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CONSUMPTION OF AFRICAN ELEMI PULP AND AFRICAN WALNUT SEED KERNEL: IMPACT ON HEPATOTOXICITY-RENAL FUNCTION INDICES OF RATS

Ernest A. A. Anyalogbu (PhD)*

Department of Biotechnology, School of Biological Science, Federal University of Technology Owerri, Imo State, Nigeria.

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***Corresponding Author**
Ernest A. A. Anyalogbu (PhD)
Department of Biotechnology, School of Biological Science, Federal University of Technology Owerri, Imo State, Nigeria.

ABSTRACT

Background: Dietary factors are critical in the maintenance of normal healthy liver and kidney. This study evaluated the effect, if any, of the consumption of the two plant foods on the hepatotoxicity-renal function indices of rats. **Method**: Flours of African elemi (AE) and African walnut (AW) samples subjected to graded heat treatments were added as sole protein sources in diets labelled: AE_0, AE_{15}, AE_{30}, AE_{45}, AW_0, AW_{45}, AW_{90}, and AW_{135} (based on heat-contact time (minutes)). The diets were used in a 28day feeding period involving 45 weanling albino rats and the serum hepatotoxicity and renal function indices of the rats were analysed using standard methods. **Results**: Similarities were observed in the relative liver and kidneys weights of animals on the test and reference diets. The serum levels of the liver function and toxicity parameters and renal function indices obtained for rats on test diets in each of the plant foods and those on the reference diet were statistically similar ($p>0.05$). On pairwise comparison, AE_{30}, AE_{45}, and diets based on processed AW gave higher serum total protein and albumin values and lower total bilirubin values, while all levels of inclusion of both plants gave lower serum AST and ALT activities than the reference diet. Also, apart from Na in AE_0, the serum levels of the renal function indices were lower in rats fed sample diets relative to those on RD. **Conclusions:** This study showed that the consumption of the plant foods do not negatively affect the liver or kidneys but on a pairwise basis, indicated hepato-tonic and reno-protective potentials.

KEYWORDS: Hepato-tonic, reno-protective, hepatotoxicity, renotoxic, hypertrophy.

INTRODUCTION

The glomerulus of the kidney interfaces directly with the blood and has a major role in maintaining body homeostasis.[1] It does this by regulating the blood levels of electrolytes, principally sodium and potassium, and removing urea and creatinine.[2] Blood Urea Nitrogen (BUN), the major nitrogenous end product of protein and amino acid catabolism, is produced by the liver and distributed throughout intracellular and extracellular fluids. It is eliminated almost entirely via urinary excretion by the kidney and may be elevated when the organ function is reduced.[3] According to Miller *et al*.[4] glomerular filtration rate (GFR), which provides the best index of overall kidney function, is most widely estimated by measuring plasma creatinine concentration. Creatinine is a breakdown product of creatine phosphate in muscle and is usually produced at a fairly constant rate by the body depending on muscle mass.[5] It is normally eliminated by the kidneys and may be elevated when kidney function is reduced.[3] Electrolytes (e.g. Na+, K+), on the other hand, are homeostatically sieved out of the blood by the kidneys. Abnormal levels may accrue under reduced kidney function. Consequently, blood levels of urea, creatinine and electrolytes (Na+, K+) are employed as markers of renal function in the routine analysis.[1,3] The liver is a complex organ and virtually affects every physiological process of the body. All the blood leaving the stomach and intestines passes through it. Some enzymes are cell, tissue or organ-specific and can be employed as their markers. Based on its enzyme constitution, the liver is the site for detoxification/biotransformation of potentially toxic substances (endogenous and exogenous compounds)[6], many intermediary metabolic processes and synthesis of several important biochemicals.[7] Such liver enzymes as alanine aminotransferase ALT and aspartate aminotransferase AST are released from the liver upon its damage or failure. Elevation in the activities of these enzymes in the serum is taken as an indicator of hepatocellular damage /injury/lesion.[8]

The liver makes glucose through gluconeogenesis; removes bilirubin, a breakdown product of haemoglobin, from the body via glucuronidation and, synthesizes the majority of plasma proteins (albumin and globulins). Consequently, alterations in blood levels of glucose, total protein, albumin and total bilirubin (total and conjugated) among others are measures of overall hepatic function.[9] Hepatotoxicity ensues when the liver injury is associated with impaired liver function.[10]

African Elemi pulp is a fruit drupe produced by African elemi or bush candle (*Canarium schweinfurthii*), an evergreen *Burseraceae* tree plant. On the other hand, the African Walnut seed kernel is a seed-borne in a thin brown shell (reminiscent of template walnut) by African walnut (*Plukenetia conophora*) plant, a climbing *Euphorbiaceae*. Macerated drupe pulp of African elemi and raw, roasted or cooked seed kernels of African walnut are consumed as masticatories/traditional snacks in parts of Nigeria and Central Africa.[11]

Several researchers have opined that dietary factors are crucial in the maintenance of normal hepatic and renal function indices required for healthy liver and kidney respectively.[12] Yakotani *et al*.[13] regretted the paucity of knowledge on the effect of plant foods on the health of the liver. Consequently, this work is intended to evaluate the effect, if any, of the consumption of the two plant foods, African elemi pulp and African walnut seed kernel, on the hepatotoxicity and renal function indices of rats.

MATERIALS AND METHODS

Sample collection and Processing

Forty-five weanling albino rats (21 - 30 days old) weighing 26.17 - 71.0 g used were obtained from the Department of Animal Health and Production, Faculty of Veterinary Medicine, University of Nigeria, Nsukka. African elemi seeds from Ngwa road market Aba, Aba South Local Government Area, Abia State, Nigeria and fresh wholesome seeds of African walnut collected from Ojoto, Idemili South Local Government Area of Anambra State, Nigeria used were processed as described by Onyeike *et al*.[14] and Anyalogbu *et al*.[11] Each of the plant samples was washed thoroughly with water and divided into four lots. The 1st lots (used raw) were labelled AE_0 and AW_0 for African elemi and African walnut respectively. The 2nd, 3rd and 4th lots; for African elemi were macerated in hot water (55^0C) for 15, 30 and 45 min and labelled AE_{15}, AE_{30}, and AE_{45} respectively and for African walnut boiled in water (99 ± 1^0C) for 45, 90, and 135 min and labelled AW_{45}, AW_{90}, and AW_{135} respectively. The pulps and kernels (respectively) were processed into flours and used as stock samples.

