

POSITIVE STIMULATORY POTENTIALS OF COCONUT (*Cocos nucifera* L.) JUICE EXTRACT ON *INVIVO* ANTIOXIDANTS, RENAL FUNCTION AND LIPID PROFILE OF MALE WISTAR RATS

Abstract

Coconut juice is a natural drink from coconut fruit. This study was aimed at evaluating its positive potential on biochemical indices. Fresh coconut fruits were de-dshelled and washed. The cotyledon was broken to extract the water which was used in blending the fruits. The extract was filtered and the filtrate was collected in a vial. Twenty healthy male Wistar rats weighing 160 -180 g, were grouped into two groups of ten rats each and were treated as follows for four weeks. Group A: control rats received oral dose of 2 mL/kg body weight of distilled water once daily for 28 days, and group B: received oral dose of 2 mL/kg body weight of coconut juice extract once daily for 28 days, both groups were, however, allowed free access to feed and water *ad-libitum*. The animals were weighed before and on completion of the experimental protocol. Blood was also collected via cardiac puncture and used for renal function and lipid profile assay, kidneys were excised and weighed, homogenized and the supernatant was used for antioxidant enzyme evaluation. Findings showed that there was no significant change ($p>0.05$) in the kidney-to-body weight ratio and renal function indices. Significant reduction ($p<0.05$) was seen in cholesterol, triglycerides, LDL but with an increase ($p<0.05$) in HDL. Antioxidant enzymes were significantly increased ($p<0.05$). The study concludes that coconut juice extract has positive stimulatory potentials on antioxidants, renal functions and lipid parameters of wistar rats.

Keywords: coconut juice extract, antioxidants, lipid profile, renal function

Introduction

Foods rich in natural antioxidants can be used as a subterfuge to reduce morbidity and mortality primarily due to oxidative stress (Zulaikhah, 2019) and the prevention of degenerative diseases (Muhammed *et al.*, 2018) such as diabetes, cancer, rheumatoid arthritis, Parkinson's, Alzheimer's, and osteoporosis. "Antioxidants are also potent chemical agents in mitigating the ageing process. Reactive oxygen species (ROS) and other free radicals generated during cellular metabolism are very unstable and react rapidly with other cellular metabolites in the body, leading to cell cellular injury and increased apoptosis" (Shahidi and Zhong 2010; Yan *et al.*, 2018). "Epidemiological evidence has implicated excessive free radicals and associated oxidative stress arising from an imbalance between prooxidants and antioxidants as the primary factor in nephrotic diseases and kidney damage" (Arhoghro *et al.*, 2012; Adwas *et al.*, 2019). "In the food industry, oxidation is a major cause of deterioration in food, these deteriorations often affect the nutritional value, and organoleptic properties of the food, leading to undesirable off-flavours and potentially toxic reaction products" (Dong *et al.*, 2008). Thus, antioxidants play a vital role in both food systems and in the human body to reduce oxidative stress. Renal function is vital for homeostasis, as the kidneys play important pleiotropic roles including removal of metabolic waste products and maintenance of water-electrolyte balance and blood pressure. Yan *et al.* (2018) posited that "synthetic antioxidants has been implicated to threaten human health, antioxidants from natural sources have attracted more attention". Coconut fruit, botanically called *Cocos nucifera*, L is a member of the Arecaceae family, it provides several health benefits outside its nutritional content. Coconut fruit juice is a natural and nutritious drink from the coconut fruit harvested from the coconut palm tree widely grown and cultivated in tropical countries like Nigeria, Indonesia, India, and the Philippines. Coconut fruit and fruit juice are rich in essential vitamins, amino acids, minerals, enzymes and growth hormones (Johnkennedy *et al.*, 2013; Lukose,

