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Original Article

EFFECTS OF POLYPHENOLS OF *Cocos Nucifera* HUSK FIBRE ON SELECTED KIDNEY FUNCTION INDICES IN MICE

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ABSTRACT

Decoction of *Cocos nucifera* husk fibre is used indigenously in Nigeria for malaria treatment. Polyphenols have been identified as the phytochemicals responsible for the antimalarial activity of *Cocos nucifera* husk fibre, though their toxicity has not been evaluated. The polyphenols of *Cocos nucifera* husk fibre were therefore evaluated for their effects on selected kidney function indices in mice. Fifty mice were randomly divided into five groups (A-E) of ten mice each. Mice in group A were orally administered 5% DMSO solution while those in groups B, C, D and E were orally administered 31.25, 62.5, 125 and 250 mg/Kg body weight of the polyphenols respectively for seven days. Serum urea, creatinine and uric acid concentrations were determined. Serum levels of sodium, potassium, chloride and calcium ions and kidney alkaline phosphatase (ALP), glutamate dehydrogenase (GDH) and gamma-glutamyltransferase (GGT) activities were also determined. The results showed that the polyphenols significantly reduced ($p<0.05$) urea concentration at 250 mg/Kg body weight and creatinine concentration at all doses compared to controls. The polyphenols caused no significant alteration ($p>0.05$) in serum uric acid concentration and kidney ALP, GGT and GDH activities compared to controls. There was significant increase ($p<0.05$) in serum sodium ion concentration at 31.25, 125 and 250 mg/Kg body weight of polyphenols whereas significant increase ($p<0.05$) in serum potassium and chloride ions was observed at 62.5 and 250 mg/Kg body weight compared to controls. Thus, polyphenols of *Cocos nucifera* husk fibre may adversely affect some osmoregulatory functions of the kidney, especially at higher concentrations.

Keywords: *Cocos nucifera*, husk fibre, kidney, polyphenols, toxicity

INTRODUCTION

Malaria is one of the most dreaded human parasitic diseases in the tropics and subtropics, especially in Africa where 90% of cases and 92% of deaths have been estimated to occur; with children under five years of age and pregnant women being most severely affected (WHO, 2016). Nigeria accounts for a quarter of all malaria cases in Africa (WHO, 2008), mostly caused by *Plasmodium falciparum* (Adebayo and Krettli, 2011), with an estimated 212 million malaria cases and over 429,000 deaths in 2015 (WHO, 2016). In addition to its direct health impact, malaria imposes a huge economic burden on afflicted individuals and nations, through high healthcare cost, missed days at work, and reduced economic output and productivity (Sachs and Malaney, 2002).

The use of plant remedies has steadily increased worldwide in recent years, as well as the search for new phytochemicals that can be developed as useful drugs for the treatment of malaria and other infectious diseases (Willcox, 1999). One of such plants used for antimalarial treatment indigenously is *Cocos nucifera* (coconut palm). *Cocos nucifera* husk fibre and white flesh are used in folk medicine for the treatment of malaria (Adebayo and Krettli, 2011; Al-Adhroey et al, 2011). Various parts of the fruit have also been reported to possess antiatherosclerotic (Esquenazi et al, 2002), anticancer (Koschek et al, 2007), antimicrobial (Alviano et al, 2008), vasorelaxant and antihypertensive (Bankar et al, 2011) properties.

Recent *in vivo* and *in vitro* studies have authenticated the acclaimed antimalarial activity of the husk fibre extract of *Cocos nucifera* (Adebayo et al, 2012; 2013). It has been suggested that the husk fibre of the West African Tall variety of *Cocos nucifera* is a potential source of novel antimalarial drug, pinpointing its ethyl acetate extract fraction as being responsible for its antimalarial activity (Adebayo et al, 2013).

Further studies have been carried out to identify the classes of phytochomicals present in the ethyl acetate extract to be alkaloids, polyphenols and tannins (Adebayo et al, 2013). The polyphenols have been indicated to possess antimalarial activity (Adebayo et al., unpublished data). The polyphenols present in the *Cocos nucifera* husk fibre have been identified to be gallic acid, procyanidin dimer, ellagic acid, epicatechin, catechin and procyanidin polymers (Silva et al, 2013). These polyphenols have been reported to be responsible for most of the pharmacological activities of the husk fibre. However, the toxicity of these polyphenols has not been evaluated. This study was therefore carried out to evaluate the toxicological effects of polyphenols of the ethyl acetate extract of *Cocos nucifera* husk fibre on renal functions.