Formulation of the experimental diets The diets was formulated precisely as described by Anyalogbu and Onyeike.[15] The sample flours were added as sole protein sources at the expense of corn starch, based on their protein contents[15,16] to give at least 10% dietary protein[17] and the diets based on the sample content respectively labelled: AE_0, AE_{15}, AE_{30}, AE_{45}, AW_0, AW_{45}, AW_{90}, and AW_{135}. Nutrend® (a commercial infant formula) was used as a control/reference diet (RD).

Experimental design

The initial weights of each of the rats were recorded and then allotted to nine groups of five rats each such that the average initial weights of the groups were as close as possible. The groups were labelled AE_0, AE_{15}, AE_{30}, AE_{45}, AW_0, AW_{45}, AW_{90}, AW_{135} and RD, and members were housed individually in all-wire cages. Sequel to 7-day acclimatization (on water and normal rat ratio), the rats were subjected to a 28-day feeding period during which water and specific diets were offered *ad libitum*. Then 6hr fast was imposed on the rats and their weights were taken to obtain the final body weights.

Organ and blood samples

At the end of the post-feeding fast, the animals were exposed to chloroform in a closed container to induce anaesthesia.[18] Then the abdomen was opened by an incision along the midventral line. The skin and musculature were folded back and an incision was made into the thoracic cavity to expose the heart. With a syringe and needle, a blood sample (about 4ml) was withdrawn by cardiac puncture, placed in a vial and allowed to stand for 45min at room temperature. Serum was obtained from the blood sample by centrifugation at 600xg for 15min and stored at 4^oC in a refrigerator before analyses. Then kidneys and liver were carefully exercised from each animal with a surgical blade, weighed on a digital weighing balance (Mettler PT 320) and the weights were recorded as absolute organ weights.

Calculation of relative organ weights

The absolute organ weights were normalized by converting them to relative organ weights as reported by Ola-Davies.[19] Relative organ weight is the per cent organ weight to body weight,

$$\text{i.e. } Relative\ organ\ weight = \frac{Organ\ weight\ (g)}{Body\ weight\ (g)}\ x\ \frac{100}{1}$$

Liver Function and Toxicity Indices Assays

Glucose concentration

This was done using the glucose oxidase method as presented by Biosystems (USA) glucose test kit. Glucose oxidase oxidizes glucose to gluconate producing a stoichiometric amount of hydrogen peroxide.

$$\text{Glucose} + \tfrac{1}{2}O_2 + H_2O \xrightarrow{\text{Glucose oxidase}} \text{Gluconate} + H_2O_2$$

In a reaction catalyzed by peroxidase, the H_2O_2 oxidizes a chromogenic substance to coloured product (pink) intensity of which is proportional to the concentration of glucose in the blood sample.

$$2H_2O_2 + 4\text{Aminoantipyrine} + \text{phenol} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine} + 4H_2O$$

In this assay, test tubes were labelled test, standard or blank and 0.02ml each of serum sample, standard glucose solution (100mg/dl), and distilled water was respectively added to the test tubes. Then 0.1ml of phenol reagent and 1.0ml of glucose oxidase-peroxidase reagent was added to each of the test tubes, mixed thoroughly by vortexing and incubated for 20min at room temperature. The absorbance of the sample or standard was read against the blank at 510nm in a spectrophotometer.

The glucose concentration of the sample was calculated as follows:

$$\text{Serum glucose conc.} = \frac{\text{Abs. of sample}}{\text{Abs. of standard}} \; x \; \text{Conc. of std.}$$

Total protein concentration

The test was carried out using the Biuret method for protein determination as presented by Biosystems (USA) protein test-kit. Peptide bonds occur with the same frequency per amino acid in a peptide. Copper (II) ions, in an alkaline medium, interact with protein peptide bonds resulting in the formation of a coloured complex. The intensity of the colour, and hence the absorption at about 500nm wavelength is directly proportional to the protein concentration, according to Beer-Lambert law.

$$\text{Proteins} + Cu^{2+} \xrightarrow{\text{Alkali}} \text{coloured complex}$$

Here test tubes were labelled, reagent blank, standard or sample. A 0.02ml each of distilled water, standard (66g/L bovine serum albumin), and serum sample was respectively pipetted into the test tubes. Then 1.0ml of Biuret Reagent solution (6mmol/L Copper (II) acetate, 12mmol/L potassium iodide, 1.15mmol/L sodium hydroxide, and detergent) was added to each of the test tubes and mixed thoroughly. The tubes were incubated for 30mins at room temperature and the absorbance of the sample and standard read against the reagent blank at 500nm wavelength in a spectrophotometer. The protein content of the serum sample was calculated using the formula:

$$\text{Total Serum Protein conc.} = \frac{\text{Abs. of sample}}{\text{Abs. of standard}} \; x \; \text{Conc. of std.}$$

Albumin concentration

The method employing bromocresol green (BCG) as described by Doumas *et al*.[20] was used. In an acidic medium (pH 4.1) BCG reacts with albumin to form an intense green complex.

$$\text{Albumin} + \text{Bromocresol} \xrightarrow{\text{pH 4.1}} \text{Green complex}$$

The absorbance of the albumin–BCG complex at 630nm wavelength is proportional to the albumin concentration in the sample.