2013; Oluwarotimi *et al.*, 2021). These documented food values make Coconut fruit and juice therapeutic in nature as it help maintain osmotic pressure inside and outside the cell (Fife, 2008; Geetha *et al.*, 2016), and Prevent oxidative stress via its antioxidant potential (Yan *et al.*, 2018; Sunil *et al.*, 2020), positive inhibitory ability in lipid peroxidation (Zulaikhah *et al.*, 2015), improved lipid profile levels (Radenahmad *et al.*, 2016), improved blood pressure (Farapti and Parlindungan, 2013), cardioprotective potentials (Agbafor *et al.*, 2015; Lima *et al.*, 2015), anti-inflammatory (Ajeigbe *et al.*, 2011), antidiabetic (Zulaikhah, 2019), haemoglobin boasting potentials and as diarrhoea therapy (Mulyanti *et al.*, 2016). With the plethora of scientific evidence validating the potential health benefits of coconut fruits and juice, this present study is therefore aimed at investigating the positive stimulatory potentials of coconut juice extract on *in vivo* antioxidants and renal function and lipid profile of male Wistar rats.

MATERIALS AND METHODS

Sample preparation

Samples of fresh *Cocos nucifera L* fruits were purchased from a local fruit market in Amasoma, Bayelsa State, Nigeria. The fruit was identified and authenticated at the Department of Plant Science and Biotechnology, Niger Delta University, Amasoma. The shell of the coconut fruit was removed and washed properly to remove debris and avoid contamination. The cotyledon was broken to extract the water which was later used in blending the fruits. The extract was filtered with Whatman No. 1 filter paper and the resulting filtrate was collected in labelled vials and kept in a refrigerator at -4°C until use.

Experimental Animals

Twenty (20) healthy adult male Wistar albino rats weighing 160-180g were used for this study. They were obtained from the animal house unit of the Department of Pharmacology, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria, and were maintained under standard housing conditions (photoperiod: 12h natural light and 12h dark). The animals were acclimatized for two weeks and were fed pelletized growers' feed and were exposed to clean tap water throughout the period of the study.

Experimental Design

The animals were grouped into two groups of ten (10) rats each in a standard plastic rat cage and were treated as follows according to their body weights for four (4) weeks. Group A: control rats received 2 mL/kg body weight of distilled water once daily, and group B: the treatment group received 2 mL/kg body weight of the coconut juice extract once daily.

Sample Collection

At the end of the experimental period, and 12 hours after the last oral drug administration, the animals were re-weighed after which they were anaesthetized in a chloroform chamber and blood samples collected via cardiac puncture into plain sample bottles. The blood samples were allowed to stand for 20 minutes for coagulation to take place, afterwards, the blood samples were centrifuged at 2000 rpm for 10 minutes and the supernatant (serum) was collected and stored in the refrigerator before biochemical assay. Furthermore the kidneys were quickly excised and weighed, after which they were homogenized with TRIS buffer and centrifuged at 3000 rpm for 20 minutes, the supernatant was collected and stored in the refrigerator until analysis.

Changes in body weight

Rats in all groups were weighed on the first day and at the end of experimental period. The percentage change in body weight was calculated using the expression below:

$$\% \text{ change in body weight} = \frac{\text{final body weight} - \text{initial body weight}}{\text{initial body weight}} \times 100\%$$

Kidney as ratio of body weight

Kidneys were removed and weighed immediately. The kidney ratio was calculated as a percentage using the expression below

$$\frac{\text{weight of kidney (g)}}{\text{body weight (g)}} \times 100\%$$

Assay kits/Reagents

Assay kits for cholesterol, triglyceride, low density lipoprotein cholesterol, high density lipoprotein cholesterol, superoxide dismutase, glutathione s-transferase and catalase are product of Biosystem, Biosystems S.A., Costa Brava, Barcelona, Spain. Assay kits for blood urea nitrogen, creatinine and uric acid are products of Fortress Diagnostic Ltd, United Kingdom. All other reagents were of analytical grade and standard suppliers.

