 2020).

Many works were reported on various effects of raw camel milk alone or raw camel urine and or their combination; to cite but few is CM exhibit hypotensive roles (Dogondaji *et al*., 2023), it has hepatoprotective effect (Dogondaji *et al*., 2024) and its effect when together with CU there is presence of some demonstrable verifiable credence, although drawn from few clinically done studies and some tests done in the laboratory pointing the two were proven to be effective agents used to remediate different pathological conditions that may include autism, viral hepatitis, type 2 diabetes, allergy involving food, tumor and other infections from bacteria, parasite and virus (Abdel Gader and Alhaider, 2016). And for long time it was acknowledged that they provide alleviation of certain ailments that include asthma, dropsy, jaundice and anti-hypertensive (Asresie and Yusuf, 2014; Yadav and Kumar, 2015).

Previous studies have also confirmed that CM distinctively possess properties that can counters fungal, viral and bacterial infections (El Agamy *et al*., 1992; Conesa *et al*., 2008), anti-hepatitis, antiplatelet (Alhaider *et al*., 2011), antioxidative factors, anticancer (Korashy *et al*., 2012), aging-protection, and other traits against thrombosis (Korish *et al*., 2015), as remedy for autoimmune diseases, possession of glucose lowering capabilities and remediation of tuberculosis condition and for processing of cleaning and cosmetic issues (Al-Juboori *et al*., 2013; Sharma and Singh, 2014).

In the light of the foregoing, this study aimed to address fatty acid containing substances that are usually found in the serum in normal setting and when hypertensive situation is evolved. Findings of this study are hoped that it will shed more light about these vital parameters in cardiometabolic screening whether or not they

become affected due to their interaction with the aforementioned camel products.

MATERIALS AND METHODS

Reagents and Known Chemicals

The L-NAME used for the hypertension induction was procured from German company (Sigma Aldrich). Kits used in measurement of these 3 lipid indices in the research and all other chemicals and reagents used were of analytical grade and are of purest quality available.

Camel Milk, CM and Camel Urine, CU samples collection and preservation

Raw milk and urine of (non-desert) healthy lactating camels (*Camelus dromedarius*) was obtained via hand milking done by some experienced camel attendants. Milk samples were collected after thorough sterilization of the udder during 6-7 hours in the morning, while urine samples was took overnight as the camels were urinating. The collected samples were packaged in the appropriate containers that were carefully labeled and transported immediately via vaccine carriers to the Laboratory where samples were kept at -80C until required further for subsequent analysis.

Preparation of experimental study animals and induction of hypertension

The Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria, Kaduna State, Nigeria has an Animal Center where different research Laboratory animals can be procured by purchase basis. 200-300g of different rat weights that were used in this research were all purchased from this center. The rats were then acclimatized for 1 week period to acquaint themselves with their new Laboratory environment under the following optimal conditions of relative humidity at 60%, room temperatures at (27-30°C) of an equally divided 24 hours (12-h light/12-h dark cycles). They were also kept and maintained on the usual laboratory feed (Optima Feeds®, Zaria, Kaduna State, Nigeria) and they get their drinking tap water without any limitation. All animal experiment conforms to the laid down guidelines corresponding to the universal procedure for laboratory animal care and use based on the National Institutes of Health (NIH) recommendations (NIH publication 85-23, revised 1985).

Rats were randomly arranged and distributed into 5 rats per each group for a maximum total of 6 groups and kept in their labeled cages at the above stated optimal laboratory conditions. The experiment commenced after one week acclimatization period. This reported research was conducted for up to 1 month; terminating on the 29th day (24h post last treatment). The schedule of the conducted tests was as described hereunder: The normotensives category comprised of 3 groups; which includes in the reverse order the 3rd group, also called the reference group or hypertensive positive control, PC) that received orally daily amlodipine (10mg/kg/day) concurrently for 4 successive weeks. There is also the second group that is hypertensive negative control or (HNC) which received orally L-NAME only (50mg/kg/day) daily for 4 successive weeks; in addition during treatment period they received commensurate volume of distilled water. Finally the first group is the normal control, NC category that received only vehicle (Distilled Water) orally daily during treatment period for 4 weeks, they neither had induction of hypertension nor any intervention or treatment given to them.

The remaining 3 groups were of the test category. Also in the reverse order were groups 6, 5, and 4 that individually received orally raw camel milk according to their body weight dosed as (CM3) for group 6 that received (500mg/kg/day), (CM2) for group 5 which got (300mg/kg/day) and (CM1) for group 4 that was given (100mg/kg/day). In the (CU) groups too, the 6, 5, and 4 groups received orally CU administered at same dose concentration of 500mg/kg/day for group 6 (CU3), 300mg/kg/day for (CU2) & 100mg/kg/day for (CU1).