Based on the protocol, test tubes were labelled blank, standard or sample. Albumin standard and serum samples (10µl each) were pipetted to the standard and sample test tubes respectively. Then 1.0ml of Bromocresol reagent (100mmol/L acetate buffer (pH 4.1), 0.27mmol/L bromocresol green, detergent) was added to all the test tubes, mixed thoroughly and incubated for 1min at room temperature. The absorbance of the sample and standard were read against the blank at 630nm wavelength in a spectrophotometer. The albumin content of the sample was calculated using the formula:

$$\text{Sample Albumin conc.} = \frac{\text{Abs. of sample}}{\text{Abs. of std.}} \times \text{Conc. of std.}$$

Total bilirubin concentration

The diazotized sulphanilic acid method described by Zoppi *et al*.[21] was used. Serum total bilirubin is composed of direct (conjugated) bilirubin and indirect (unconjugated) bilirubin. In the presence of solubilizing agent (such as a surfactant), unconjugated bilirubin is separated from its carrier (albumin) and then together with conjugated bilirubin couples with diazotized compound and transform into azobilirubin (azodye) – giving total bilirubin. The addition of alkaline solution causes the colour of the azobilirubin (pink) to change to greenish-blue with the intensity being proportional to the concentration of bilirubin in the sample.

$$\text{Bilirubin} + \text{diazotized compound} \xrightarrow[\text{Alkaline solution}]{\text{Surfactant}} \text{Azobilirubin (total)}$$

In this assay, four test tubes were labelled, reagent blank, sample blank, sample and standard. A 100µl distilled water was placed in the reagent blank, 100µl of serum sample in each of the sample blank and sample test tubes and 100µl of bilirubin standard (4.3g/L concentration) in standard. Then 1.0ml of Reagent AT (a mixture of 29mmol/L sulphanilic acid, 0.2mmol/L HCl and 50mmol/L cetrimide) was pipetted into the sample blank, 1.0ml of working reagent

(a mixture of 11.6mmol/L sodium nitrate and 4ml of reagent AT) into each of the Reagent blank, sample and standard test tubes. The content of the test tubes was mixed thoroughly by vortexing and incubated for 2min at room temperature.

The absorbance of the sample blank was read in a spectrophotometer at 540nm wavelength against distilled water. The absorbance values of sample and standard were read at the same wavelength (as the sample blank) against the reagent blank.

The total serum bilirubin (mg/dl) was calculated as follows:

$$\text{Serum total bilirubin} = \frac{\text{Abs. of sample} - \text{Abs. of sample blank}}{\text{absorbance of std}} \times \frac{\text{Conc. of std}}{1}$$

Aspartate aminotransferase (AST) activity

The method as described by Awodele *et al.*[22] was adapted for the determination of aspartate aminotransferase activity. AST catalyses the transfer of the alpha-amino group from L-aspartate to alpha-ketoglutarate (transamination reaction) forming oxaloacetic acid and glutamic acid.

$$\alpha - \text{ketoglutarate} + \text{L} - \text{aspartate} \xrightarrow{\text{AST}} L - \text{glutamate} + \text{Oxaloacetate}$$

AST activity is monitored by measuring the concentration of oxaloacetate hydrazone complex produced when the oxaloacetic acid is reacted with 2,4-dinitrophenylhydrazine.

$$\text{Oxaloacetate} + 2{,}4 - \text{dinitrophenylhydrazine} \longrightarrow \text{Hydrazone complex}$$

Upon addition of sodium hydroxide, the complex forms a colour whose intensity is related to enzyme activity

A mixture of 100mmol/L phosphate buffer (pH 7.4), 100mmol/L of L-aspartate and 2mmol/L L-ketoglutarate (Reagent 1) and, 2mmol/L 2, 4-dinitrophenyl hydrazine (Reagent 2) were made ready by allowing to equilibrate at room temperature. With a micropipette, 0.1ml of the serum sample was placed in a test tube, 0.5ml of the Reagent 1 was added and mixed. The mixture was incubated for 30min in a water bath set at 37^{o}C. Then 0.5ml of the Reagent 2 was added and the mixture was incubated for 20 min at 25^{o}C. Then, 5.0ml of 4% sodium hydroxide was added and the solution was mixed thoroughly by vortexing. A reagent blank was also prepared. The absorbance of the sample was read in a digital spectrophotometer against the reagent blank at 510nm wavelength after 5min.

The activity of AST in the serum sample was read from the AST activity calibration curve.

Alanine aminotransferase (ALT) activity

The alanine aminotransferase activity was also determined based on the method described by Awodele *et al*.[22] The enzyme ALT catalyses the transfer of amino group (-NH2) from L-alanine to alpha-ketoglutarate forming pyruvate and L-glutamate.

$$\alpha - \text{ketoglutarate} + \text{L} - \text{alanine} \xrightarrow{\text{ALT}} \text{glutamate} + \text{pyruvate}$$

ALT activity is quantified by measuring the concentration of pyruvate hydrazone complex formed when the pyruvate is reacted with 2,4-dinitrophenylhydrazine.

$$\text{pyruvate} + 2,4 - \text{dinitrophenylhydrazine} \longrightarrow \text{pyruvate} - \text{hydrazone complex}$$

This produces a red-coloured complex on the addition of sodium hydroxide. The intensity of the colour is related to enzymatic activity.

The protocol used for ALT activity assay was the same as that for AST except that 200mmol/L of L-alanine substituted 100mmol/L of L-aspartate in Reagent 1.

RENAL FUNCTION INDICES ASSAYS

Sodium (Na^+) concentration

The sodium content of the serum sample was determined colourimetrically using the method described by Guillaumin and DiBartola.[23] Sodium is precipitated as the triple salt, sodium magnesium uranyl acetate. The excess uranium is then reacted with ferrocyanide, producing a chromophore whose absorbance varies inversely from the concentration of sodium in the test sample.

Procedure

The determination of serum sodium concentration was done in two stages: Filtrate preparation, and colour development.