Biochemical estimations of lipid profile

The biochemical estimation of total cholesterol and triglyceride were determined by **Colorimetric** method as described by Ochei and Kolhatkar (2008), low-density lipoprotein cholesterol and high-density lipoprotein cholesterol were quantitatively determined spectrochemically by adopting the method described by Grove (1979) and Burstein et al. (1970) respectively, the ratio of LDL to HDL was determined from the expression stated below Kpomah and Arhoghro (2012).

$$\text{The ratio of LDL to HDL (Atherogenic index)} = \frac{\text{Level of LDL}}{\text{Level of HDL}}$$

Biochemical Estimation of Antioxidant Enzymes and Renal function parameters

SOD was assayed by the method described by Misra & Fridovich (1972). GST activity was assayed by the method of Habig *et al.* (1974). CAT activity was quantitatively determined by the method of Cohen *et al.* (1970). BUN was evaluated by the modified Berthelot method according to Tobacco *et al.* (1979). CRT was assayed by the Colorimetric kinetic method of Bartels *et al.* (1971). UA was assessed using the enzymatic **Colorimetric** method of Duncan *et al.* (1982).

Statistical Analysis

Data were expressed as Mean \pm Standard Deviation for ten (10) replicate determinations. Mean differences between the treated group and the control group were compared using student's t-test. Data were analyzed using SPSS version 16 for windows (IBM Corp, USA). $p < 0.05$ was set as the level of significance. The charts were plotted using Graphpad Prism 8.

RESULTS

The effect of Coconut juice extract on changes in body weight

The results of the effect of coconut juice extract on changes in body weight is depicted in Figure 1.0. **The oral administration of the coconut juice extract caused significant changes ($p < 0.05$) on the final body weight (FBW) and percentage changes in body weight (CBW), however, non-significant changes ($p > 0.05$) was not observed with respect to kidney weight (KW) and relative kidney weight (RKW)**

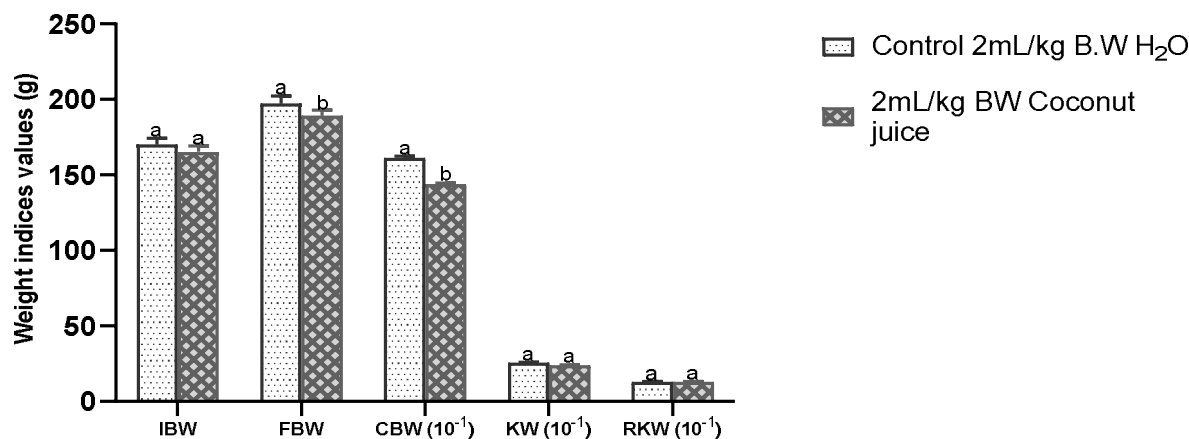


Fig. 1.0: effect of coconut juice on body weight indices (IBW-Initial body weight, FBW- Final body weight, %CBW- % change in body weight, KW- Kidney weight, RKW- Relative kidney weight).

Column of bars of same parameter having same letter identification are not significantly different ($p > 0.05$) while those with different letters are significantly different ($p < 0.05$)

The effects of coconut juice extract on the lipid profile of male Wistar rats

The results of the effect of coconut juice extract on lipid profile is shown in Figure 2.0. The result indicated significant reduction ($p < 0.05$) in the concentrations of total cholesterol (T.Chol), triglycerides (TG), low density lipoprotein cholesterol (LDL) and the ratio of LDL/HDL. The coconut juice extract also brought about significant increase ($p < 0.05$) on the value of high density lipoprotein cholesterol (HDL).

