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Adiposity, Atherogenic Index and Lipid Profile of Male Wistar Rats Administered with Kunun-Zaki

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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

Aim: *Kunun-zaki* (KZ) is a highly refreshing local beverage that is commonly consumed both in urban and rural areas. The objective of the study was to investigate the effect of KZ on lipid profile and other markers of cardio-vascular risks in Wistar rats.
Methods: Fourteen adult male Wistar rats (mean weight of 150 g) used for the study were randomly divided into 2 groups (Groups A & B) of 7 rats each. The experimental rats in Group A and B were administered daily with 5 mL of distilled water and *kunun zaki* respectively for a period of 2 weeks. All experimental animals were allowed access to standard feed and water *ad libitum*. Blood samples were collected and analyzed for total cholesterol (TC), triglyceride (TG), low density lipoprotein-cholesterol (LDL-C), high density lipoprotein- cholesterol (HDL-C), and very low density lipoprotein cholesterol (VLDL-C) using standard biochemical methods. The body mass index (BMI), atherogenic index, and adiposity index were also determined. Liver, kidney and heart were harvested and weighed to determine organ weights. The results were statistically analyzed using Student's 't'test and by Pearson's correlation coefficient. All values were presented as mean ± standard error of mean. $P< 0.05$ was considered significant.
Results: While TC, LDL-C, HDL-C concentrations were not significantly different, both TG and VLDL-C concentrations in KZ administered rats were significantly higher compared with control.

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BMI, antherogenic index and adiposity were not significantly different also when both groups were compared.

Conclusion: the results of the study indicate that *kunun-zaki* does cause modulatory elevation in lipid profile.

Keywords: Kunun-zaki; atherogenic index; adiposity index; lipid profile; body mass index.

1. INTRODUCTION

The Nigerian soft drink industry has been heavily dependent on imported raw materials for many years. In order to conserve foreign exchange, emphasis is now on the development of indigenous beverages and the country's attention has begun to shift toward local sourcing of raw materials for economic development [1]. The drink- *Kunun-zaki* has its origin in the northern parts of Nigeria but is now popular in almost all the states of the nation [2]. This non-alcoholic beverage is becoming more widely accepted in other parts of Nigeria, owing to its refreshing qualities [2, 3]. In recent times, the consumption of ready-to-eat food has been on a high side, this is probably due to their convenience. Ready-to-eat foods occur in varieties of forms, e. g. fruits, fruit juices, nutritional drinks, snacks etc. Generally, they are food items consumed on purchase from vendor, hawkers and consumed immediately without any further preparation. Most ready-to-eat foods are found in public places e. g. markets, motor parks, streets, outside school premises, hospitals and even highways [4]. The beverage (*kunun-zaki*) is widely found not only in the local market but also at resorts [5]. Of the nutritional indigenous drinks consumed by a large number of the populace in Nigeria, *kunun-zaki* and zobo are most common.

Kunun-zaki is a Hausa word meaning sweet beverage and it is a cheap traditional drink widely consumed especially during the dry season [1, 4]. Preparation methods amongst different peoples are determined by taste and cultural preferences. It can be produced either from millet (*Pennisetum typoidum*), Sorghum (*Sorghum bicolor*), or maize (*Zea mays*) [6]. Aside grains and sweet potato (Ipomoea batatas), phytochemical and antioxidant rich spices are utilized for the preparation of *kunun-zaki* [7, 8]. Examples being ginger (*Zingiber officinale*), black pepper (*Piper-niger*), red pepper (*Piper-annum*), cloves (*Syzgium aromaticum*) and garlic (*Allium sativa*) [8]. According to data obtained from past studies, there is sufficient evidence that some of these spices may modulate lipid metabolism; significant enough to elevate high density lipoprotein (HDL), cholesterol and lower density lipoprotein (LDL) [9]. Yet there is dearth of information about the effects of *kunun-zaki* on biochemical parameters in mammals. Hence this study was designed to examine the effect of *kunun-zaki* on lipid parameters, and other risk factors of cardiovascular disease such as atherogenic index, adiposity index and body mass index.