Filtrate preparation

Test tubes were labelled blank, standard and sample and, 1.0ml of filtrate reagent (2.1mM uranyl acetate and 20mM magnesium acetate in ethyl alcohol) pipetted to each of them. Then 50µl of each distilled water, standard (150mEq/L sodium chloride solution) and serum sample were respectively added to the test tubes. The tubes were vigorously shaken for 3 min

to mix and then centrifuged at 1500G for 10min and the supernatant was collected into appropriately labelled test tubes.

Colour development

To each of the tubes, 1.0ml of Acid reagent (dilute acetic acid) was added. Then 50µl of colour reagent (potassium ferrocyanide, non-reactive stabilizers, and fillers) was added to all the tubes and mixed by vortexing. The absorbances (A) of the tubes were read against distilled water at 550nm in a spectrophotometer and the concentration of sodium in the serum sample was calculated as follows:

$$\text{Sodium in sample} = \frac{\text{A(blank)} - \text{A(sample)}}{\text{A(blank)} - \text{A(std)}} x \text{ Conc. of std (mmol/L).}$$

Potassium (K^+) concentration

The Tetraphenylboron – sodium method as described by Henry[24] was used in this determination. The solid particle in the serum sample is sedimented in an alkaline medium with trichloroacetic acid (TCA). The potassium ions in the supernatant precipitated with sodium tetraphenylboron (Na-TPB) giving rise to a turbid and stable potassium tetraphenylborate suspension whose turbidity is proportional to the potassium concentration of the sample.

Test tubes were labelled blank, sample and standard. In another test tube, 0.1ml of serum sample and 1.0ml of reagent A (0.3m Trichloroacetic acid) were pipetted, mixed thoroughly, centrifuged at 2000xg for 5mins and the supernatant collected. Then 2.0ml of the working reagent (0.1m Na-TPB/0.1m NaOH (1:1)) was added to each of the labelled test tubes and 0.2ml each of the supernatants and standard (Aqueous solution of potassium equivalent to 5mmol/L) added to sample and standard respectively. The content of the test tubes was mixed thoroughly to obtain homogenous turbidity and allowed to stand for 5min. Then the mixture was mixed again (by shaking) and the absorbance of the sample and standard read at 580nm against the blank. The potassium concentration (mmol/L) of the sample was calculated using the formula.

$$\text{Potassium conc.} = \frac{\text{Abs. of sample}}{\text{Abs. of std.}} x \text{ Conc. of std.}$$

Urea concentration

The urease/salicylate method described by Osman *et al.*[25] was used. Urease stoichiometrically converts urea in the serum to ammonium.

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} 2NH_4^+ + CO_2$$

Through coupled reaction with the salicylate, the ammonium is converted to coloured compound – indophenols and measured by spectrometry.

$$NH_4^+ + \text{Salicylate} + \text{NaClO} + \text{Nitroprusside} \longrightarrow \text{coloured compound (Indophenols)}$$

Three test tubes were labelled blank, standard and sample. A 10µL each of Urea standard (50ml/dL) and serum sample was pipetted into standard and sample test tube respectively. Then 1.0ml of reagent A (mixture of 62mmol/L sodium salicylate, 3.4mmol/L sodium nitroprusside, 20mmol phosphate buffer (pH6.9), >500U/L Urease) was added to each of the test tubes, mixed thoroughly and incubated for 10min at room temperature. Subsequently, 1.0ml of reagent B (7mmol/L sodium hypochlorite, 150mmol/L sodium hydroxide) was pipetted to each of the test tubes mixed thoroughly and again incubated for 10min at room temperature. The absorbance of the standard and sample was read at 600nm wavelength against the blank. Urea concentration in the sample was calculated using the formula:

$$\text{Urea Conc.} = \frac{Abs.\,of\ sample}{Abs.\,of\ std.}\ x\ Conc.\,of\ std.$$

Creatinine concentration

The Creatinine Alkaline Picrate method described by Fabiny and Ertingshausen[26] was used.

In an alkaline medium, creatinine in the sample reacts with picrate forming a coloured complex intensity of which is an index of the creatinine concentration in the sample and is measured spectrophotometrically.

For this assay, a working reagent prepared by thoroughly mixing 0.4mol/L sodium hydroxide and 25mol/L picric acid (1:1) was placed in a water bath set at 37^0C. The temperature of the spectrophotometer was brought to 37^0C also. Cuvettes were labelled standard or sample and 1.0ml of the working reagent pipetted to each of the cuvettes. Then 0.1ml of standard (2mg/dl) or serum sample was pipetted to the standard or sample cuvette, mixed and inserted into the spectrophotometer. A stopwatch was immediately started. Absorbance at 500nm wavelength was recorded after 30seconds (A_1) and after 90seconds (A_2).

Creatinine in the sample was calculated using the general formula:

$$\text{Creatinine in sample} = \frac{(A2 - A1)\ \text{sample}}{(A2 - A1)\text{std.}} x\ \text{Conc. of std.}$$

Effects of samples on serum concentration of parameters

This was calculated from respective values of sample and control groups using the general formula:

$$\text{Effect (\%)} = \frac{\text{Sample} - \text{Control}}{Sample} x \frac{100}{1}$$

Statistical analyses

Data from the assays were tested for statistical significance using one-way analysis of variance (ANOVA) and treatment means (means ± SDs) compared by the PostHoc-Least significance Difference – Duncan's multiple range tests using Statistical Package for Social Sciences (SPSS) version 20. Differences were considered significant at $p \leq 0.05$ in all case.

