



RENOTOXICOLOGICAL EVALUATION OF WATER HYACINTH LEAF PROTEIN CONCENTRATE USING RAT MODEL

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ABSTRACT

This study evaluated the toxicological impact of water hyacinth leaf protein concentrate (WHLPC) on the kidney of albino rats. Rats were fed with various formulations (7.73, 15.46, 23.19, and 30.92) %w/w of WHLPC over a period of 20 weeks. A control feed contains soybean (15.46%w/w) in place of WHLPC. Rats were grouped into 5 groups in accordance with the feed formulations and the control. Each group contains 20 rats, 4 rats were removed from each group and sacrificed at the start of the experiment and on week 5, 10, 15 and 20 respectively. The kidney was isolated, a portion was fixed in buffered neutral formalin for histology studies and the other portion was homogenized for toxicology tests. Levels of malondialdehyde (MDA) and reduced glutathione (GSH) were determined in the kidney. Standard enzyme assays were conducted for catalase (CAT), superoxide dismutase (SOD), alkaline phosphatase (ALP), acid phosphatase (ACP), alanine transaminases (ALT), aspartate transaminases (AST), lactate dehydrogenase (LDH), glutathione-S-transferase (GST) and γ -glutamyl transpeptidase (GGT). Results showed no significant difference ($p>0.05$) among all treatment groups including the control. Histology photomicrograph showed normal cellular architecture lending credence to the biochemical assays. Conclusively, WHLPC, at nutritional concentration used in this study may be considered safe for the kidney.

KEYWORDS: kidney, water hyacinth, rats, toxicology, histology, soybean, assay.

INTRODUCTION

Water hyacinth is an aquatic vascular plant with rounded, upright and shiny green leaves and lavender flowers similar to orchids. Its individual rosette is erect and free floating with numerous stolons. Each rosette carries six to eight spirally arranged succulent leaves that are produced sequentially on a short vertical stem. The inflorescence consists of ten to thirty flowers with six violet blue or violet pink petals. The top petal has gold yellow spot bordered with blue line which resembles the pattern of peacock eye. The root system of water hyacinth is dark blue in colour with numerous stolons. New plants are formed at the end of these stolons. Measured from flower top to root top, water hyacinth usually reaches height of 1.5 m and more. When grown in wastewaters, water hyacinth is smaller and it often reaches heights that are no more than 0.5 to 1.2 m (Center *et al.*, 2002).

From a socio-economic perspective, water hyacinth invasion into freshwater systems presents a problem for many human uses. The most direct impacts are to boating access, navigability, and recreation. Access to

fishing grounds and fish catchability are also affected (Kateregga and Sterner, 2009). Furthermore, evapotranspiration from water hyacinth can exceed open-water evaporation rates by a factor of ten in some areas. This can be a serious concern in water limited areas and small water bodies. Water hyacinth can greatly affect a fishery if it induces changes in fish community composition, or if catchability of harvested species is changed. The economic cost of controlling water hyacinth infestations is a function of the rate of removal, cost of labor, cost of equipment, and the frequency of treatment. Mechanical shredding of water hyacinth is cheaper than harvesting (Greenfield *et al.*, 2006), but there are significant consequences of allowing the plant to die and decompose within the system.

In spite of these problems, a critical study of the plant physiology indicated that it could have potential application in agriculture and in homes. There must be a concerted effort therefore, at harnessing this beneficial biological resource rather than just spending huge financial and human resources at just eradicating it. In our previous study, edible form of water hyacinth leaf

protein concentrate (WHLPC) was extracted using an efficient and cost effective method (Adeyemi and Osubor, 2016). The effect of the extracted WHLPC on the blood of rats was evaluated in a separate study and experimental evidence revealed that it had no adverse effect (Adeyemi and Osubor, 2017). The present study is to evaluate the toxicology of WHLPC on kidney using rats as a model.

The environment of the kidney, both inside and outside the nephron varies along its length, and this influences the types of toxic effect produced by nephrotoxic agents. The kidney is a target organ for the reasons of renal blood flow, its concentrating ability, active transport of compounds by the tubular cells and metabolic activation. It is clear that the tissues of the kidney are often exposed to higher concentrations of potentially toxic compounds than most other tissues. The toxic effects caused may be due to a variety of mechanisms ranging from the simple irritant effects to enzyme inhibition and tubular damage. There are a variety of ways in which kidney damage can be detected ranging from simple qualitative tests to more complex biochemical assays. The present study employed both biochemical and pathological techniques.