2. MATERIALS AND METHODS

2.1 Materials

Ingredients for the preparation of *kunun zaki* included millet, ginger (*Zingiber officinale*), black pepper (*Piper-niger*), red pepper (*Piper-annum*), cloves (*Syzgium aromaticum*), garlic (*Allium sativa*) and table sugar (sucrose). *Kunun zaki* was freshly prepared each day according to the procedure reported by Gaffa et al*.* [8]. There are several methods employed in the production of millet based *kunun zaki* (MKZ). Methods such as the pre-fermentation, post-fermentation, and malting are always used in the preparation of MKZ [8]. For the purpose of this study the post-fermentation method was employed. Two hundred and fifty grams of millet was sorted, cleaned and steeped in a potable water before it was wet-milled, the resulting slurry was divided into two portions (i.e., 250 g and 50 g), boiled water was poured on one slurry (250 g) and allowed to cool before it was mixed vigorously with uncooked slurry (50 g), the mixture was allowed to ferment for 14 hours at 40°C, then sieved with clean muslin- cloth (0.0025 mm) and was sweetened with table sugar.

2.2 Study Site

The Animal House of the Physiology Department, Ladoke Akintola University of Technology, Ogbomoso, Oyo State.

2.3 Study Design

This is an experimental study.

2.4 Animal Handling

Fourteen Adult Male Wistar rats with average weight 150 g were purchased from the animal house of Ladoke Akintola University of Technology, Ogbomoso, Oyo State. The rats were kept in cages and made to acclimatize for 7 days and maintained under standard conditions of temperature and humidity. The rats were randomly divided into Groups A and B, which constituted the control and test groups respectively; each group consisted of 7 rats. They were allowed access to standard feed and tap water *ad libitum*. All animals received humane care in accordance with the principle of Laboratory Animal care of the National Society of Medical Research and Guide for the care and use of Laboratory Animals of the National Academy of Sciences (National Institutes of Health Publication no. 80-23). The animals in the control group [GROUP A] were administered with distilled water, whereas those in test group [GROUP B] received *kunun-zaki* throughout the 14-day experiment.

2.5 Administration of *kunun zaki*

Administration of *kunun-zaki* and distilled water was carried out using gavage needles. Each of the experimental animals in GROUP B was administered with a total volume of 5 mL *kunun-zaki*, 2.5 mL in the morning and 2.5 mL in the evening. The animals in test GROUP A received distilled-water as described above.

2.6 Clinical Observations and Body Weight Measurement

Every rat in each of the group was observed twice daily (before and after exposure) for signs of clinical toxicity in the appearances of the skin and fur, eyes and mucous membrane, behavioural pattern, respiratory system and morbidity /mortality. The body weight and length of each animal in the treatment and control groups were measured at the beginning of the experiment and once weekly until the end of the study to obtain the body mass index (BMI).

2.7 Protocol for Blood Collection and Organ Weight

At the end of exposure period, after an overnight fast of 12 hours, animals were weighed prior to blood collection and then euthanized by cervical dislocation. Blood was collected through cardiac puncture for estimation of plasma levels of total cholesterol, HDL, LDL, VLDL and triglycerides. It was dispensed into lithium heparin bottle, centrifuged at 3000 rpm for 10 minutes to obtain plasma and stored at -20°C prior to biochemical analyses. The organs (liver, kidney and heart) of the animals was surgically removed, rinsed with ice-cold physiological saline, blotted dry and weighed.

2.8 Methods for Determination of Plasma Lipid Profile

Estimation of Total Cholesterol (TC), Triglyceride, High density lipoprotein (HDL) and Low density lipoprotein (LDL) was done using Randox kits. VLDL-C was estimated using the method as described by Friedwald et al. [10]:

VLDL-C (mmol/l) = Total cholesterol – (HDL-C + LDL-C)

2.9 Determination of Adiposity and Assessment of Body Mass Index (BMI) of Experimental Rats

BMI of experimental rats was calculated as described by Erejuwa et al. [11]. The body weight and body length measurements were used for the estimation of BMI and adiposity index. The BMI was determined using the formula: BMI = Body weight (g) / body length2 (cm2). Body mass index was classified as follows: (Normal BMI: male [0.45 ± 0.02–0.68 ± 0.05] g/cm2 [12].

2.9.1 Adiposity index

Adiposity index was calculated using the formula for Lee index of obesity: Adiposity index = cube root of body weight (g) / body length (cm) nose-to-anus length (cm) [12].

2.9.2 Determination of atherogenic index

Atherogenic index was calculated by using the formula:

log10 (TG/HDL-C). [13].

It can be classified according to the values obtained: −0.3 to 0.1 for low risk, 0.1 to 0.24 for medium, and more than 0.24 for high risk of cardiovascular disease [14].