RESULTS

Absolute and relative organ weights of rats fed diets based on raw and macerated African elemi pulp flours are presented in Table 1, while those of rats fed diets formulated with raw and cooked AW seed kernel flours are presented in Table 2. The absolute liver and kidneys weights of rats fed reference/control diet (Nutrend) were significantly ($p \leq 0.05$) higher than those adapted on each of the plant food-based diets. Relative to the RD group, while the absolute organ weights from rats on each of the plant food-based diets were generally decreased (-3.49 - -44.83%) the relative organ weights were variously affected by AE but increased (3.33 – 10.82% in AW (Figures 1 and 2). Tables 3 and 4 present the serum values of liver function (glucose, total protein, albumin and total bilirubin) and toxicity (aspartate aminotransferase AST and alanine aminotransferase ALT) indices of rats fed raw and macerated AE pulp and, raw and cooked AW seed kernel flours-based diets respectively. The values obtained from test groups in both plant foods, though statistically similar ($p>0.05$) were decreased except for T. protein and albumin (Figures 3 and 4).

The serum concentration of renal function indices of rats fed diets based on raw and macerated AE pulp flour are presented in Table 5. Those rats fed diets based on raw and cooked AW seed kernel flour are shown in Table 6. Apart from the value of Na in AE_0 and creatinine in AW_0 diet groups, the serum concentration of all the renal function indices were

lower in rats fed with the two plant foods-based diets than the RD (Figures 5 and 6) with the values for K in AE_0, AE_{45} and AW_{135} being statistically significant ($p<0.05$).

DISCUSSION

This study was designed to evaluate the effect, if any, of the consumption of the two plant foods, African elemi pulp and African walnut seed kernel, on the liver function and toxicity, and renal function indices of rats. The relative organ weight shows the weight in gramme of organ per 100g body weight. The conversion of absolute organ weight to relative weight normalizes the absolute weight[19] clarifying treatment-related organ weight changes and eliminates variation due to bodyweight differences.[27] Patrick-Iwuanyanwu *et al*.[28] showed an increase in relative weights of liver and kidney in rats exposed to toxicants: gasoline vapours, and kerosene and petrol-contaminated diets respectively. Consequently, the observed similarity in this study in the relative liver and kidneys weights of animals on the test and reference diets despite the apparent significant ($p<0.05$) variation in absolute weights could be an indication that there were no pronounced incriminating factors that could cause hypertrophy or hypotrophy of these organs in the diets offered.[29] The kidney as well as the liver plays important roles in the detoxification and excretion of most toxic materials from the body. As indicated by Babatuyi *et al*.[18], the observed non-significant relative weight changes in animals on both test diets (-13.73% to 10.82%) may have resulted as the organs tried to metabolize the toxicants such as phytochemicals present in the samples.[30] This may reflect the ability of the rats to adequately handle and tolerate antinutritional factors at the levels in test diets and could be an indication of the hepatoprotective nature of the test samples.[31]

Table 1: Absolute and relative organ weights of rats fed diets based on raw and macerated African elemi pulp flour.

Diet	Liver (g)		Kidneys (g)	
	Abs	Rel	Abs	Rel
RD	3.86 ± 0.86^{a}	2.72 ± 0.05^{a}	0.84 ± 0.18^{a}	0.58 ± 0.05^{a}
AE_0	3.15 ± 0.43^{b}	2.76 ± 0.12^{a}	0.68 ± 0.14^{b}	0.59 ± 0.08^{a}
AE_{15}	2.93 ± 1.42^{b}	2.75 ± 0.26^{a}	0.58 ± 0.27^{b}	0.54 ± 0.05^{a}
AE_{30}	3.04 ± 1.13^{b}	2.71 ± 0.37^{a}	0.65 ± 0.24^{b}	0.58 ± 0.10^{a}
AE_{45}	3.41 ± 0.97^{b}	2.70 ± 0.27^{a}	0.65 ± 0.16^{b}	0.51 ± 0.04^{a}

Values are means and standard deviation (n = 5). Means in the same column with a different superscript letter are significantly different from one another at a 5% level ($p \leq 0.05$). Abs = Absolute organ weight; Rel = relative organ weight (g of organ per 100g body weight); RD = Reference diet (Nutrend^{R}).

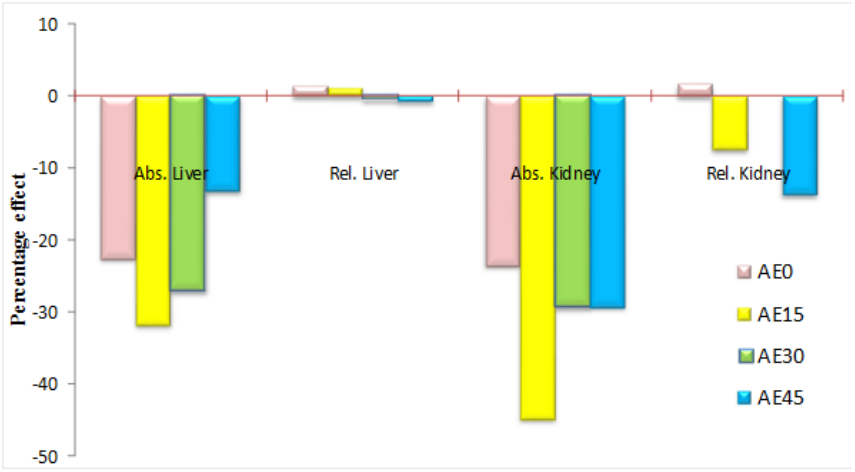

Fig. 1: Effect of sample on absolute and relative organ weights of rats fed diets based on raw and macerated AE pulp flour.

Table 2: Absolute and relative organ weights of rats fed diets based on raw and cooked African walnut seed kernel flour.

Diet	Liver (g)		Kidneys (g)	
	Abs	Rel	Abs	Rel
RD	3.86±0.86^{a}	2.72±0.05^{a}	0.84±0.18^{a}	0.58±0.05^{a}
AW_0	3.42±0.19^{b}	2.82±0.06^{a}	0.71±0.11^{b}	0.57±0.05^{a}
AW_{45}	3.54±1.53^{b}	2.87±0.27^{a}	0.75±0.20^{b}	0.61±0.07^{a}
AW_{90}	3.73±0.98^{b}	3.05±0.26^{a}	0.72±0.18^{b}	0.60±0.10^{a}
AW_{135}	3.51±0.71^{b}	2.98±0.25^{a}	0.73±0.12^{b}	0.63±0.06^{a}

Values are means and standard deviation (n = 5). Means in the same column with a different superscript letter are significantly different from one another at a 5% level ($p<0.05$).