MATERIALS AND METHODS

Reagents

Reagents and solvents used in this research are products of British Drug House, Poole, England and were of analytical grade.

Study Area

The study area, where Water hyacinth samples were collected from, is River Ijana located within longitude 5.54°E and 5.70°W and latitude 5.31°N and 5.6°S in Warri, Delta State, Nigeria (Adeyemi and Osubor, 2016).

Sample Collection and Extraction

Extraction of the water hyacinth leaf protein concentrate (WHLPC) was done as described in our previous study (Adeyemi and Osubor, 2016; 2017).

Feed Formulation and Animal Management

Feedstuff formulated were five types according to the composition of source materials (WHLPC) and the daily nutrient requirements. The formulated diets were designated Control and WHLPC1, WHLPC2, WHLPC3, WHLPC4, respectively as earlier described (Adeyemi and Osubor, 2017).

Albino rats (*Rattus novergicus*) ($N = 100$) were purchased from the Animal Holding of the Department of Anatomy University of Benin, Benin-City, Nigeria. The experimental animals were kept inside 5 plastic cages containing 20 animals each. The rats were categorised into 5 groups namely; Group I (control rats fed with soybean as protein source), Group II (rats fed with WHLPC1 as protein source), Group III (rats fed with WHLPC2 as protein source), Group IV (rats fed

with WHLPC3 as protein source), Group V (rats fed with WHLPC4 as protein source).

Feeding period

In this study, the feeding exercise was over a period of 20 weeks and experimental rats were placed on respective diet. Rats ($n = 4$) were randomly selected from each group on the first day and sacrificed to determine basal enzymes activities to be monitored. This was repeated at week 5, 10, 15 while the remaining rats were sacrificed at the 20th week. After the sacrifice, the rats were anaesthetized by placing them in a jar containing cotton wool soaked with chloroform before being sacrificed by jugular puncture. The rats were quickly dissected and the kidney was excised, freed of fat, blotted with clean tissue paper and weighed. A portion of the kidney was fixed in buffered neutral formalin for histology studies while the other portion was homogenized for biochemical studies and enzyme assays.

Biochemical Studies and Enzyme Assay

The malondialdehyde (MDA) concentration in the serum and tissues of rats experimental was determined following the method described by Bird *et al.* (1982). Reduced Glutathione (GSH) concentration in the tissues of experimental rats was determined following the method described by Jollow *et al.* (1974).

Superoxide dismutase (SOD) activity of the kidney of experimental animals was determined following the method described by Misra and Fridovich (1972). The catalase activity was determined following the method described by Sinha (1971). The activities of alkaline phosphatase (ALP) and acid phosphatase (ACP) were determined following the method described by Bessey *et al* (1946) as modified by Wright *et al* (1972). The activities of aspartate transaminase (AST) and alanine transaminase (ALT) in the kidney of experimental animals were determined following the procedure reported by Reitman and Frankel (1957) as modified by Schmidt and Schmidt (1963). The method used for assaying lactate dehydrogenase is based on that of Wroblewski and La Due (1955) in which pyruvate is reversibly reduced to lactate in the presence of nicotinamide adenine dinucleotide (reduced) as co-enzyme. The cytosolic glutathione s-transferase activity was determined spectrophotometrically at 37°C (340nm) by the procedure described by Habig *et al* (1974). The activity of Gamma glutamyl transpeptidase (GGT) was determined following the method described by Tietz (1990).

Histological Study

Histological study on tissues obtained from experimental rats was carried out following the method described by Drury and Wallington (1973).

Statistical Analyses

All numerical results were obtained from the five (5) groups (control and treated). Data were presented as mean \pm SEM and analysed using one way analysis of variance (ANOVA) and Duncan Multiple Range Test using SPSS-18.0 (Statistical packages for social Scientists – version 18.0) statistical program. P values <0.05 were considered significant.

