

Hepatotoxicological Evaluation of Water Hyacinth Leaf Protein Concentrate using Rat Model

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Abstract: In these days of diminishing natural resources, it has become necessary to investigate the existing resources in water hyacinth for production of food and food additives. The aim of this study was to evaluate the toxicological effect water hyacinth leaf protein concentrate (WHLPC) on rats' liver. Water hyacinth leaf protein concentrate (WHLPC) was used to formulate feed using different concentrations (7.73, 15.46, 23.19, and 30.92) %w/w. A control feed was formulated with soybean (15.46%w/w) in place of WHLPC. The resulting feeds were fed to different groups of rats. The feeding exercise was for a period of twenty (20) weeks. The first day of experiment was taken as basal. Rats (4 in number) were removed on the first day, week 5, 10, 15 and 20 respectively. These rats were sacrificed and liver was collected for biochemical and histological analyses. Activities of selected enzymes (AST, ALT, ALP, ACP, GGT, LDH, CAT, SOD) of liver of treated rats compared favourably with those of control rats. Evidence from this study is that WHLPC is acutely non-toxic and supports normal liver development. WHLPC was also found to be well tolerated and therefore may be a good raw material for food and beverage industries.

Keywords: Water hyacinth; Leaf protein concentrate; Liver; Enzymes; Rats.

Date of Submission: 18-09-2019

Date of Acceptance: 05-10-2019

I. Introduction

Nowadays, toxicological studies are contributing to human health more than ever. Reports on the toxicological studies of plant proteins, which are continuously growing in number in the literature, have been reviewed^{1,2,3,4} Two important aspects are discussed: dietary safety evaluation, including toxicity tests and the maximum daily intake allowance, and the appropriate proportion in our daily diets of proteins from traditional foods and of new proteins from plant sources not traditionally employed as foods. Water hyacinth belongs to the class of plants not traditionally employed as foods.

Water hyacinth leaf protein concentrate (WHLPC) may be used as supplementary food. It is likely to be nutritious because of the high protein content and the content of unsaturated fats, carotenes, xanthophylls, starch and minerals such as iron, calcium and phosphorus^{3, 5}. Leaf protein concentrate (LPC) is a concentrated form of the proteins found in the leaves of plants. It has been examined as a human or animal food source, because it is potentially the cheapest, most abundant source of available protein. Although humans can derive some protein from the direct consumption of leaves as leaf vegetables, the human digestive system would not be able to deal with the enormous bulk of leaves needed to meet dietary protein requirements with leaf vegetables alone⁶.

Extraction of WHLPC in edible form has been scanty in literature⁵; toxicological investigation of WHLPC especially its effect on the liver is scarce. The present study has delved into the examination of the impact of WHLPC on liver enzymes and the cells of the liver as a whole.

II. Materials and methods

Reagents

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

Study Area

Water hyacinth samples were collected from River Ijana located within longitude 5.54⁰E and 5.7⁰W and latitude 5.31⁰N and 5.6⁰S in Warri, Delta State, Nigeria. More detailed information is as reported⁵.

Sample Collection and Extraction

The *Eichhorniacrassipes* (Mart.) Solms samples were collected and the water hyacinth leaf protein concentrate (WHLPC) was extracted based on the method described in our previous study^{4,5}.

Feed Formulation and Animal Management

Five kinds of diets were prepared in accordance with the composition of source materials and the daily nutrient requirements, which were named Control and WHLPC1, WHLPC2, WHLPC3, WHLPC4, respectively. Feedstuff formula had been previously reported⁴. The experimental animals were handled in accordance with the principles guiding the use and handling of experimental animals as stipulated by the Animal Research Ethics Committee of the College of Science.

Hundred Albino rats (*Rattus norvegicus*) 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 as follows;

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

Experimental rats were placed on respective diet over a period of 20 weeks. However, 4 rats 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 whole liver was excised, freed of fat, blotted with clean tissue paper and weighed. A portion of each organ was fixed in buffered neutral formalin for histology studies while the other portions were homogenized for biochemical studies and enzyme assays.

Determination of Malondialdehyde (MDA) Concentration

The MDA concentration in the serum and tissues of rats experimental was determined following the method described by Bird *et al.*⁷.

Determination of Reduced Glutathione (GSH) Concentration

The GSH concentration in the tissues of experimental rats was determined following the method described by Jollow *et al.*⁸.