All the rats regardless of their cage or grouping they have had their food and water without any restriction. The dissolved L-NAME for hypertension induction was orally administered to all the rat groups with the exception of vehicle

or distilled water-only treated normotensive group at (50 mg/kg/day) during experimental period. The rat experiments were approved by the University Research and Ethics Committee (UREC) for Animal Care and Use and had followed guidelines stipulated by the National Animal Research Center (Taipei, Taiwan).

Lipid Parameters Assays

(a) Preparation of serum

After 1 day from the last treatment given to the rats which was also followed by 8-10 h of fasting, the rats were humanely killed through light anesthesia application by squeezing of cotton wool that was dipped into a (mixture of ether/chloroform) unto the rats' nostrils. The aim was for induction of rapid unconsciousness and subsequent death of the rats with minimal distress or pain. Thereafter, the rats were later killed by their slaughtering with a sharp blade. The blood oozing from the lower vena cava of the slaughtered rats was collected into test tubes containing anticoagulant. The blood samples were then centrifuged at 4000rpm/10min to separate serum from the plasma. The obtained serum was later used for the lipid parameters measurement, and the resultant plasma was stored at −80°C until it is needed for subsequent future use.

(b) Biochemical Analyses

The measured plasma lipid parameters [the trio of Triglycerides, Total Cholesterol content (TC), and cholesterol lipoproteins of High Density (HDL)] was determined by enzyme colorimetric methods using kits manufactured by (Accurex Biomedical limited Pvt. Ltd, India) as per the manufacturer's instruction manual along with other standard commercial kits. All the performed assays were made as duplicates and in each batch quality control sera were also analyzed. The 4^{th} or last index; the lipoprotein cholesterol content levels of Low-density category (LDL) was calculated using Friedewald *et al*., 1972 formula that minuses the determined values of the previous 3 parameters from the TC lipoprotein values.

Analysis of Statistical Data

The Fisher's least significant difference test was deployed to do the analysis of variance through the one-way (ANOVA) for all the obtained data. Statistical Analysis System software (SAS) developed by the SAS Institute, Cary, USA was made use of. Results were presented as mean ± SEM (Standard Error of Mean). A statistical significance is indicated when p values stood at <0.05.

RESULTS

The results obtained from the research are apprehended as given hereunder presented in the accompanying Tables 1 and 2. The obtained results compared between all the 5 research groups showed TRIG, HDL and TC values from both graded milk and urine tests have align much with their NC and PC peers in the dual study. The HNC however have differed with some significance from both the CM/CU tests and the other instituted controls; which is the (NC/PC) too.

DISCUSSION

In scientific research works that involves hypertension induction in animals especially the rodents an almost a *fait accompli* was the use of L-NAME and it is characterized by generalized deficiency of NO and increase in BP progressively if such L-NAME use is prolonged (Abdel-Rahman *et al*., 2017). This L-NAME use leads to the obstruction of NO synthase which ultimately caused elevation of serum cholesterol amount in rats (Khedara *et al*., 1996).

Foregoing from this study, though L-NAME was used for chronic hypertension induction in rats, it was only done to give room in knowing the effect and impact of CM and or CU interaction on the key lipid measurement indices within both non-hypertensive and hypertensive settings. SBP of rats measured from the tail-cuff method was found to have become significantly raised ($p < 0.001$) through the oral gavage administration of

(L-NAME); a key NOS inhibitor for up to four weeks period. Worthy of noting here is a fruit, *Lagenaria siceraria* (Molina) Standley (*Cucurbitacae*) which is another natural product; invariably from plant origin that has also displayed antihyperlipidemic activity (although distinct from animal source) in a high fat diet induced hyperlipidemia. Thus, even though not belonging from same natural kingdom; to some extent this research finding has agreed with (Ghule *et al*., 2009) earlier reports for antidyslipidemic activity.

Table 1. Lipid indices of hypertensive rats (inducted by L-NAME) administered raw camel milk only.

Lipids	NC	HNC	PC	CM1	CM2	CM3
TC (mg/dl)	94.2±0.7	114.6±0.6*	105.2±1.1	93.9±0.7#	94.1±1.3#	95.5±1.3
Trig (mg/dl)	74.8±1.4	107.4±0.9*	78.7±1.1	76.8±1.1#	72.5±0.8#	78.7±0.8#
HDL (mg/dl)	43.4±0.5	83.6±1.0*	45.0±0.9#	48.6±0.5#	47.1±1.0#	45.0±0.9#
LDL	10.2±0.6	27.9±1.1	18.6±1.1	10.1±0.6	10.5±0.5	9.6±0.7#

Values are mean ± SEM, (n=5). Bonferroni post hoc tests followed the One-way ANOVA. PC:-positive control= L-NAME+ amlodipine at 10mg/kg body weight/day; while HNC:-hypertensive negative control = L-NAME only (50mg/kg body weight/day); and NC:-normal control; = rats treated with just distilled water during treatment time. CM1:-raw camel milk treatment group at 100mg/kg body weight/day; CM2:-raw camel milk test given 300mg/kg body weight/day; CM3:-raw camel milk administered at 500mg/kg body weight/day.