2.10 Statistical Analysis

Data obtained were expressed as mean ± standard error of mean. Data were subjected to

Student's t-test was analyzed with Statistical Package for Social sciences (SPSS IBM 23) version 9. Pearson's correlation coefficient was used to detect association between different parameters. Significance differences was established at $p<0.05$.

3. RESULTS

Results of the study are presented below. Tables 1 and 2 contain the results of lipid profile and body mass index respectively. Meanwhile, the results of organ weights, atherogenic index and adiposity can be obtained in Table 3.

Results of the lipid profile presented in Table 1 below showed that the plasma levels of total cholesterol, HDL-C and LDL-C were not significantly different when both groups were compared ($p>0.05$). Meanwhile, triglycerides and VLDL-C were significantly higher in *kunun-zaki* administered rats compared with control ($p<0.05$). Table 2 results revealed that BMI of the *kunun-zaki* administered group showed no significant difference when compared to the control group for the 2-week period of the experiment ($p>0.05$). Correlation study between BMI, adiposity or atherogenic index and lipid profile showed significant difference only for BMI and TC ($p= 0.024$, $r= 0.536$), all others were not significantly different.

4. DISCUSSION

Administration of *kunun-zaki* to male wistar rats resulted in dyslipidemia as the levels of TG and VLDL-C were higher compared with control. Dyslipidemia is said to occur when there is abnormality in one or more components of lipid profile [13]. The changes in lipid profile in wistar rats upon administration of *kunun-zaki*, may not be unassociated with impact of its main ingredient (i.e. millet) on these important markers of cardiovascular disease. According to literature, millet has high carbohydrate content, which may sometimes be as high as 70% depending on the variety [15]. This means it can easily predispose to increased lipid production when there is consistent/excessive intake. While initial metabolic process triggered by excess carbohydrate consumption is glycogenesis, but the capacity of mammals to store carbohydrates in this form is limited, therefore excessive intake

Table 1. Comparison of lipid profile of rats in groups A and B

Groups	TC (unit)	TG (unit)	HDL-C (unit)	LDL-C (unit)	VLDL-C (unit)
Group A	2.4±0.14	0.81±0.02	1.14±0.03	0.91±0.10	0.38±0.01
Group B	2.5±0.1	1.12±0.08	1.1±0.05	0.9±0.12	0.44±0.02
p-value	0.75	*0.01	0.51	0.93	*0.04

*Data are expressed as Mean ± SEM. *P < 0.05 is considered significant. Groups A- Control; Group B- Kunun-zaki administered rats*

Table 2. Comparison of Body mass index of the experimental animals

Groups	Body mass index		
	Week 0	**Week 1**	**Week 2**
A	0.45±0.04	0.46±0.05	0.47±0.03
B	0.45±0.01	0.47±0.04	0.48±0.01
P value	0.96	0.70	0.87

Data are expressed as Mean ± SEM. Groups A- Control; Group B- Kunun-zaki administered rats

Table 3. Comparison of organ weights, atherogenic index and adiposity of the experimental animals

Groups	Organ weights			Atherogenic index	Adiposity
	Liver (g)	**Kidney (g)**	**Heart (g)**		
A	6.29±0.28	2.14±0.14	1.15±0.14	0.13±0.01	0.76±0.10
B	6.43±0.20	2.29±0.18	1.29±0.18	0.13±0.02	0.70±0.02
p-value	0.69	0.55	0.57	0.90	0.53

Data are expressed as Mean ± SEM. Groups A- Control; Group B- Kunun-zaki administered rats of carbohydrate results in fat formation especially triglycerides which was significantly higher in *kunun-zaki* administered rats compared with control group.

Excessive carbohydrate translates not only to changes in lipid profile (especially dyslipidemia) [16], additionally it is usually accompanied by increase in weight and BMI, as the triglycerides so produced are stored in adipose tissue. Interestingly, this was not the case with respect to *kunun-zaki* administered rats, as higher TG and VLDL-C levels were not accompanied by increased BMI. This may be attributed to short period (14 days) of administration.