Liver Enzyme Assays

Superoxide dismutase (SOD) activity of the tissues of experimental animals was determined following the method described by Misra and Fridovich⁹. The catalase activity of the tissue homogenate obtained from the experimental animals was determined following the method described by Sinha¹⁰. The activities of alkaline phosphatase (ALP) and acid phosphatase (ACP) in tissues of the experimental rats were determined following the method described by Bessey *et al.*¹¹ as modified by Wright *et al.*¹². The activities of aspartate transaminase (AST) and alanine transaminase (ALT) in tissues of experimental animals were determined following the procedure reported by Reitman and Frankel¹³ as modified by Schmidt and Schmidt¹⁴. The method used for assaying lactate dehydrogenase is based on that of Wroblewski and La Due¹⁵ 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.*¹⁶. The activity of Gamma glutamyltranspeptidase (GGT) was determined following the method described by Tietz¹⁷.

Histological Study

Histological study on tissues obtained from experimental rats was carried out following the method described by Drury and Wallington¹⁸.

Statistical Analyses

All numerical results were obtained from the five (5) groups (control and treated). Data were presented as mean±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.

III. Results

Activity of selected enzymes and levels of MDA and GSH of liver of experimental rats are presented in Tables 1-5. No significant difference (p>0.05) was found in the specific activity of the selected liver enzymes and levels of MDA and GSH among the five groups of rats over the 20 week duration of the experiment. Relative to the control group (Group I) the activity of the selected liver enzymes as well as levels of MDA and GSH followed a similar pattern. Notable was the ACP activities of Group V which was significantly lower (p<0.05) than the other treatment groups at weeks 15 and 20.

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

Group of Rats									MDA	GSH	GST
	AST	ALT	ALP	ACP	GGT	LDH	CAT	SOD	(nmol/mg)	(µg/mg)	
I	24.8±1.18 ^a	15.7±2.01 ^a	6.3±0.48 ^a	146.7±5.88 ^a	76.5±4.34 ^a	16.2±1.04 ^a	0.82±0.04 ^a	1.1±0.02 ^a	12.5±2.1 ^a	1.22±0.05 ^a	1.24±0.02 ^a
	25.0±2.21 ^a	16.1±1.77 ^a	7.9±1.10 ^a	140.5±6.01 ^a	73.4±4.11 ^a	15.8±0.89 ^a	0.79±0.03 ^a	1.1±0.02 ^a			
II	25.2±1.34 ^a	15.4±1.93 ^a	7.5±1.08 ^a	143.4±4.87 ^a	75.5±3.98 ^a	16.0±1.01 ^a	0.85±0.05 ^a	1.3±0.02 ^a	11.6±1.8 ^a	1.2±0.02 ^a	1.23±0.03 ^a
	24.7±2.03 ^a	16.3±1.57 ^a	6.8±0.86 ^a	146.5±5.25 ^a	72.8±4.24 ^a	15.7±0.88 ^a	0.81±0.04 ^a	1.2±0.03 ^a			
III	25.1±1.68 ^a	15.9±1.88 ^a	6.5±0.89 ^a	141.8±6.02 ^a	74.9±4.88 ^a	15.8±0.91 ^a	0.85±0.04 ^a	1.2±0.02 ^a	11.9±2.5 ^a	1.22±0.04 ^a	1.25±0.04 ^a
	25.2±1.34 ^a	15.4±1.93 ^a	7.5±1.08 ^a	143.4±4.87 ^a	75.5±3.98 ^a	16.0±1.01 ^a	0.85±0.05 ^a	1.3±0.02 ^a			

Each value represents mean ± SEM of two determinations of liver 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 liver of rats placed on feed formulated with water hyacinth leaf protein concentrate (WHLPC) over a period of 5 weeks.

Group of Rats									MDA	GSH	GST
	AST	ALT	ALP	ACP	GGT	LDH	CAT	SOD	(nmol/mg)	(µg/mg)	
I	24.9±1.19 ^a	15.8±2.02 ^a	6.3±0.48 ^a	147.4±5.91 ^a	76.9±4.36 ^a	16.28±1.05 ^a	0.82±0.04 ^a	1.11±0.02 ^a	12.56±2.1 ^a	1.23±0.05 ^a	1.25±0.02 ^a
	25.1±2.22 ^a	16.2±1.78 ^a	7.9±1.11 ^a	141.2±6.04 ^a	73.8±4.13 ^a	15.88±0.89 ^a	0.79±0.03 ^a	1.11±0.02 ^a			
II	25.3±1.35 ^a	15.5±1.94 ^a	7.5±1.09 ^a	144.1±4.89 ^a	75.9±4.00 ^a	16.08±1.02 ^a	0.85±0.05 ^a	1.31±0.02 ^a	11.66±1.8 ^a	1.21±0.02 ^a	1.24±0.03 ^a
	24.8±2.04 ^a	16.4±1.58 ^a	6.8±0.86 ^a	147.2±5.28 ^a	73.2±4.26 ^a	15.78±0.88 ^a	0.81±0.04 ^a	1.21±0.03 ^a			
III	25.2±1.69 ^a	16.0±1.89 ^a	6.5±0.89 ^a	142.5±6.05 ^a	75.3±4.90 ^a	15.88±0.91 ^a	0.85±0.04 ^a	1.21±0.02 ^a	11.96±2.5 ^a	1.23±0.04 ^a	1.26±0.04 ^a
	25.2±1.34 ^a	15.4±1.93 ^a	7.5±1.08 ^a	143.4±4.87 ^a	75.5±3.98 ^a	16.0±1.01 ^a	0.85±0.05 ^a	1.3±0.02 ^a			

