

ROASTED CASHEW NUT KERNEL (*ANACARDIUM OCCIDENTALE L.*) SUPPLEMENTATION IMPROVES HEPATIC AND RENAL STATUS BUT NOT LIPID PROFILE IN RATS

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ABSTRACT

Objective: Although studies suggest that nut consumption is associated with a variety of beneficial health outcomes, however, there is a dearth of data in the literature to document this effect for roasted cashew nut kernel (RCNK). Our objective was to determine whether dietary consumption of RCNK could improve lipid profile, hepatic and renal status in rats.

Methods: A total of 24 rats were randomly divided into four groups: A control and three experimental groups fed with roasted cashew nut-supplemented diet at different concentrations for 28 consecutive days. After the experimental period, rats were anesthetized with ether and retro-orbital blood samples were withdrawn. Serum samples were obtained to analyze lipid profile, markers of oxidative stress, hepatic and renal status using standard methods.

Results: The supplemented diet significantly decreased the liver function parameters in rats. All the concentrations of RCNK in diets significantly reduced serum creatinine and urea levels. However, total cholesterol and low-density lipoprotein-cholesterol (LDL-C) were significantly increased, whereas oxidative stress markers and malondialdehyde were improved by the supplemented diet, although insignificantly, as compared with the control.

Conclusions: These results suggest that RCNK may have beneficial health effects on the liver and kidney status although marked improvement was not demonstrated in oxidative stress markers. However, the significant increase in serum total and LDL-C indicates the need for further studies.

Keywords: Roasted cashew nut, *Anacardium occidentale*, Lipid profile, Liver enzymes, Oxidative stress.

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INTRODUCTION

Nut consumption is an important source of nutrition and increasingly consumed for health benefits in different climes of the world. Nuts are dry fruits with one seed in which the ovary wall becomes hard at maturity [1]. They are reported to be nutrient-dense foods with complex matrices rich in monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids, dietary fibers, high-quality proteins, micronutrients, magnesium, potassium, arginine, and bioactive phytochemicals [1-3]. The popular edible nuts such as almonds, Brazil nuts, hazelnuts, walnuts, peanuts, pecans, pistachios, kola nuts, and cashew nuts are consumed by humans, although individual intake varies remarkably [4]. Epidemiological findings indicate that frequent nut consumption offers protection from fatal and non-fatal cardiovascular disease events, reduction in the risk of chronic diseases and improvement in body weight gain [3,4]. Studies have associated nut consumption with several health benefits and demonstrated its antioxidant, hypocholesterolemic, anticancer, antiobesity, anti-inflammatory and antidiabetic effects, among other functional properties mediated by several different mechanisms [2,4,5].

Cashew nut (*Anacardium occidentale L.*), the heart-like fruit of the family Anacardiaceae, plays an important role in human diets as an edible nut worldwide. It is native to Brazil, and introduced about two centuries ago to Goa, India, which became one of the major producers of cashew nuts, accounting for almost 50% of the total world export [6], with commercial recognition being observed in other developing countries, including Indonesia and some African countries [6]. The kernels of cashew nuts are rich in lipids (42.6%), proteins (20.0%), and fiber (5.9%) and comprised 575 kcal of energy per 100 g [7]. In addition, 100 g of edible cashew provide 3.96 g of sugar, 2.82 mg of vitamin B, 37 mg of calcium, 292 mg of magnesium, 593 mg of phosphorus, and 660 mg of potassium [7]. Recently, the antioxidant activities of various

bioactive compounds such as phenolics, flavonoids, phospholipids, sphingolipids, sterols, and tocopherols were reported in cashew nut samples [7]. The cashew nut is roasted over open fires or in red hot charcoal in most Nigerian rural communities to obtain the nutritious kernel from the tough shell and consumed in large quantities all year round [8]. During the process, the cashew nut becomes crunchy and brittle leading to an overall increased taste, flavor, sensory qualities, and palatability [9].