Abs = Absolute organ weight; Rel = relative organ weight (g of organ per 100g body weight); RD = Reference diet (Nutrend^{R}).

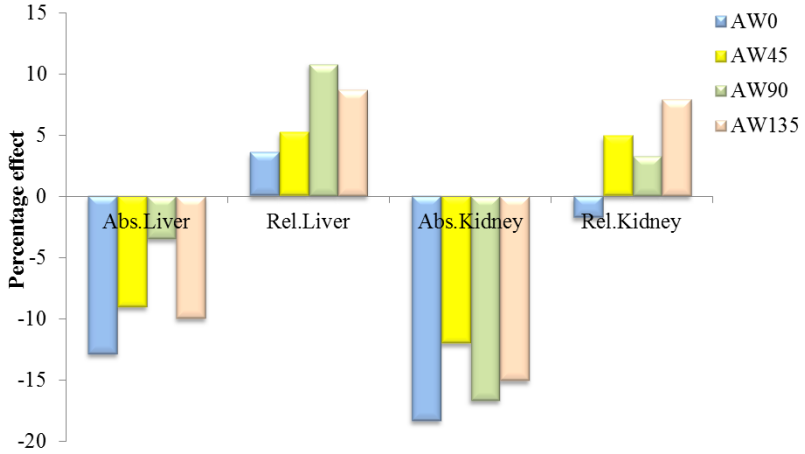

Fig. 2: Effect of sample on absolute and relative organ weights of rats fed diets based on raw and cooked AW seed kernel flour.

Table 3: Serum concentrations of liver function and toxicity indices of rats adapted on raw and macerated African elemi pulp-based diets.

Parameter	RD	AE_0	AE_{15}	AE_{30}	AE_{45}
Glucose (mg/dl)	124.97±22.76[a]	120.61±8.75[a]	119.13±4.69[a]	119.65±8.22[a]	122.43±6.52[a]
Total protein (g/L)	72.66±9.17[a]	69.79±6.03[a]	72.16±2.86[a]	72.91±2.75[a]	73.46±2.46[a]
Albumin (g/L)	33.70±4.02[a]	32.02±8.10[a]	32.62±0.68[a]	33.87±1.89[a]	33.75±3.56[a]
Total bilirubin (mg/dl)	1.89±0.13[a]	1.66±0.34[a]	1.47±0.69[a]	1.68±0.19[a]	1.74±0.54[a]
AST (IU/L)	0.17±0.05[a]	0.14±0.06[a]	0.14±0.04[a]	0.15±0.01[a]	0.14±0.02[a]
ALT (IU/L)	0.24±0.02[a]	0.22±0.06[a]	0.23±0.03[a]	0.21±0.03[a]	0.24±0.05[a]

Values are means and standard deviation (n = 5). Means in the same row with a different superscript letter are significantly different from one another at a 5% level ($p<0.05$). RD = Reference diet (Nutrend[R]). AST = aspartate aminotransferase, ALT = alanine aminotransferase.

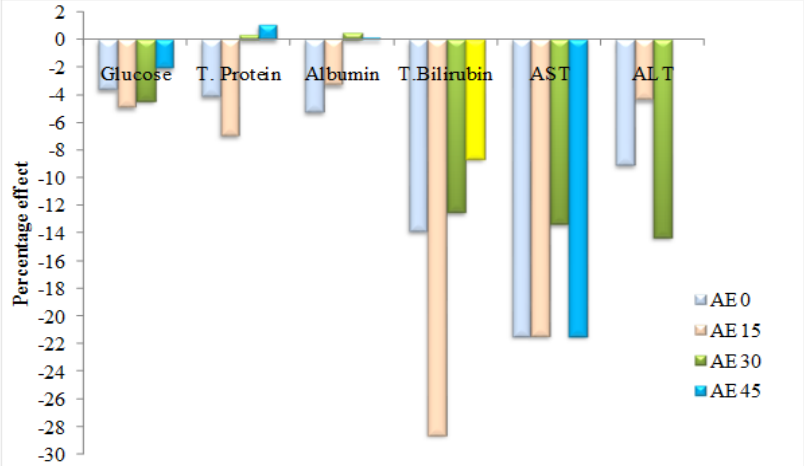

Fig. 3: Effect of sample on serum concentrations of liver function and toxicity indices of rats adapted on raw and macerated AE pulp-based diets.

Table 4: Serum level of liver function and toxicity indices of rats fed with raw and cooked African walnut seed kernel-based diets.

Parameter	RD	AW_0	AW_{45}	AW_{90}	AW_{135}
Glucose (mg/dl)	124.97±22.76[a]	120.49±9.78[a]	124.61±46.15[a]	123.25±6.70[a]	121.41±16.14[a]
Total protein (g/L)	72.66±9.17[a]	72.40±3.01[a]	73.96±2.52[a]	74.31±11.24[a]	74.59±3.14[a]
Albumin (g/L)	33.70±4.02[a]	32.0±0.24[a]	34.37±1.58[a]	34.65±1.88[a]	35.13±6.67[a]
Total bilirubin (mg/dl)	1.89±0.13[ab]	2. 03±1.78[a]	1.11±0.47[b]	1.02±0.10[b]	0.99±0.57[b]
AST (IU/L)	0.17±0.05[a]	0.11±0.01[a]	0.11±0.05[a]	0.11±0.02[a]	0.12±0.01[a]
ALT (IU/L)	0.20±0.02[a]	0.15±0.02[a]	0.15±0.02[a]	0.16±0.02[a]	0.18±0.03[a]

Values are means and standard deviation (n = 5). Means in the same row with a different superscript letter are significantly different from one another at a 5% level ($p<0.05$). RD = Reference diet (Nutrend^{R}). AST = aspartate aminotransferase, ALT = alanine aminotransferase.