RESULTS

Table 1 presents specific activity of selected kidney enzymes and concentrations of malondialdehyde (MDA) and reduced glutathione (GSH) of experimental rats before the commencement of the feeding exercise. Activity of selected enzymes and concentration of MDA and GSH among treatment groups including Control were not significantly different ($p\geq0.05$). Similarly, 5, 10, 15 and 20 weeks into the feeding exercise, no significant difference ($p\geq0.05$) existed in the enzyme activity and concentration of MDA and GSH of kidney of rats among the treatment groups relative to Control (Tables 2-5). The response of the kidney to formulated feed and control feed as measured by the activity of the selected kidney enzymes and levels of MDA and GSH suggests no significant difference ($p\geq0.05$). The formulated and control feed were found to be tolerated

by the kidney of experimental rats in a similar manner. No concern was raised by either the enzyme activity or the indices of oxidative stress (MDA and GSH) of the kidney of the experimental rats.

Plates 1 to 5 present the photomicrograph of kidney of rats placed on the experimental diets over a period of twenty weeks. Histology results showed no cellular lesion, normal cellular architecture and no feature of pathological concern. Specifically, however, distinct renal tubules (black arrow) and corpuscles (brown arrow) with prominent macula densa (blue arrow) was observed in kidney of Control. In WHLPC1, dense rounded glomeruli (black arrow) and tubules (brown arrow) was seen. Seen in the kidney of rats placed on WHLPC2 over a period of 20 weeks, was distinct glomeruli (black arrow) and renal tubules (brown arrow). Dense rounded structure of renal corpuscles (black arrow) surrounded by narrow Bowman's space (blue arrow) was observed in rats placed on WHLPC3 while Prominent glomeruli (black arrow) and tubules (blue arrow) with one distinct intralobular vein (red arrow) around 5 O' Clock and prominent nuclei (black arrows), central vein (blue arrow) and the distinct hepatocytes which are well venuleserated by sinusoidal space (yellow arrow) were observed in WHLPC4 and WHLPC5 respectively.

Table 1: Specific activity (U/mg protein) of selected enzymes of the kidney of rats to be placed on feed formulated with water hyacinth leaf protein concentrate (WHLPC) over a period of 20 weeks.

Group of Rats	AST	ALT	ALP	ACP	GGT	LDH	CAT	SOD	MDA (nmol/mg)	GSH (μmol/mg)	GST
I	4.41±0.51 ^a	3.5±0.67 ^a	192±7.32 ^a	23.5±1.22 ^a	43.5±4.84 ^a	20.3±1.55 ^a	1.02±0.01 ^a	1.02±0.01 ^a	15.65±1.4 ^a	1.00±0.01 ^a	0.89±0.01 ^a
II	4.32±0.50 ^a	3.67±0.54 ^a	187±8.44 ^a	21.5±2.44 ^a	40.45±5.2 ^a	20.5±1.67 ^a	1.02±0.01 ^a	1.03±0.01 ^a	15.77±1.7 ^a	1.01±0.01 ^a	0.86±0.03 ^a
III	4.23±0.48 ^a	3.55±0.47 ^a	190±5.87 ^a	22.5±1.88 ^a	43.25±4.76 ^a	19.8±1.58 ^a	1.02±0.01 ^a	1.02±0.01 ^a	15.23±1.8 ^a	1.01±0.01 ^a	0.88±0.03 ^a
IV	4.40±0.50 ^a	3.64±0.55 ^a	184±9.20 ^a	23.0±2.00 ^a	42.11±4.88 ^a	20.1±2.00 ^a	1.01±0.01 ^a	1.02±0.01 ^a	15.48±1.5 ^a	1.02±0.01 ^a	0.88±0.02 ^a
V	4.39±0.49 ^a	3.52±0.50 ^a	186±6.33 ^a	24.0±1.54 ^a	42.50±5.00 ^a	20.7±1.98 ^a	1.01±0.01 ^a	1.03±0.01 ^a	15.55±1.4 ^a	1.01±0.01 ^a	0.89±0.03 ^a

Each value represents mean ± SEM of two determinations of kidney from four different animals. Values in the same column bearing different superscripts are significantly different (p<0.05).

Table 2: Specific activity (U/mg protein) of selected enzymes of the kidney of rats placed on feed formulated with water hyacinth leaf protein concentrate (WHLPC) over a period of 5 weeks.