Although some of the reasons why *kunun-zaki* as a beverage was welcome by all strata of Nigerian society is because the raw materials are locally sourced from cheap easily accessible ingredients and it is a highly refreshing drink. Another appeal for its consumption nationally, is the fact that the spices [garlic, ginger, black pepper and red pepper] used for its preparation are highly nutritious, to the extent that in some quarters, *kunun-zaki* is rapidly replacing both carbonated and energy drinks. Unfortunately, it seems from the results of the study that the effects of this local beverage on lipid parameters are not profoundly different from some other beverages available in the market.

For instance, 3 mL/100 mg bw of Coca-cola caused significant lower levels of cholesterol, TG, HDL-C, but no change in LDL-C level. Meanwhile administration of the same dose level of Schweppes resulted in significantly higher levels of TC, TG, HDL-C and lower level of LDL-C compared with distilled water administered rats. While Sprite showed no differences in all lipid parameters when compared with control, Fayrouz resulted in higher level of TG, low LDL-C, low TG and high HDL-C and administration of Fanta at the same dosage level showed high TC, low levels of TG and LDL-C and normal levels of HDL-C [17]. It was not only carbonated drinks that resulted in dyslipidemia, Famurewa et al. [18] reported that administration of caffinated energy drink to rats resulted in decreased total cholesterol, LDL-C, but higher triglyceride levels compared with unexposed rats. HDL-C was also significantly reduced. The implication of these results is that like many other beverages in the market, *kunun-zaki* may result in some of the clinical features of hyperlipemia such as glucose intolerance, non-alcoholic hepatic disease which are characteristics of metabolic syndrome [19] but obesity may not be an accompanying feature since BMI was not elevated. Furthermore, since hypertriglyceridemia also affect the heart and blood vessels, this may mean an increase risk of pancreatitis or inflammation of the pancrease. No such information though has been obtained from several communities where humans have consumed *kunun-zaki* for generations.

The common component/ingredient to these three categories of beverages [*kunun-zaki*, carbonated drinks, energy drink] is sucrose. i.e. the local beverage used for the study and others in the market which have shown dyslipidemic effects contain sucrose as sweetener, meanwhile sucrose had always been linked with abnormal lipid parameters [20]. Otero-Losada et al. [20] observed that sucrose sweetened beverage featured significant weight gain and higher levels of triglycerides compared with control. This is aside the fact that studies carried out on sucrose alone is also linked with increase in weight [21]. *Kunun-zaki* is made in a variety of ways, although sucrose is used as a sweetener in the study, sweet potato (*Ipomoea batatas*) is another important sweetener in many local communities in many parts of Nigeria. Whether the results would have been different with the use of sweet potato as the sweetener remains to be determined.

It was not only the body weight of *kunun-zaki* administered rats that were not significantly different compared with control, such non-significant differences were observed with respect to organ weights as well i.e. kidney, liver and heart. While Munawar et al. [21] also observed that consumption of caffinated soft drink (Coca cola) and energy drink [Red Bull] did not result in significant changes in body weight compared with control, but they reported significantly increase in the relative tissue weight index of Coca Cola and Red Bull administered rats. The difference in the present results and other beverages in the market with respect to impact on organ weight may not be unconnected with the amount consumed and the effects of their uncommon ingredients. The dose of coca cola administered by Munawar et al. [21] was 11 ml which is equivalent to consumption of 3 cans by a seventy kilogram (70 kg) man but 5 mL of *kunun-zaki* was administered/daily to rats in the present study. And while *kunun-zaki* contains the following naturally occurring spices: ginger (*Zingiber officinale*); black pepper (*Piper-niger*); red pepper (*Piper-annum*); and cloves (*Syzgium aromaticum*); aside sucrose other ingredients of

Coca Cola are caffeine, ceramel coloring, lime and coca extract, phosphoric acid, citric acid, cinnamon, nutmeg, vanilla, and orange.

According to Cao et al. [22], chronic administration of cola beverages to rats caused no changes in body weight and kidney weight while their observation is in agreement with the results of the study, it differs from the inference of Munawar et al. [21], this may be due to differences in duration of exposure as well as differences in the species of experimental animals used for each study. Weight, length and BMI of *kunun-zaki* administered rats were not significantly different compared with control.

The results suggest that *kunun-zaki* is not a healthy beverage, unlike green tea, soy milk, oat drinks, tomato juice, and coca drinks which have been reported to reduced LDL-C cholesterol. Green tea contains catachins and other antioxidants that lower LDL-C. Oat drinks contain beta-glucans which create a gel-like substance in the gut and interact with bile salts, reducing cholesterol absorption. Tomato juice is rich in lycopene and can improve lipid levels by reducing LDL-C while cocoa drinks contains antioxidants called flavanols that decreases LDL-C and increasing HDL-C. Meanwhile, the results of lipid profile of *kunun-zaki* administered rats, are similar to those of sweetened drinks which are known for altered levels of TG, VLDL-C, or TC [17, 18].