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MATERIALS AND METHODS

Chemicals and Reagents

n-Hexane and ethyl acetate were obtained from Eagle Scientific Limited, Beeston, Nothingham, UK. The assay kits for the determination of plasma concentrations of urea, creatinine and uric acid and that for GGT activity were obtained from Randox Laboratory Ltd., Co-Atrim, UK. All other chemicals used were of analytical grade and were prepared in all glass distilled water.

Plant material

Coconut husk fibre was obtained from the Nigerian Institute for Oil Palm Research (NIFOR), Badagry, Lagos State, in January, 2013. It was taxonomically authenticated at the Herbarium in the Department of Plant Biology, where a voucher specimen was deposited with a Voucher Number UIH001/508.

Experimental animals

Fifty adult Swiss albino mice with an average weight of 20.0 ± 0.57g were obtained from the small Animal Breeding Unit of the Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria. The animals were housed in standard plastic cages and were acclimatized for two weeks. They were maintained under standard conditions of 12 h light and dark cycle and were fed with standard rat chow and water *ad libitum*. The animals were handled according to the guidelines stipulated for animal care (Clark et al, 1997; Garber et al, 2011).

Extract Preparation

The extract was prepared according to the method of Adebayo et al (2003). The coconut husk fibre was dried under shade at room temperature and pulverized into powder. The powder (2000 g) was percolated in 13 L of n – hexane for 72 h in a tightly stoppered glass container. This was shaken at intervals of 2 h. The resulting mixture was filtered with Whatmann No. 1 filter paper. The filtrate was then concentrated under pressure in a rotary evaporator at 40 ^{o}C, thereby generating the crude hexane extract. The residue was air dried, to allow complete evaporation of the n-hexane from the sample. This residue was again percolated in 13 L of ethyl acetate for another 72 h. This was filtered using Whatmann No. 1 filter paper and the filtrate was likewise concentrated using rotary evaporator at 40 ^{o}C to generate the ethyl acetate extract, with a percentage yield of 0.26%.

Extraction of Polyphenols

Polyphenols were extracted using the method described by Bohm and Abyazan (1994). The ethyl acetate extract (5g) was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatmann No. 42 filter paper and the filtrate was later transferred into a crucible and evaporated to dryness over a water bath at 37 ^{o}C and weighed to a constant weight and the yield was 10.6% of the ethyl acetate extract. The polyphenols obtained was used for this study and further confirmatory phytochemical tests were carried out to ascertain that only polyphenols were present.

Animal Grouping and Drug Administration

Fifty mice were randomly assigned into five groups (A – E) of ten mice each. Each mouse in the control group (A) was orally administered 0.2 ml of 5% DMSO solution for seven days. The polyphenols were dissolved in 5% DMSO solution and doses of 31.25, 62.5, 125 and 250 mg/kg body weight of polyphenols were orally administered to mice in groups B, C, D and E respectively for seven days.

Collection and Preparation of Samples

At the end of the experimental period, the mice were slightly anaesthetized with diethyl ether, the neck area was quickly cleared of fur and skin to expose the jugular veins, from which blood was then collected into sample bottles. The blood samples were allowed to stand for 10 min at room temperature for clot formation and subsequently centrifuged at 3000 rpm for 5 min, after which the serum was pipetted out and stored frozen at -20 ^{o}C till needed for analysis.

Also, the mice were quickly dissected and the organ of interest (kidney) was isolated, cleaned of blood stains, weighed, suspended in an ice-cold 0.25 M sucrose solution (1:5 w/v) and homogenised. The homogenates were frozen overnight to allow complete cell lysis and maximum release of enzymes (Akanji et al, 1993).