However, some reports have associated conventional shelling methods with the loss of heat-sensitive bioactive compounds in cashew nut kernel [6,10,11], as previous studies suggest that processing method may affect nutritional quality and health benefits of foods [9,12]. To our knowledge, there is a paucity of data on the association of roasted cashew nut kernel (RCNK) with health benefits through dietary consumption. Although a line of evidence suggests that nut consumption may beneficially impact on health outcomes [1,2,4,13], recent observations indicate that health benefits from nut consumption may be contextually specific, depending on the type of nut consumed [13]. For instance in the previous studies, the beneficial health impacts of consumption of walnut, almonds and peanut have been associated with improvement in glycemic control, plasma lipid profile, lipid peroxidation markers, diabetes, risk of colorectal cancer, and cardiovascular diseases [14-18], whereas the unsalted cashew nut intervention diets had no significant effect on lipid profile, serum fructosamine, serum high-sensitivity C-reactive protein, blood pressure, and serum uric acid concentrations when compared with the control diet [3]. The nuts most frequently studied have been almonds and walnuts. Currently, studies on the health impact of consumption of RCNK are scarce in scientific literature, although studies on composition analysis reported beneficial nutritional profile [6,8,9]. The assessment of the possible health effects of RCNK consumption will enhance and provide evidence for its importance in diet. This

study, therefore, investigated the effect of RCNK on lipid profile, oxidative stress markers, hepatic and renal status in rats.

METHODS

Chemicals and reagents

All chemicals and reagents used were of analytical grade. Commercial reagent kits for the determination of serum lipid profile, alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea, creatinine, and enzyme makers of oxidative stress were supplied by Randox Diagnostic Laboratory, Crumlin, UK. 2-thiobarbituric acid (TBA) was obtained from Sigma Chemicals Co. (Taufkirchen, Germany). Diethyl ether was supplied by Guanghua Chemical Factory Co., Ltd., China.

Experimental animals

A total of 24 Wistar albino rats of either sex weighing 120-140 g were obtained from the Animal House, Biochemistry Department, University of Nigeria, Nsukka and acclimatized for 1 week in a well-ventilated experimental room of the Department of Biochemistry, Ebonyi State University, Abakaliki before the experiment. The animals were housed in metal cages and maintained under standard environmental conditions with a range temperature 21-23°C and photoperiod of 12 hrs light/12 hrs dark cycle with free access to normal rat chow and clean water. Handling of animals was in accordance with the relevant institutional and ethical guidelines as approved for scientific study.

RCNK

RCNK was purchased from the commercial sellers and ground manually to the size of normal rat feed to enhance homogeneity and free consumption of the supplemented diet.

Experimental design

After the acclimatizing period, animals were randomly segregated into four different groups of six animals each. The control group was fed with a normal rat chow; Group 2 fed with 10% RCNK supplemented diet; Group 3 fed with 20% RCNK supplemented diet, while Group 4 was fed with 30% RCNK supplemented diet (w/w). The normal rat chow and RCNK were mixed until there was homogeneity and served to the animals *ad libitum*. The preparation of the supplemented diet was done on regular demand. Before the consumption of the diet, initial weight of rats in control and experimental groups was determined and recorded. The rats were exposed to daily consumption of the supplemented diet for 28 consecutive days. After the experimental period, final weights of rats were taken and recorded. All the groups of rats were fasted overnight and subjected to mild diethyl ether anesthesia on day 29. Blood was quickly withdrawn via retro-orbital puncture into clean plain tubes. The blood was allowed to stand for 35 minutes and then centrifuged at 3500 rpm for 15 minutes to obtain serum. The sera were separated and stored (at -20°C) until biochemical analyses.

Biochemical analyses

Serum lipid profile, ALT, AST, superoxide dismutase (SOD), glutathione peroxidase (GPx) activities, urea, and creatinine were estimated with commercially available kits from Randox Laboratories Ltd. (Crumlin, UK) via spectrophotometer, model SPM721 (Biotrust Diagnostics Inc., USA). Catalase (CAT) activity was determined by the method of Sinha [19]. Lipid peroxidation product, malondialdehyde (MDA) was estimated spectrophotometrically by measuring TBA-reactive substances, as described by Wallin *et al.* [20].

Statistical analysis

The data were expressed as mean±standard error of means based on the indicated number in the experiment (n=6). A statistical analysis was performed with SPSS 17.0 for windows. The differences between the means of data were compared by one-way ANOVA test followed by Tukey's test. The differences were considered significant at p<0.05.

RESULTS

Body weight change

At the end of the 28 days, change in body weight was significantly higher (p<0.05) in animals that consumed supplemented diets in comparison to control group (Table 1).

Lipid profile

The serum lipid profile parameters are shown in Table 2. The daily consumption of the diet by rats demonstrated non-significant (p>0.05) concentration-dependent increases in triglyceride. Similar trends were observed for total cholesterol (TC) and low-density lipoprotein-cholesterol (LDL-C), although with significant increases (p<0.05) in rats fed with 10% and 30% RCNK diets as compared to control. However, the supplemented diets (20% and 30% RCNK) decreased high-density lipoprotein-cholesterol (HDL-C) non-significantly as compared to control.