Group of Rats	AST	ALT	ALP	ACP	GGT	LDH	CAT	SOD	MDA (nmol/mg)	GSH (μmol/mg)	GST
I	4.43±0.51 ^a	3.52±0.67 ^a	192.86±7.35 ^a	23.61±1.23 ^a	43.70±4.86 ^a	20.39±1.56 ^a	1.02±0.01 ^a	1.02±0.01 ^a	15.72±1.41 ^a	1.00±0.01 ^a	0.89±0.01 ^a
II	4.34±0.50 ^a	3.69±0.54 ^a	187.84±8.48 ^a	21.60±2.45 ^a	40.63±5.22 ^a	20.59±1.68 ^a	1.02±0.01 ^a	1.03±0.01 ^a	15.84±1.71 ^a	1.01±0.01 ^a	0.86±0.03 ^a
III	4.25±0.48 ^a	3.57±0.47 ^a	190.86±5.90 ^a	22.60±1.89 ^a	43.44±4.78 ^a	19.89±1.59 ^a	1.02±0.01 ^a	1.02±0.01 ^a	15.30±1.81 ^a	1.01±0.01 ^a	0.88±0.03 ^a
IV	4.42±0.50 ^a	3.66±0.55 ^a	184.83±9.24 ^a	23.10±2.01 ^a	42.30±4.90 ^a	20.19±2.01 ^a	1.01±0.01 ^a	1.02±0.01 ^a	15.55±1.51 ^a	1.02±0.01 ^a	0.88±0.02 ^a
V	4.41±0.49 ^a	3.54±0.50 ^a	186.84±6.36 ^a	24.11±1.55 ^a	42.69±5.02 ^a	20.79±1.99 ^a	1.01±0.01 ^a	1.03±0.01 ^a	15.62±1.41 ^a	1.01±0.01 ^a	0.89±0.03 ^a

Each value represents mean ± SEM of two determinations of kidney from four different animals. Values in the same column bearing different superscripts are significantly different (p<0.05).

Table 3: Specific activity (U/mg protein) of selected enzymes of the kidney of rats placed on feed formulated with water hyacinth leaf protein concentrate (WHLPC) over a period of 10 weeks.

Group of Rats	AST	ALT	ALP	ACP	GGT	LDH	CAT	SOD	MDA (nmol/mg)	GSH (μmol/mg)	GST
I	4.72±0.55 ^a	3.75±0.72 ^a	205.44±7.83 ^a	25.15±1.31 ^a	46.55±5.18 ^a	21.72±1.66 ^a	1.09±0.01 ^a	1.09±0.01 ^a	16.75±1.50 ^a	1.07±0.01 ^a	0.95±0.01 ^a
II	4.62±0.54 ^a	3.93±0.58 ^a	200.09±9.03 ^a	23.01±2.61 ^a	43.28±5.56 ^a	21.94±1.79 ^a	1.09±0.01 ^a	1.10±0.01 ^a	16.87±1.82 ^a	1.08±0.01 ^a	0.92±0.03 ^a
III	4.53±0.51 ^a	3.80±0.50 ^a	203.30±6.28 ^a	24.08±2.01 ^a	46.28±5.09 ^a	21.19±1.69 ^a	1.09±0.01 ^a	1.09±0.01 ^a	16.30±1.93 ^a	1.08±0.01 ^a	0.94±0.03 ^a
IV	4.71±0.54 ^a	3.89±0.59 ^a	196.88±9.84 ^a	24.61±2.14 ^a	45.06±5.22 ^a	21.51±2.14 ^a	1.08±0.01 ^a	1.09±0.01 ^a	16.56±1.61 ^a	1.09±0.01 ^a	0.94±0.02 ^a
V	4.70±0.52 ^a	3.77±0.54 ^a	199.02±6.77 ^a	25.68±1.65 ^a	45.48±5.35 ^a	22.15±2.12 ^a	1.08±0.01 ^a	1.10±0.01 ^a	16.64±1.50 ^a	1.08±0.01 ^a	0.95±0.03 ^a

Each value represents mean ± SEM of two determinations of kidney from four different animals. Values in the same column bearing different superscripts are significantly different (p<0.05).

Table 4: Specific activity (U/mg protein) of selected enzymes of the kidney of rats placed on feed formulated with water hyacinth leaf protein concentrate (WHLPC) over a period of 15 weeks.