Determination of Biochemical Parameters

The concentrations of urea, creatinine and uric acid in the serum were determined using methods described by Venianim and Vakirtzi (1970), Bartels and Bohmer (1972) and Tiezt (1995) respectively. The activities of alkaline phosphatase, glutamate dehydrogenase and gamma – glutamyltransferase in the kidney were determined using methods described by Wright et al (1972), Szasz (1969) and Shimizu et al. (1979). The serum levels of Na^{+}, K^{+}, Cl^{-} were determined using methods described by Tiezt (1995) while the serum level of Ca^{2+} was determined using the methods described by Barnett et al (1973).

Statistical analysis

Experimental data are presented as means ± SEM. Statistical analysis was implemented using computer software, 20.0 version of SPSS statistical package program (SPSS, Chicago, IL). One-way analysis of variance was used to compare variables among the different groups while Duncan's Multiple Range Test was used as the post hoc test. Differences among the various treatment groups at $p<0.05$ were considered statistically significant.

RESULTS

No significant change ($p>0.05$) in kidney-body weight ratio was caused by polyphenols at all doses administered compared to control (Table 1). There was no significant change ($p>0.05$) in serum urea concentration in all the

animals administered various doses of polyphenols except the significant reduction ($p<0.05$) observed at 250 mg/kg body weight compared to control (Table 2). The polyphenols caused significant decrease ($p<0.05$) in serum creatinine concentration of the animals at all doses compared to control (Table 2). Relative to control, there was no significant alteration ($p>0.05$) in serum uric acid concentration at all doses of polyphenols administered (Table 2).

There was no significant alteration ($p>0.05$) in serum sodium ion concentration of mice administered 62.5 mg/kg body weight of polyphenols but the level was increased significantly ($p<0.05$) at other doses compared to control (Table 3). The polyphenols did not significantly alter ($p>0.05$) serum concentrations of potassium and chloride ions at 31.25 and 125 mg/kg body weight but caused significant increase ($p<0.05$) at 62.5 and 250 mg/kg body weight compared to controls (Table 3). The polyphenols caused significant increase ($p<0.05$) in serum calcium ion concentration at 62.5 mg/Kg body weight compared to control (Table 3). Alkaline phosphatase, gamma-glutamyl transferase and glutamate dehydrogenase activities in the kidney were not significantly changed ($p>0.05$) at all doses of polyphenols compared to controls (Table 4).

Table 1. Kidney – Body Weight Ratios of Mice Administered Polyphenols of *Cocos nucifera* Husk Fibre

GROUP	Kidney/Body Weight Ratio
CONTROL	0.033 ± 0.017 [a]
31.25 mg/Kg b.wt	0.011± 0.002 [a]
62.5 mg/Kg b.wt	0.013± 0.003 [a]
125 mg/Kg b.wt	0.026± 0.014 [a]
250 mg/Kg b.wt	0.011± 0.003 [a]

Values are expressed as Means ± SEM (n=10). Values in each column with the same superscript are not significantly different ($p>0.05$). b.wt – body weight

DISCUSSION

Serum urea, creatinine and uric acid concentrations are used for the assessment of renal sufficiency (Whelton et al, 1994). Serum urea, creatinine and uric acid concentrations higher than normal are indicators of deficiency in renal function (Whelton et al, 1994; Oyewole et al, 2012). Moreover, increase in serum urea level may also be due to increase in protein catabolism (Whelton et al, 1994) while decreased serum urea level may be attributed to impairment of the urea cycle leading to reduced production of urea (Adebayo et al, 2003). The polyphenols, at various doses administered, caused no significant alteration ($P>0.05$) in serum urea concentration except the reduction ($P<0.05$) observed at 250 mg/kg body weight, suggesting that there was impairment in the urea cycle at this dose in the liver. The decrease in serum creatinine concentration of all the treated groups suggests impairment in creatinine production in the muscle caused by the polyphenols. Uric acid is the major product of purine catabolism. The polyphenols, at all doses administered, caused no significant alteration ($P>0.05$) in serum uric acid level, suggesting that the normal excretion of the end product of purine catabolism was not adversely affected. It also suggests that the contribution of uric acid to the antioxidant status of the blood was not compromised.