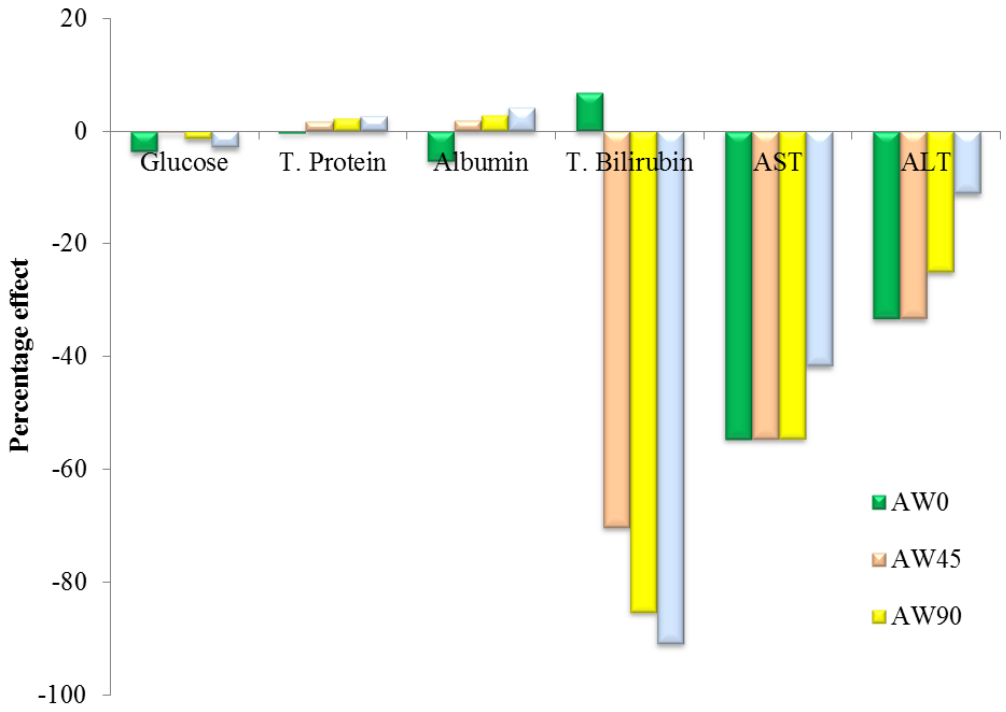

Fig. 4: Effect of sample on the serum level of liver function and toxicity indices of rats fed with raw and cooked AW seed kernel-based diets.

Table 5: Serum concentrations of renal function indices of rats fed diets based on raw and macerated African elemi pulp flour.

Parameter	Serum concentration				
	RD	AE_0	AE_{15}	AE_{30}	AE_{45}
Na (mmol/L)	72.68±24.34^{a}	77.78±23.95^{a}	70.27±30.62^{a}	62.21±21.03^{a}	46.06±24.35^{a}
K (mmol/L)	8.76±3.81^{b}	5.89±0.73^{a}	8.16±1.19^{ab}	6.31±0.83^{ab}	5.99±0.51^{a}
Urea(mg/dl)	54.85±19.74^{a}	44.69±1.81^{a}	40.92±1.99^{a}	37.93±3.34^{a}	37.23±12.23^{a}
Creatinine (mg/dl)	1.28±0.27^{a}	1.22±0.15^{a}	1.21±0.30^{a}	1.20±0.20^{a}	1.20±0.33^{a}

Values are means and standard deviation (n = 5). Means in the same column with a different superscript letter are significantly different from one another at a 5% level ($p<0.05$). RD = Reference diet (Nutrend^{R}).

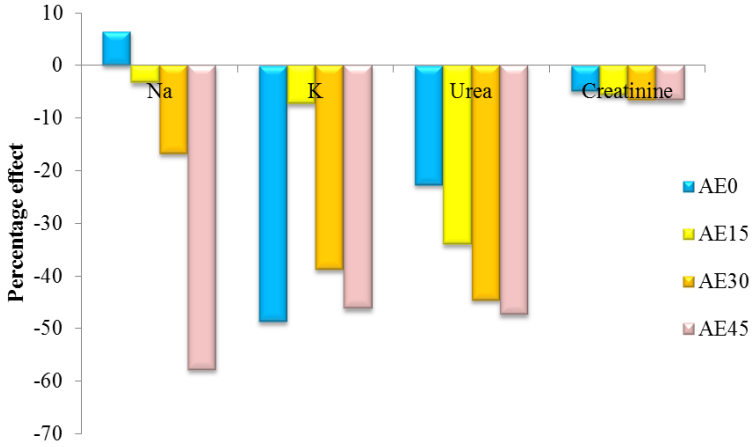

Fig. 5: Effect of sample on serum concentrations of renal function indices of rats fed diets based on raw and macerated African elemi pulp flour.

Table 6: Serum concentration of renal function indices of rats adapted on diets formulated with raw and cooked African walnut seed kernel flour.

Parameter	Serum concentration				
	RD	AW_0	AW_{45}	AW_{90}	AW_{135}
Na (mmol/L)	72.68±24.34[a]	55.39±20.23[a]	47.04±51.86[a]	46.68±21.10[a]	54.86±37.82[a]
K (mmol/L)	8.76±3.81[a]	7.60±1.64[ab]	6.81±0.27[ab]	6.27±1.31[ab]	5.68±0.49[b]
Urea (mg/dl)	54.85±19.74[a]	51.59±1.14[a]	49.59±1.91[a]	51.32±17.07[a]	46.45±6.01[a]
Creatinine (mg/dl)	1.28±0.27[a]	1.28±0.39[a]	1.26±0.12[a]	1.26±0.48[a]	1.27±0.41[a]

Values are means and standard deviation (n = 5). Means in the same column with a different superscript letter are significantly different from one another at a 5% level ($p<0.05$). RD = Reference diet (Nutrend[R]).