The results of the present study are not sufficient to call for decrease in *kunun-zaki* consumption, as in many local communities the most commonly used sweetner is sweet potato, a crop that is rich in fibre and does not contain appreciable refined sugar like sucrose. It is reasonable to exercise caution as this local beverage has been taken for countless generations in many Nigerian communities and its consumption has not been linked to increased risk of cardiovascular disease. Hyperlipidemia is a modifiable risk factor of cardiovascular disease. Additionally, another important reason while a change in method of *kunun* preparation rather than a total ban on its consumption should be preferred is that during many months in northern Nigeria, both fruits and fresh vegetables are not readily available leaving the grains such as millet as an important source of minerals e.g. calcium, iron, zinc, copper and manganese.

More interestingly, the hypolipidemic effects of garlic, ginger, black pepper and red pepper widely reported in literature seems to be lacking in *kunun-zaki* administered rats. Akanfe et al. [9] observed that ginger modulated lipid metabolism, significant enough to elevate high density lipoprotein (HDL-C), cholesterol and lower low density lipoprotein (LDL-C). Black pepper, one of the spices used for *kunun* preparation has been reported to show remarkable reduction in plasma and tissue lipid profile by reducing total cholesterol in its free and esterified forms, free fatty acids, phospholipids and triglycrides and that in high fat diet fed rats, black pepper increased HDL-C and lowered LDL-C [23]. The pepper was administered at dosage level of 250 mg/kg. Cloves were also found to reduce total cholesterol, triglycerides, LDL, VLDL, and increase HDL-C [24] while Akanfe et al. [9] administered ginger at a dosage level of 400 mg/kg BW which is much higher than level administered to *kunun-zaki* fed rats. Weight loss was significantly higher in garlic group although it did not support beneficial effects on LDL-C [25] but garlic elevated levels of TG, and HDL and but lowered those of TC and LDL-C in wistar rats.

Atherogenic index of plasma (AIP) is one of the lipid ratios defined as $\log_{10}$ (TG/HDL-C). It is use as a marker of plasma atherogenicity. Atherogenic index of plasma (AIP) is a novel index composed of triglycerides and high-density lipoprotein-cholesterol [26]. It has been used to quantify blood lipid levels and commonly used as indicator of dyslipidemia and associated diseases e.g., cardiovascular diseases [27]. The mean atherogenic index of plasma of the *kunun* group showed non-significant difference compared to the control group.

Meanwhile it is important to be cautious in extrapolating these results to humans. The species differences in response to an agent is a factor that cannot be discounted. Additionally, the sweetener used in making *kunun-zaki* for the present study is not the one commonly adopted for *kunun* preparation in local communities where unerves not only as a refreshing local beverage for adults but also a popular weaning food for babies.

5. CONCLUSION

The finding of this study showed that lipid profile was altered with *kunun-zaki* administration, especially as *kunun-zaki* administered rats featured higher levels of triglycerides and very low-density lipoprotein. This might imply that *kunun-zaki* is a dyslipidemic agent.

6. RECOMMENDATION/FURTHER STUDY

It seems appropriate therefore, that since *kunun-zaki* has not been linked with cardiovascular disease in humans in communities where sweet potato is used as sweetener, it may be appropriate to carry out a study in which the effects of *kunun-zaki* sweetened with either sweet potato or refined sugar- sucrose are compared, so as to establish the contributing impact of sucrose to the results of the present study.

7. CONTRIBUTION TO KNOWLEDGE

Kunun-zaki has been consumed for generations in many parts of Nigeria, yet there is no significant empirical biochemical information about it on several metabolic processes vital to health and well being. The dyslipidemic effects of this local beverage have helped to clarify if *kunun-zaki* consumption may play a role in increased risk of cardiovascular risk commonly reported among the elites. It may help to deal with or address the millions of deaths and several more millions of disability-adjusted life years globally (GBD, 2015) described to occur from cardiovascular disease especially among the categories of people recently adopting *kunun* and other local drinks as snacks

ETHICAL APPROVAL

Animal Ethic committee approval has been taken to carry out this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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