Liver and kidney status

Table 3 presents the effect of the diet on markers of the liver and kidneys status. The diet decreased the serum markers of the liver and kidney status in this study. Markers of the liver status, AST and ALT activities were found to be significantly decreased (p<0.05) by 10% RCNK and 20% RCNK diets, respectively. Moreover, all supplemented diets significantly decreased serum levels of urea and creatinine (p<0.05).

Oxidative stress and lipid peroxidation

Table 4 shows that the diets had no significant effect on SOD, CAT, and GPx in the current study. Although we observed some increases in serum levels of these markers of oxidative stress (SOD and GPx) but not significantly different (p>0.05) when compared with control. Lipid peroxidation marker, MDA, decreased with the diet but only significantly (p<0.05) for the treatment with 20% RCNK.

DISCUSSION

Numerous studies have provided evidence that nuts constitute a good source of vital nutritional compounds that could elicit beneficial outcomes in health and disease [1,4]. However, emerging evidence indicates that the beneficial effects may depend on the type of nut consumed [13]. Cashew nut is rarely studied in association with health

Table 1: Effects of RCNK-supplemented diet on body weight of rats

Parameter	Control	10% RCNK	20% RCNK	30% RCNK
Initial body weight (g)	120.0±6.37	138.6±4.20	128.1±9.53	136.2±5.81
Final body weight (g)	149.8±6.62	181.4±6.52	174.2±15.08	180.6±8.70
Weight change (g)	28.4±3.17	42.8±3.73*	40.9±6.91	44.5±3.02*

Values are mean±SEM (n=6). *Values with superscripts in the same column differ significantly (p<0.05) from the control. SEM: Standard error of mean, RCNK: Roasted cashew nut kernel

Table 2: Effect of RCNK-supplemented diet on lipid profile of rats (mmol/L)

Treatment	Triglyceride	TC	LDL-C	HDL-C
Control	1.90±0.09	2.77±0.11	2.38±0.79	1.77±0.14
10% RCNK	1.83±0.14	2.73±0.19	4.30±0.14 ^a	1.78±0.17
20% RCNK	2.02±0.05	2.93±0.15	3.52±0.71	1.60±0.19
30% RCNK	2.04±0.09	3.06±0.07 ^a	4.46±0.42 ^a	1.74±0.42

Values are mean±SEM (n=6). Values with superscripts in the same column differ significantly (p<0.05) from the control. SEM: Standard error of mean, RCNK: Roasted cashew nut kernel, TC: Total cholesterol, LDL-C: Low-density lipoprotein-cholesterol, HDL-C: High-density lipoprotein-cholesterol

Table 3: Effect of RCNK-supplemented diet on markers of liver and kidney status of rats

Treatment	AST (U/L)	ALT (U/L)	Urea (mg/dl)	Creatinine (mg/dl)
Control	73.7±10.16	107.0±21.05	50.8±6.65	1.64±0.11
10% RCNK	43.5±5.32 ^a	95.5±20.76	21.0±4.04 ^a	0.91±0.07 ^a
20% RCNK	51.0±3.62	60.0±6.76 ^a	44.67±9.39	1.28±0.09 ^a
30% RCNK	48.8±5.09	66.2±7.74	40.6±10.63	1.14±0.04 ^a

Values are mean±SEM (n=6). Values with superscripts in the same column differ significantly (p<0.05) from the control. SEM: Standard error of mean, RCNK: Roasted cashew nut kernel, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase

Table 4: Effect of RCNK-supplemented diet on markers of oxidative stress and MDA in rats

Treatment	SOD (U/ml)	CAT (U/ml)	GPx (U/ml)	MDA (mg/ml)
Control	11.20±0.12	2.73±0.34	30.57±7.23	1.96±0.06
10% RCNK	11.41±0.19	3.03±0.25	42.85±7.92	1.74±0.03
20% RCNK	11.29±0.07	2.07±0.19	54.72±11.86	1.47±0.25 ^a
30% RCNK	11.19±0.14	2.38±0.16	48.56±13.85	1.85±0.05

Values are mean±SEM (n=6). Values with superscripts in the same column differ significantly (p<0.05) from the control. SEM: Standard error of mean, RCNK: Roasted cashew nut kernel, SOD: Superoxide dismutase, CAT: Catalase, GPx: Glutathione peroxidase, MDA: Malondialdehyde

benefits, in spite of its robust antioxidant and fatty acid profiles (21.6% saturated fatty acid and 78.1% unsaturated fatty acid) reported [21]. To our knowledge, however, very little research has been done on cashew and limited data are currently available on the beneficial role of RCNK in biological system.