Electrolyte concentrations in the serum, being governed by diet, renal excretion and pathological conditions, could serve as indices of kidney function (Kamath, 1972; Guyton and Hall, 2000). Sodium ion plays important roles in maintaining the salt/water balance in the body, thus protecting the body against excessive fluid loss and neuronal dysfunction (Flanigan, 2000). The observed increase in serum concentrations of sodium and chloride ions at various doses of polyphenols suggests increased reabsorption of these ions in the kidney, possibly through the activation of renin-angiotensin system, which has been reported to regulate the concentrations of these ions in the blood (McCormick and Bradshaw, 2006).

Table 2. Selected Kidney Function Indices in Serum of Mice Administered Polyphenols of *Cocos nucifera* Husk Fibre

GROUP	Urea Concentration (mmol/L)	Creatinine concentration (mmol/L)	Uric Acid concentration (mmol/L)
CONTROL	188.40 ± 0.21 [a]	4324.00 ± 1308.64[a]	2875.53 ± 379.01[a]
31.25 mg/Kg b.wt	190.76± 1.00 [a]	1380.00 ± 92.00 [b]	2467.03± 502.96[a]
62.5 mg/Kg b.wt	188.79± 0.35 [a]	1564.00 ± 184.00 [b]	2555.14± 614.41[a]
125 mg/Kg b.wt	187.72± 1.69 [a]	1392.27 ± 203.33 [b]	3067.77± 419.96[a]
250 mg/Kg b.wt	178.62± 4.71 [b]	1165.33 ± 30.67 [b]	2558.21± 1348.85[a]

Values are expressed as Means ± SEM (n=10). Values in each column with different superscripts are significantly different ($p<0.05$). b.wt – body weight

Table 3: Concentrations of Serum Electrolytes in Mice Administered Polyphenols of *Cocos nucifera* Husk Fibre

GROUP	Na^+(mmol/L)	K^+ (mmol/L)	Cl^-(mmol/L)	Ca^{2+} (mmol/L)
CONTROL	134.50 ± 1.55[a]	3.93 ± 0.15[a]	68.75 ± 19.99[a]	2.04 ± 0.03[a]
31.25 mg/Kg b.wt	145.75± 1.11[c]	4.20 ± 0.11[a,b]	96.75 ± 2.06[a,b]	2.06 ± 0.06 [a]
62.5 mg/Kg b.wt	137.50± 0.96[a,b]	4.60 ± 0.2[b,c]	104.50 ± 1.71[b]	2.20 ± 0.01[b]
125 mg/Kg b.wt	139.75± 1.11[b]	4.18 ± 0.09[a,b]	95.00 ± 1.29[a,b]	2.16 ± 0.04[a,b]
250 mg/Kg b.wt	145.00± 0.91[c]	4.80 ± 0.27[c]	108.50 ± 0.96[b]	2.10 ± 0.06[a,b]

Values are expressed as Means ± SEM (n=10). Values in each column with different superscripts are significantly different ($p<0.05$). b.wt – body weight

Table 4: Activities of Selected Enzymes in Kidney of Mice Administered Polyphenols of *Cocos nucifera* Husk Fibre

GROUP	ALP (nmol/min/mg protein)	GDH (U/mg protein)	GGT (IU)
CONTROL	25.67 ± 6.47[a]	2.77 ±0.46[a,b]	19.86 ± 1.35[a,b]
31.25 mg/Kg b.wt	24.29± 4.88[a]	4.39± 1.82[b]	28.10± 4.73[b]
62.5 mg/Kg b.wt	16.26± 2.27[a]	0.27± 0.04[a]	23.45± 3.66[a,b]
125 mg/Kg b.wt	27.10± 2.31[a]	0.93± 0.42[a]	13.19± 0.87[a]
250 mg/Kg b.wt	20.45± 3.42[a]	0.74± 0.23[a]	21.39 ± 5.22[a,b]

Values are expressed as Means ± SEM (n=10). Values in each column with differentsuperscripts are significantly different ($p<0.05$). ALP – Alkaline phosphatase; GDH – Glutamate dehydrogenase, GGT – gamma-glutamyltransferase, b.wt – body weight