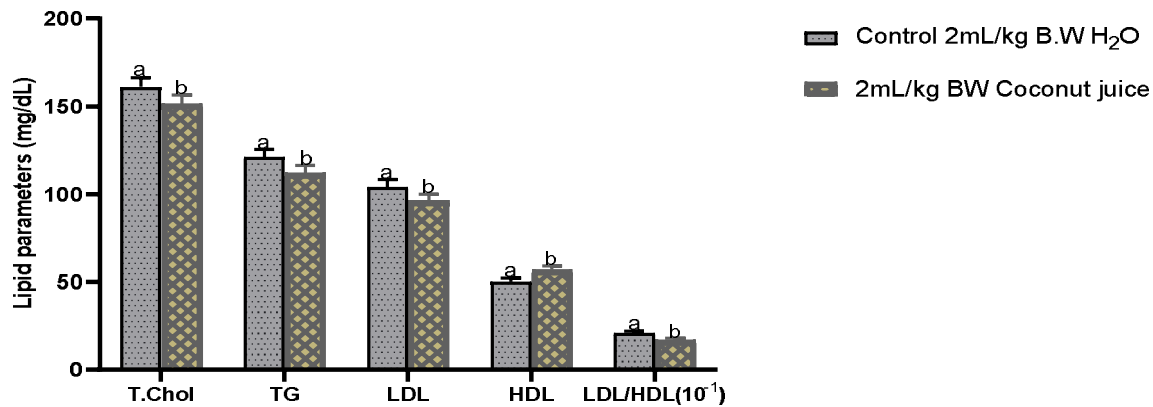


Fig.2.0: effect of Coconut juice extract on Lipid profile of male Wistar rats
Column of bars of same parameter having different letter notation are significantly different ($p < 0.05$)

The effect of coconut juice extract on renal function of male Wistar rats

The effect of coconut juice extract on renal function of male Wistar rats in mg/dL after 28 days treatment regimen is presented in Figure 3.0. The results obtained indicated a non-significant change ($p > 0.05$) on the concentrations of blood urea nitrogen (BUN), creatinine (CRT) and uric acid (UA).

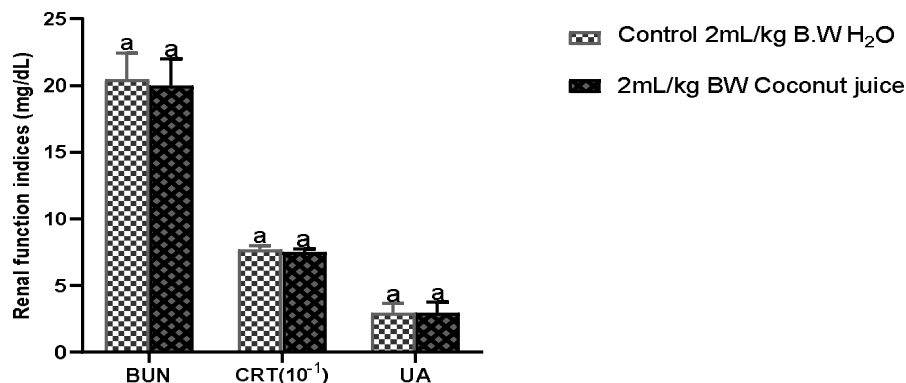


Fig. 3.0: the effect of coconut juice extract on kidney function parameters of male Wistar rats (BUN- Blood urea nitrogen, CRT- Creatinine, UA- Uric acid)

The effect of coconut juice extract on antioxidant enzymes of male Wistar rats

The effect of coconut juice extract on antioxidant enzymes of male Wistar rats in unit/mg tissue is presented in Figure 4.0. Findings from the result indicated a significant increase ($p < 0.05$) in concentrations of antioxidant parameters of superoxide dismutase (SOD), Glutathione-S-transferases (GST) and catalase (CAT)