In this context, the present experimental study evaluates whether dietary consumption of RCNK could improve lipid profile, hepatic and renal status in rats. As deciphered by the present investigation, the dietary treatments improve the liver and kidney status but not lipid profile in albino rats. Our results showed that at the end of the 4th week, there was a statistically significant increase in body weight gain when the RCNK-supplemented diet groups were compared to control (Table 1). This could be due to an increase in diet consumption by rats which may be caused by increased palatability, taste and flavor associated with RCNK [9]. However, some studies found that long-term consumption of nuts is associated with reduced risk of weight gain and obesity [4,22]. Although our study was done in experimental animals and specifically in RCNK, our findings suggest that different nuts may have different effects on health indices as reported by a previous study [13].

In previous studies, walnut and almonds - the frequently studied nuts provide evidence that nut diet displays beneficial cholesterol-lowering effect usually without any significant effect on triglycerides or HDL-C in healthy subjects [23]. In this study, RCNK seems to increase lipoprotein cholesterol including triglyceride although some increases were not statistically remarkable. Of note in this study is the 10% and 30% RCNK-supplemented diets that significantly increase TC and LDL-C levels without favorable improvement in HDL-C levels (Table 2). This is in agreement with two separate studies that used diet high in cashew or walnut [3,24] compared to control diets in obese patients with the metabolic syndrome that failed to show a significant effect on the TC, LDL-C, and HDL-C levels. In contrast, a study reported beneficial effect of cashew nut on lipoprotein cholesterol and triglyceride [25]. Earlier studies have reported that roasting of cashew nut may reduce bioactive compounds and exhibits increase in total fats and fatty acids [6,9]. For example, Kosoko *et al.* [9] reported significant increases in total fats and fatty acids in open pan and halogen oven RCNK when compared with unprocessed cashew nut kernel. Heating is known to adversely affect fatty acids [26], although an important fraction of fat contained in most

nuts is MUFA, which is not a substrate for oxidation [1]. However, the fatty acids may be affected by heating, a practice common in cashew nut processing, which may increase susceptibility to lipid oxidation that deteriorates the quality of dietary oils and fats [26], and this may be important for cholesterol levels observed in this study.

Our study demonstrated beneficial impact of the supplemented diet on the liver and kidney status. The activities of serum AST and ALT are the most sensitive biomarkers that indicate hepatic damage and toxicity. Elevation of AST has been reported to be an index of hepatocellular injury in rats, while ALT elevation is more associated with the necrotic state [27]. In this study, the 10% and 20% RCNK diet treatments induced the opposite effect and markedly reduced the serum levels of AST and ALT. These findings demonstrate the non-toxic and membrane stabilizing potential of the RCNK. Although we observed non-significant increases in antioxidant enzyme activities, they may play a positive role in the beneficial outcome associated with the kernel on hepatocytes. Similarly, serum creatinine and urea levels were significantly decreased in every group treated with supplemented diet. Marked elevations of serum creatinine and urea concentration are known as markers of significant functional impairment of kidney, although serum creatinine concentration is a more potent indicator than urea in the first phase of kidney injury [28,29]. Limited data are currently available on RCNK, and we found no study that evaluated consumption of cashew nut for the liver and kidney health.

Our study showed that MDA levels were decreased while SOD, CAT, and GPx levels were increased insignificantly in the serum. In physiological condition, endogenous generation of reactive oxygen species (ROS) could rapidly be detoxified by antioxidant enzymes including SOD and CAT. Walnut and Brazil nuts have been shown to contribute to a significant antioxidant effect in experimental rats and disease conditions, including hypertension [30-32]. Analysis of the effects of cashew nut in the diet on antioxidant status of human subjects with metabolic syndrome resulted in an increased antioxidant capacity [24]. The 28 days of cashew kernel consumption show insignificant improvement in serum antioxidant system, however, it is not known whether long-term consumption of RCNK would demonstrate significant antioxidant effect against ROS damage and generation of MDA, an indicator of free radical generation and end-product of lipid peroxidation. As suggested by earlier studies [9-12], the bioactive compound profile might have been reduced in the roasted cashew nut by the roasting method, and this may account for the observation of non-significant antioxidant effect in this study.

CONCLUSION

This study has shown the beneficial effects of RCNK on the liver and kidney status, although marked improvement was not demonstrated in oxidative stress markers. However, the supplemented diets contributed significantly to increase serum cholesterol levels with important health implications. The potential health benefits or harms of nuts may depend on the type of nut consumed. Nevertheless, future research on roasted cashew nut is worthy of exploration.

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