Group of Rats	AST	ALT	ALP	ACP	GGT	LDH	CAT	SOD	MDA (nmol/mg)	GSH (μ mol/mg)	GST
I	4.81 \pm 0.56 ^a	3.82 \pm 0.73 ^a	209.28 \pm 7.98 ^a	25.62 \pm 1.33 ^a	47.42 \pm 5.28 ^a	22.13 \pm 1.69 ^a	1.11 \pm 0.01 ^a	1.11 \pm 0.01 ^a	17.06 \pm 1.53 ^a	1.09 \pm 0.01 ^a	0.97 \pm 0.01 ^a
II	4.71 \pm 0.55 ^a	4.00 \pm 0.59 ^a	203.83 \pm 9.20 ^a	23.44 \pm 2.66 ^a	44.09 \pm 5.67 ^a	22.35 \pm 1.82 ^a	1.11 \pm 0.01 ^a	1.12 \pm 0.01 ^a	17.19 \pm 1.85 ^a	1.10 \pm 0.01 ^a	0.94 \pm 0.03 ^a
III	4.61 \pm 0.52 ^a	3.87 \pm 0.51 ^a	207.10 \pm 6.40 ^a	24.53 \pm 2.05 ^a	47.14 \pm 5.19 ^a	21.58 \pm 1.72 ^a	1.11 \pm 0.01 ^a	1.11 \pm 0.01 ^a	16.60 \pm 1.96 ^a	1.10 \pm 0.01 ^a	0.96 \pm 0.03 ^a
IV	4.80 \pm 0.55 ^a	3.97 \pm 0.60 ^a	200.56 \pm 9.03 ^a	25.07 \pm 2.18 ^a	45.90 \pm 5.32 ^a	21.91 \pm 2.18 ^a	1.10 \pm 0.01 ^a	1.11 \pm 0.01 ^a	16.87 \pm 1.64 ^a	1.11 \pm 0.01 ^a	0.96 \pm 0.02 ^a
V	4.79 \pm 0.53 ^a	3.84 \pm 0.52 ^a	202.74 \pm 6.90 ^a	26.16 \pm 1.68 ^a	46.33 \pm 5.45 ^a	22.56 \pm 2.16 ^a	1.10 \pm 0.01 ^a	1.12 \pm 0.01 ^a	16.95 \pm 1.53 ^a	1.10 \pm 0.01 ^a	0.97 \pm 0.03 ^a

Each value represents mean \pm SEM of two determinations of kidney from four different animals. Values in the same column bearing different superscripts are significantly different (p<0.05).

Table 5: Specific activity (U/mg protein) of selected enzymes of the kidney of rats placed on feed formulated with water hyacinth leaf protein concentrate (WHLPC) over a period of 20 weeks.

Group of Rats	AST	ALT	ALP	ACP	GGT	LDH	CAT	SOD	MDA (nmol/mg)	GSH (μ mol/mg)	GST
I	4.83 \pm 0.56 ^a	3.83 \pm 0.73 ^a	210.33 \pm 8.02 ^a	25.74 \pm 1.34 ^a	47.65 \pm 5.30 ^a	22.24 \pm 1.70 ^a	1.12 \pm 0.01 ^a	1.12 \pm 0.01 ^a	17.14 \pm 1.53 ^a	1.10 \pm 0.01 ^a	0.97 \pm 0.01 ^a
II	4.73 \pm 0.55 ^a	4.02 \pm 0.59 ^a	204.85 \pm 9.25 ^a	23.55 \pm 2.67 ^a	44.31 \pm 5.70 ^a	22.46 \pm 1.83 ^a	1.12 \pm 0.01 ^a	1.13 \pm 0.01 ^a	17.28 \pm 1.86 ^a	1.11 \pm 0.01 ^a	0.94 \pm 0.03 ^a
III	4.63 \pm 0.53 ^a	3.89 \pm 0.51 ^a	208.14 \pm 6.43 ^a	24.65 \pm 2.06 ^a	47.38 \pm 5.21 ^a	21.69 \pm 1.73 ^a	1.12 \pm 0.01 ^a	1.12 \pm 0.01 ^a	16.68 \pm 1.97 ^a	1.11 \pm 0.01 ^a	0.96 \pm 0.03 ^a
IV	4.82 \pm 0.55 ^a	3.99 \pm 0.60 ^a	201.56 \pm 9.08 ^a	25.20 \pm 2.19 ^a	46.13 \pm 5.35 ^a	22.02 \pm 2.19 ^a	1.11 \pm 0.01 ^a	1.12 \pm 0.01 ^a	16.96 \pm 1.64 ^a	1.12 \pm 0.01 ^a	0.96 \pm 0.02 ^a
V	4.81 \pm 0.54 ^a	3.86 \pm 0.55 ^a	203.75 \pm 6.93 ^a	26.29 \pm 1.69 ^a	46.56 \pm 5.48 ^a	22.68 \pm 2.17 ^a	1.11 \pm 0.01 ^a	1.13 \pm 0.01 ^a	17.03 \pm 1.53 ^a	1.11 \pm 0.01 ^a	0.97 \pm 0.03 ^a