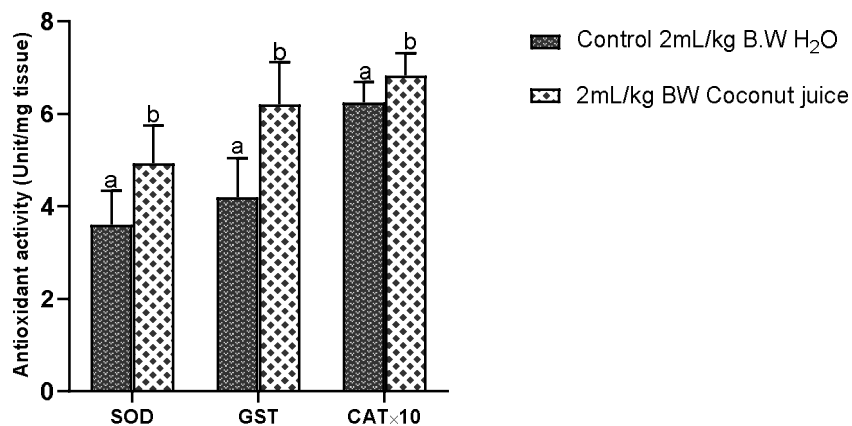


Fig.4.0:effects of coconut juice extract on antioxidant enzymes of kidney of male Wister rats

Discussion

“Change in body weight is a subtle index of the general health status and well-being of animals” (Arthur *et al.*, 2011, Arhoghro and Kpomah, 2022). The results obtained showed that all rats in the respective groups experienced an increase in body weight after the treatment regimen. These increases in body weight were more pronounced in the control group with a 16.12 % increase and 14.4 % for the coconut water extract treated group. Coconut water although low in calories (Mohamad *et al.*, 2017) is rich in bioactive enzymes like acid phosphatase, dehydrogenase, diastase, peroxidase and RNA polymerases (Zulaikhah, 2019), these enzymes can boost metabolic activities like digestion, the increased metabolism is directly proportional to the rate at which fat and fat-associated nutrients burn (Vaishnavi, 2019), hence the difference between the percentage increase between among the control group and the coconut extract treated groups but could be seen to cause an enhanced and optimal serum lipid profile of the Wistar rats when compared to the control group. “Levels of lipid parameters, such as total cholesterol, triglyceride, low density lipoprotein cholesterol, and high density lipoprotein cholesterol are key indices of cardiovascular health” (Chu *et al.*, 2016). “The oral route administration of the coconut juice extract caused a significant decrease ($p < 0.05$) in total cholesterol, triglyceride, LDL and LDL/HDL with a concomitant significant increase ($p < 0.05$) in HDL levels. This positive stimulation potential of lipid parameter induced by the coconut juice extract has been linked with reduced cardiovascular risk” (Hedayatnia *et al.*, 2020). “Weight gain is a function of excessive adipogenesis, which is often regulated by the sterol regulatory element-binding proteins (SREBP) which are a family of transcription factors that mediate lipid homeostasis through its action in the expression of successive enzymes needed for the endogenous synthesis of cholesterol, fatty acids, triacylglycerol and phospholipids. SREBP expression results in an accumulation of lipid droplets, cholesterol homeostasis, and an increase in high levels of the serum lipid profile” (Ahn *et al.*, 2013). Lazic *et al.*, (2020) posited that “changes in organ weight of animals are a positive indicator of chemically induced organ damage. The effect of the coconut water extract from the study had no significant change ($p > 0.05$) in the

relative weight of the kidneys when compared to the control group, an indication of proportional growth with no adverse or toxicity effect”.

“The kidneys play a vital role in the everyday metabolism of the cell via its role in the excretion of waste products and toxins like urea, uric acid and creatinine, metabolic regulation of extracellular fluid volume, serum osmolality and electrolyte balance, coupled with the production of hormones like erythropoietin and 1,25 dihydroxy vitamin D and renin” (Nwankpa *et al.*, 2018; Tejchman *et al.*, 2021). “Evaluation of renal function activity is key in the diagnosis, management and treatment of patients with kidney disease and pathologies affecting renal function. Urea, the principal nitrogenous waste formed during protein metabolism and whose concentration in blood is dependent upon the relationship between its production and excretion, increased value above the normal range may indicate kidney disease, shock, dehydration, diabetes, acute myocardial infarction while a decreased value below normal may portend liver failure, impaired absorption and overhydrating” (Zulaikhah & Sampurna, 2016). Creatinine is a breakdown product of creatine phosphate, which occur primarily as a result of muscle metabolic activities and is then excreted by glomerular filtration during normal regular renal function. Higher values of creatinine above 1.5 mg/dL is an indication of impairment in liver function. Uric acid is a breakdown metabolite from purine metabolism, abnormally high levels of uric acid is associated with a condition called gout. The non-significant changes ($p \geq 0.05$) in values of urea, creatinine and uric acid, when compared to the control, is a positive indication that the coconut water extract had no adverse effect on the renal function indices of the rats.