Chloride ion plays roles in maintaining proper body water distribution, osmotic pressure, and normal anion-cation balance in the extracellular fluid compartment (Burtis and Ashwood, 1994). The observed significant increase ($p<0.05$) in serum level of chloride ion of mice administered 62.5 and 250 mg/Kg b.wt of polyphenols could be as a result of loss of body fluids (dehydration) and high levels of blood sodium observed in this study. It could also be as a result of hyperactivity of the parathyroid glands. This can lead to depression of myocardial function and reduce cardiac output, renal and intestinal perfusion (Waters et al, 2001). Potassium ion is important in muscle contraction and the transmission of nerve impulses in animals through action potentials and also influences osmotic balance between cells and the interstitial fluid. The observed significant increase ($p<0.05$) in serum potassium ion levels in mice administered 62.5 and 250 mg/kg body weight of polyphenols could be as a result of its reduced excretion by the kidney which could lead to fatal heart rhythm disturbances (arrhythmias) including ventricular tachycardia and ventricular fibrillation.

Calcium ion is essential for living organisms as a signal for many cellular processes. The significant increase ($p<0.05$) observed in the serum level of calcium ion of mice administered 62.5 mg/Kg b.wt of polyphenols could be as a result of decreased excretion by the kidney or hyperactivity of the thyroid gland. This could lead to dehydration because the kidneys will automatically start excreting more water which would lead to nausea, loss of appetite, vomiting and acute state of confusion associated with apathy, depression, or lethargy. Personality changes and psychosis have also been implicated in subjects with increased serum calcium ion concentration (Tonner and Schlechte, 1993; Chan et al, 1997).

Alkaline phosphatase (ALP) has been reported to be the marker enzyme for plasma membrane integrity (Wright and Plummer, 1974; Akanji et al, 1993), such that any alteration in the activity of the enyzme in tissue, caused by interaction of membrane with xenobiotics (Molina et al, 2005), would indicate likely damages to the plasma membrane of the cells (Yakubu et al, 2006). Decrease in ALP activity caused by stressors probably indicates an altered production and transport of inorganic phosphate (Engstrom, 1974) and an inhibitory effect on cell growth and proliferation (Goldfischer, 1964). Inhibition of ALP activity results in reduction in protein synthesis and uncoupling of oxidative phosphorylation (Verma et al, 1984). At various doses of polyphenols administered, alkaline phosphatase activity in kidney was not significantly changed, suggesting the preservation of the integrity of the plasma membrane of cells in this tissue and availability of inorganic phosphate for cellular processes.

Glutamate dehydrogenase catalyzes the reversible oxidative deamination of L– glutamate to α– ketoglutarate and ammonia. It plays an important role in catabolism of amino acids (Murray et al, 2007) and the kidney has been reported to be the secondary site of protein catabolism. This reaction also serves as a link between protein and carbohydrate metabolism through TCA cycle. It also plays a major role in glutathione metabolism and reabsorption of amino acids from glomerular filtrate and intestinal lumen (Kaplan and Pesce, 1996). Glutamate dehydrogenase is a useful biochemical indicator of injury to the mitochondria (Henley et al, 1966). An increase in its activity in the tissue indicates induced synthesis of the enzyme (Lehninger, 1978), whereas reduction in its activity indicates that the mitochondrion has been adversely affected or suppression of its synthesis

(Akanji et al, 2008). Glutamate dehydrogenase activity in the kidney of mouse was not significantly affected at all doses of polyphenols administered, suggesting that there was no significant mitochondrial damage in the kidney and its role in catabolism of amino acids in the kidney was not compromised.

Gamma-glutamyltransferase is a membrane bound glycoprotein found mainly in cells of liver, kidney, pancreas and prostate (Mayne, 1998). It catalyzes the transfer of glutamate from one molecule to another. This enzyme acts only on peptides or peptide-like compounds containing a terminal glutamate residue joined to the remainder of the compound through the terminal carboxyl group (Tietz, 1995). At various doses of polyphenols administered, gamma glutamyltransferase activity in kidney was not significantly changed, suggesting the preservation of the integrity of the plasma membrane of cells in this tissue.

The results of this study suggest that the polyphenols of *Cocos nucifera* husk fibre, to some extent, may adversely affect some osmoregulatory functions of the kidney, especially at higher concentrations.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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