Each value represents mean \pm SEM of two determinations of kidney from four different animals. Values in the same column bearing different superscripts are significantly different (p<0.05).



Plate 1: Photomicrograph (H&E, x40) of kidney of Control rats. Distinct renal tubules (black arrow) and corpuscles (brown arrow) with prominent macula densa (blue arrow).



Plate 2: Photomicrograph (H&E, x40) of kidney of rats placed on WHLPC1 over a period of 20 weeks. Dense rounded glomeruli (black arrow) and tubules (brown arrow).



Plate 3: Photomicrograph (H&E, x40) of kidney of rats placed on WHLPC2 over a period of 20 weeks. Distinct glomeruli (black arrow) and renal tubules (brown arrow).



Plate 4: Photomicrograph (H&E, x40) of kidney of rats placed on WHLPC3 over a period of 20 weeks. Dense rounded structure of renal corpuscles (black arrow) surrounded by narrow Bowman's space (blue arrow).

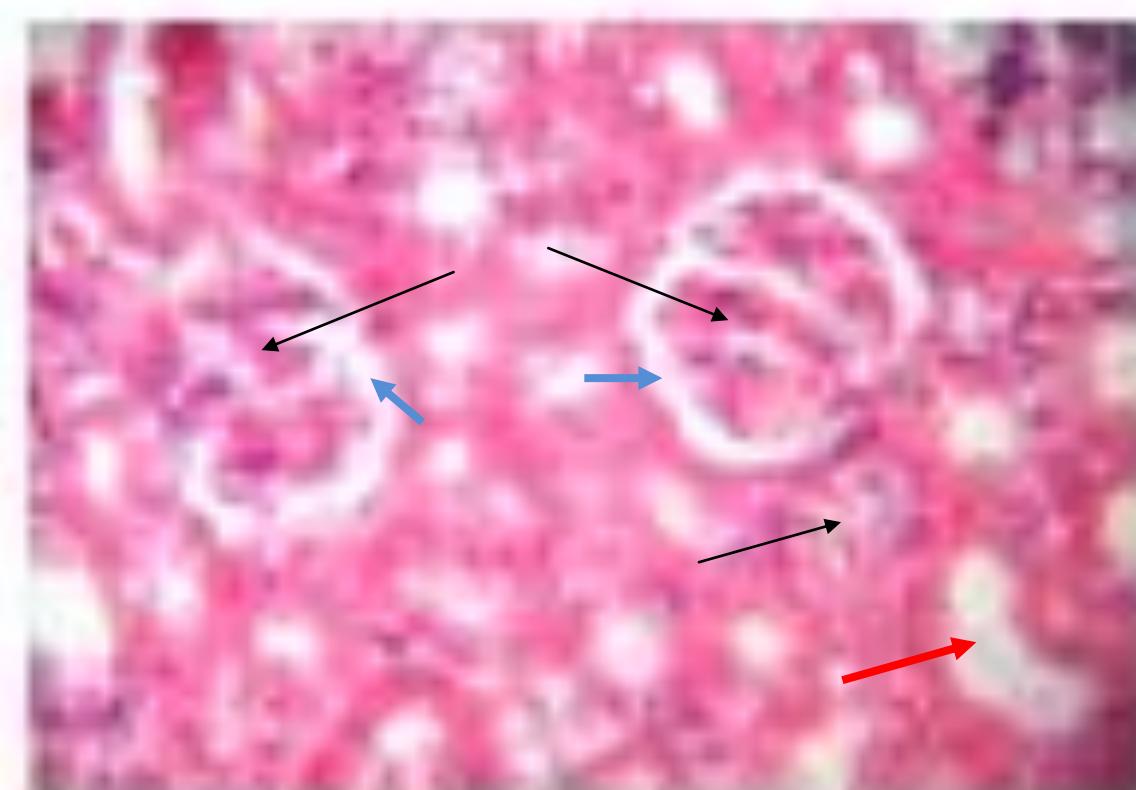


Plate 5: Photomicrograph (H&E, x40) of kidney of rats placed on WHLPC4 over a period of 20 weeks. Prominent glomeruli (black arrow) and tubules (blue arrow) with one distinct intralobular vein (red arrow) around 5 O'Clock.