TC:-serum cholesterol in totality; Trig:-Tri esters of glycerides, HDL:-serum lipoprotein cholesterol of high density; LDL:-serum lipoprotein content of low density category. * At when ($P<0.05$) denotes to significantly different from Normotensive control by using analysis of variance (ANOVA), (n=5), # ($p < 0.05$), statistically different with significance from L-NAME (50 mg/kg), ## ($p < 0.001$), much significantly different from L-NAME (50 mg/kg body weight/day).

Table 2. Lipid values of hypertensive rats (inducted by L-NAME) administered only raw camel urine.

Lipids	NC	HNC	PC	CU1	CU2	CU3
TC (mg/dl)	94.2±0.7	114.6±0.6*	105.2±1.1	93.5±0.4#	95.5±1.2	96.7±0.9
Trig (mg/dl)	74.8±1.4	107.4±0.9*	78.7±1.1	78.3±0.8#	75.3±0.8#	81.3±1.0#
HDL (mg/dl)	43.4±0.5	83.6±1.0*	43.7±0.7	45.3±1.5#	32.5±1.5##	38.0±2.0#
LDL	10.2±0.6	27.9±1.1	18.6±1.1	9.3±0.7	9.8±0.6	9.9±0.6

Values are mean ± SEM, (n=5). Bonferroni post hoc tests that was followed by One-way ANOVA. HNC:-hypertensive negative control; L-NAME only group at (50mg/kg body weight/day); NC:-normal control; that have not received any intervention; PC:-positive control, a dissolved L-NAME+amlodipine at (10mg/kg body weight/day); CU1:-raw camel urine test given at 100mg/kg body weight/day; CU2:-raw camel urine treatment group that received CU at 300mg/kg body weight/day; CU3:- treatment group that received raw camel urine at 500mg/kg body weight/day.

TC:-serum cholesterol in totality; HDL:-serum lipoprotein cholesterol content belonging to high density; LDL:-serum lipoprotein cholesterol content of low density. * At when ($P<0.05$) denotes to significantly different from Normotensive control by using analysis of variance (ANOVA), (n=5), # ($p < 0.05$), statistically different with significance from L-NAME (50 mg/kg), ## ($p < 0.001$), much significantly different from L-NAME (50 mg/kg body weight/day).

At end of four weeks of hypertension induction and CM/CU separate concurrent treatments; HDL cholesterol, triglycerides and total cholesterol indices were found to eventually increase with statistical significance in the HNC (L-NAME only) group, when compared to their normotensives. All the three graded CM and CU interventions were tilting towards maintaining the lipid values that synchronizes with not only NC but also the PC values. The antihyperlipidemic effect displayed by CU was herewith brought to the fore. At 100mg/kg dose there is sharp decrease ($p < 0.001$) in only triglyceride and cholesterol from the hypertensive negative control group (L-NAME only) compared to both NC, PC and other treatment groups. But at 300mg/kg there was significant decrease in all lipid indices of HDL, triglycerides and cholesterol compared to (L-NAME only) group. Even 500mg/kg dose of CU decreases ($p < 0.001$) only two parameters of HDL and cholesterol in the adversely affected negative control group.

The investigation reported in this research has confirmed another advantageous effect of raw

CM and raw CU towards countering and normalizing the abnormal high lipid values in the hypertensive situation except for the few result outliers comparing PC result for TC with that of NC treatment. In the present L-NAME model of hypertension used; a reduction in cholesterol in CM+L-NAME and CU+ L-NAME treated rats have further brought to the fore the antihyperlipidemic effect of CM/CU. Thus, as reported in details in this investigation CM/CU on one hand when compared to the amlodipine drug both showed concurrence displaying antihypertensive and antihyperlipidemic effects respectively.

CONCLUSION

It is safe to make a bold conclusion based on what was reported in the research that simultaneous and dual administration of CM or CU or amlodipine with L-NAME have reduced with significance the observed elevated blood pressure. Not only that also there is no any untoward repercussions detected that affects the serum lipid levels among all the studied rats at the adopted experimental conditions. The raw crude protein earlier detected in proximate composition analysis of both CM and CU separately published may appear to have contributed to this antihyperlipidemic activity. It is thus concluded that CM and CU individually or acting together in synergy possesses antihyperlipidemic activity.

CONFLICT OF INTEREST

The authors hereby state that they do not have any conflicts of interest to declare.

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