Cells of organisms are fortified with antioxidant defense systems that mitigate the effect of relative oxidative stress induced by exposure to toxicants. Adwas *et al.*, (2019) posited that “oxidative stress occurs when the balance between reactive oxygen species (ROS) formation and detoxification favours an increase in ROS levels leading to disturbed cellular function. ROS causes injury to cellular macromolecules leading to lipid peroxidation, nucleic acid, and protein alterations”. “The formation of lipid peroxidation and the subsequent alteration of nucleic acid and protein are primary etiological factors in the initiation and progression of various metabolic and neurodegenerative diseases” (Azab *et al.*, 2017). “Oxidative stress is associated with disrupted redox regulation mechanism and cellular signalling pathways that can lead to the formation of numerous types of cancer cells and oncogenic initiation and propagation” (Gill *et al.*, 2005; Miranda-Vilela *et al.*, 2011). Antioxidants in cells include catalases, superoxide dismutases (SOD), and glutathione peroxidases (GPX), their induction is usually in response to specific toxicants and pollutants that can induce oxidative stress (Cheung *et al.*, 2001; Azab *et al.*, 2019). SOD scavenges superoxide radicals converting them to H_2O_2 . (Arhoghro & Kpomah, 2022). GPx brings about the reduction in levels of hydrogen peroxide, lipid hydroperoxides and other organic hydroperoxides (Azab *et al.*, 2019). “Glutathione-S-transferases (GST) a representative of the major class of detoxifying enzymes (Hayes & Pulford, 1995), which form a family of multifunctional proteins implicated in the cellular detoxification of cytotoxic and genotoxic xenobiotic compounds and the protection of tissues against oxidative damage” (Pickett & Lu, 1989; Christophe *et al.*, 2017). Catalase is an enzyme present in virtually all aerobic cells, it shields such cells from oxidative stress by catalyzing the speedy decomposition of hydrogen peroxide in two forms of reactions depending on its peroxidative and catalytic activities

(Al-Abrash *et al.*, 2000). The oral administration of coconut juice extract, however, had a significant increase in the level of antioxidant enzymes studied, as evidenced by the increased value of SOD, GST and CAT activity. These increases may be attributed to the antioxidant boasting capacity of phytochemicals and nutritional ingredients present in coconut juice extract (Agbafor *et al.*, 2015; Muhammad, 2018). These nutrients include the amino acid L-arginine which from literature can significantly reduce the rate of generation of free radicals, increase glutathione peroxidase (GPx) activity and with potent antioxidant activity (Lima *et al.*, 2015; Zulaikhah, 2019), the sulphur containing amino acid methionine, is a key player in the biosynthesis of glutathione. Coconut juice extract is also rich in ascorbic acid, which decreases lipid peroxidation in rats (Lima *et al.*, 2015). Other active components in coconut juice extract include cytokines, selenium, zinc, manganese and copper (Zulaikhah, 2019). Cytokines is a potent antioxidant against free radical-induced cell damage (Ajeigbe *et al.*, 2011). Selenium is one of the micronutrients that form the enzyme GPx (Bhagya *et al.*, 2012).

Conclusion

Findings from this study established that the oral administration of 2 mL/kg body weight of coconut juice extract administered for twenty-eight days had positive stimulatory potential on cell antioxidants (SOD, GST and CAT). Boost in antioxidants has been shown in literature to prevent oxidative stress, the primary cause of most metabolic and neurodegenerative diseases. Findings from this study also indicated that the coconut juice extract had a positive health-promoting effect on lipid profile with no attendant adverse effect on renal function as indicated by no significant change ($p < 0.05$) in values of urea, uric acid and creatinine.

Ethical Approval

All animal experimental protocols were approved by the Committee of Scientific Ethics at Niger Delta University, Wilberforce Island, and were carried out by its guidelines for animal use.

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