DISCUSSION

The kidney has been reported to be very sensitive to food toxicity responding with abnormal enzyme activities, anomalous concentrations of antioxidants and some serum metabolites (Adeyemi *et al.*, 2010a). The kidney is a target organ for toxicity for the reasons of renal blood flow, concentrating ability, active transport of compounds by the tubular cells and metabolic activation. The observation in this study is that the activity of enzymes and concentrations of various indices of oxidative stress of rats placed on feed formulated with WHLPC compared favourably with those of rats placed on feed formulated with soybean (Tables 1-5). This observation portends that WHLPC may not be toxic to the kidney. Alkaline phosphatases (ALP) are a group of isoenzymes which catalyse the hydrolysis of organic phosphates at alkaline pH. They are found principally in bone, liver, kidney, intestinal wall, lactating mammary gland and placenta. The primary importance of measuring alkaline phosphatase activity is to check the possibility of damage to plasma membrane of tissues such as kidney (Adeyemi *et al.*, 2010b). Tissues including bone, liver, spleen, kidney, and red blood cells and platelets have acid phosphatase (ACP). Damage to these tissues causes increase or decrease in acid phosphatase activity. Abnormal values of serum ALT and AST have been observed for different diseased conditions (Trevor, 2001). In this study, no abnormal enzyme activity observed in the kidney of rats fed with WHLPC.

Glutathione (GSH) has a protective role against noxious chemicals (Kohen and Nyska, 2002). Glutathione is responsible for the regulation of intracellular levels of lipid peroxidation and also act as a reactant in conjugation with electrophilic substances, therefore a change in GSH level may be a very important indicator of the detoxification ability of an organism (Vijayavel *et al.*, 2004). GSH is an effective protectant capable of detoxifying oxyradicals (Ross, 1998). Evidence from various pathological and toxicological conditions such as chemical induced oxidative injury, aging and degenerative disease indicate that GSH is a primary component of the protection system of cells against oxidative and free radical damage (Kohen and Nyska, 2002). Lipid peroxidation is one of the major mechanisms involved in oxidative cell injury and an increase in Malondialdehyde (MDA) level is frequently observed during oxidative stress and has generally been used as a marker of oxidative damage (Yildirin *et al.*, 2011). It is also a major oxidation product of peroxidized polyunsaturated fatty acids and increased MDA content can be related to degradation of an environment due to poor water quality (Charissou *et al.*, 2004). SOD is a group of metalloenzymes that play crucial antioxidant role and constitute a defense system against natural and chemical pollutants by catalyzing the dismutation of the highly reactive superoxide anion radical (O_2^-) which is an important agent of oxygen toxicity to the less reactive species H_2O_2 (Ozmen, 2005). CAT is a peroxisomal haemoprotein that catalyzes the removal of H_2O_2 formed

during the reaction catalyzed by SOD. Earlier studies established that antioxidants enzymes are induced during conditions of oxidative stress as a defense mechanism (Farombi et al., 2007; Doherty, et al., 2010).

Glutathione-S-transferases are cytosolic enzymes, although also detectable in the microsomal fraction, and are found in many tissues but particularly liver, kidney, gut, testis and adrenal gland. **γ -glutamyltranspeptidase (glutamyltransferase)** is a membrane-bound enzyme found in high concentrations in the kidney. Abnormal activities of these enzymes had been associated with toxic effect. In the present study, both GST and GGT activities compared favourably among all treatment groups and control. This portends a condition of safety.

CONCLUSION

It could be inferred from the growing experimental evidence that WHLPC is not toxic to the kidney but rather supports normal kidney health. In addition, a condition of oxidative stress in the kidney as a result of WHLPC is not likely to occur as the levels of indices of oxidative stress determined are within normal range in rats placed on WHLPC formulated feed relative to the Control.

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