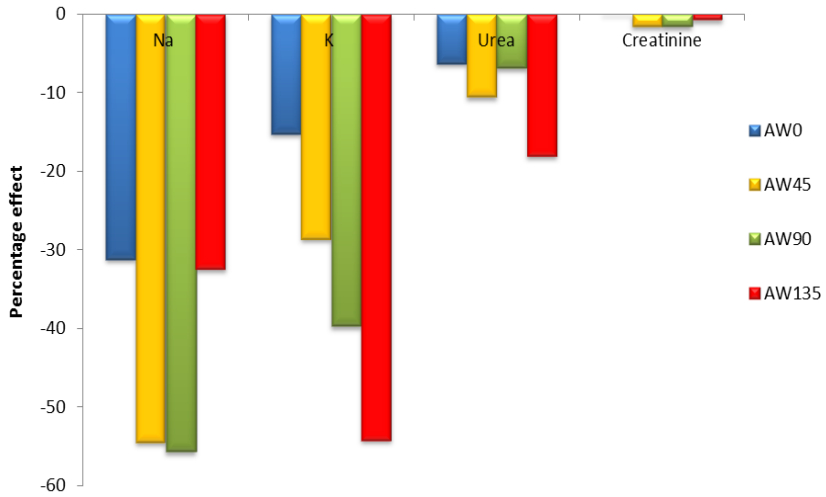

Fig. 6: Effect of sample on serum concentration of renal function indices of rats adapted on diets formulated with raw and cooked African walnut seed kernel flour.

The liver performs the normal metabolic homeostasis in the body as well as biotransformation, detoxification and excretion of many endogenous and exogenous compounds.[6] The organ can produce glucose via gluconeogenesis, produces the majority of plasma proteins (e.g. albumin)[7] and also is responsible for clearing the blood of bilirubin (unconjugated).[18] Consequently, the levels of serum glucose, total protein, albumin and total bilirubin index the degree of functionality of the liver.[9] Ajiboye *et al*.[32] reported that an increase in the concentration of serum bilirubin indicates liver damage.

The statistical similarity between the liver function parameters obtained for rats on test diets in each of the plant foods and those on the reference diet could suggest that the functionality of the liver was not compromised by the inclusion of the plant foods in the diets. The fact that AE_{30} and AE_{45}, and processed AW (Fig. 3 and 4) gave higher total protein and albumin values and lower total bilirubin values relative to the reference diet could mean that the plant foods enhanced the protein synthetic and bilirubin clearing abilities of the animals. This may be reflections of the hepatotonic properties of the samples.

The enzymes AST and ALT are produced mainly by the liver. Consequently, their serum activities are useful biomarkers of hepatocellular integrity[33] and their elevation is indicative of cellular leakage (resulting from hepatocellular damage /lesion) and loss of functional integrity of the hepatic cell membrane.[8] Patrick-Iwuanyanwu *et al.*[28] reported elevated serum activity levels of AST and ALT in rats subjected to kerosene and petrol-contaminated diets. The absence of any significant difference in this study between the serum AST and ALT activity levels of test groups of both plants and RD group could be an indication that the integrity of the liver was not affected by the samples in the diets.[34] According to Ajiboye *et al*.[32], a reduced level of serum AST and ALT activities could suggest conferment of protection on the liver tissues against damage by the test diet.

In this study, the magnitude of the values of these parameters in both samples was lower in the test groups than in the reference group (Fig. 3 and 4). This could mean that the hepatocellular integrity was better maintained in the presence of the plant foods and therefore indicative of their hepatoprotective potentials.[32] From Table 3 and 4, and Fig. 3 and 4, the plant foods at the level studied could be absolved of any risk of hepatotoxicity which according to Vagace and Gervasini[10] arises when a liver injury is associated with impaired liver function.

The kidney (via its glomerulus) plays a major role in maintaining body homeostasis. It sieves electrolytes (principally Na+, K+) out of the blood, eliminates urea, the major nitrogenous end product of protein and amino acid catabolism from the blood via urinary excretion.[3] It also eliminates creatinine whose blood concentration in addition to urea indicates glomerular filtration rate, GFR[32] - the best index of overall kidney function.[4] Abnormal levels of these may accrue in the blood under reduced kidney function. Consequently, their blood concentrations are employed as indices of renal function in routine analyses.[1,3] Imo *et al*.[35] posited that elevation of serum levels of electrolytes, urea and creatinine are signs of severe kidney impairment.

The absence of any significant difference between the serum sodium, urea and creatinine levels of test groups and reference group (RD) could be an indication of the absence of any renotoxic substance in the test samples.[31,35] On pair-wise comparison, the serum levels of the renal function indices except for Na in AE_0 and creatinine in AW_0 diet groups, were lower in rats fed with the two plant foods-based diets relative to RD (Figures 5 and 6). This finding is suggestive of renal tonic or reno-protective effects of the plant foods especially when processed.[35]

CONCLUSION

Based on the observed similarity in the relative liver and kidneys weights, liver function parameters, serum AST and ALT activity levels, the serum sodium, urea and creatinine levels, between animals on test and reference groups, it could be concluded that the plant foods neither contain factors that could cause noticeable hypertrophy or hypotrophy of the liver or kidneys, compromise the functionality and integrity of the liver, nor contain any renotoxic substance. Rather on a pairwise basis the consumption of the samples especially when processed showed hepato-tonic / hepato-protective and reno-protective potentials.

Conflict of interests: The author declared no conflict of interest.

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Ethical Considerations

Compliance with ethical guidelines

The animals (albino rats) used were obtained from the Department of Animal Health and Production, Faculty of Veterinary Medicine, University of Nigeria, Nsukka Nigeria. The study protocol was approved by the Ethics Committee of the University which has an institutional ethical permit for the use of animals in research.

Authors' contributions

The research was carried out by the author.

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