Each value represents mean ± SEM of two determinations of liver 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 liver of rats placed on feed formulated with water hyacinth leaf protein concentrate (WHLPC) over a period of 10 weeks.

Group of Rats									MDA	GSH	GST
	AST	ALT	ALP	ACP	GGT	LDH	CAT	SOD	(nmol/mg)	(µg/mg)	
I	26.78±1.27 ^a	16.96±2.17 ^a	6.80±0.52 ^a	158.44±6.35 ^a	82.62±4.69 ^a	17.50±1.12 ^a	0.89±0.04 ^a	1.19±0.02 ^a	13.50±2.2 ^a	1.32±0.05 ^a	1.34±0.02 ^a
	27.00±2.39 ^a	17.39±1.91 ^a	8.53±1.19 ^a	151.74±6.49 ^a	79.27±4.44 ^a	17.06±0.96 ^a	0.85±0.03 ^a	1.19±0.02 ^a			
II	27.22±1.45 ^a	16.63±2.08 ^a	8.10±1.17 ^a	154.87±5.26 ^a	81.54±4.30 ^a	17.28±1.09 ^a	0.92±0.05 ^a	1.40±0.02 ^a	12.53±1.9 ^a	1.30±0.04 ^a	1.33±0.03 ^a
	26.68±2.19 ^a	17.60±1.70 ^a	7.34±0.93 ^a	158.22±5.67 ^a	78.62±4.58 ^a	16.96±0.95 ^a	0.87±0.04 ^a	1.30±0.03 ^a			
III	26.73±1.79 ^a	16.93±2.00 ^a	6.92±0.95 ^a	151.02±6.41 ^a	79.77±5.20 ^a	16.83±0.97 ^a	0.91±0.04 ^a	1.28±0.02 ^a	12.67±2.6 ^a	1.30±0.04 ^a	1.33±0.04 ^a
	27.22±1.45 ^a	16.63±2.08 ^a	8.10±1.17 ^a	154.87±5.26 ^a	81.54±4.30 ^a	17.28±1.09 ^a	0.92±0.05 ^a	1.40±0.02 ^a			

Each value represents mean ± SEM of two determinations of liver 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 liver of rats placed on feed formulated with water hyacinth leaf protein concentrate (WHLPC) over a period of 15 weeks.

Group of Rats	AST	ALP	ACP	GGT	LDH	CAT	SOD	MDA (nmol/mg)	GSH (µg/mg)	GST	
I	26.92±1.28 ^a	17.04±2.18 ^a	6.84±0.52 ^a	159.23±6.38 ^a	83.03±4.71 ^a	17.58±1.13 ^a	0.89±0.04 ^a	1.19±0.02 ^a	13.57±2.28 ^a	1.32±0.05 ^a	1.35±0.02 ^a
II	27.14±2.40 ^a	17.47±1.92 ^a	8.57±1.19 ^a	152.50±6.52 ^a	79.67±4.46 ^a	17.15±0.97 ^a	0.86±0.03 ^a	1.19±0.02 ^a	11.72±2.60 ^a	1.29±0.07 ^a	1.32±0.04 ^a
III	27.35±1.45 ^a	16.72±2.09 ^a	8.14±1.17 ^a	155.65±5.29 ^a	81.95±4.32 ^a	17.37±1.10 ^a	0.92±0.05 ^a	1.41±0.02 ^a	12.59±1.95 ^a	1.30±0.02 ^a	1.34±0.03 ^a
IV	26.81±2.20 ^a	17.69±1.70 ^a	7.38±0.93 ^a	159.01±5.70 ^a	79.02±4.60 ^a	17.04±0.96 ^a	0.88±0.04 ^a	1.30±0.03 ^a	13.13±2.28 ^a	1.34±0.05 ^a	1.37±0.02 ^a
V	24.60±1.65 ^a	15.58±1.84 ^a	6.37±0.87 ^a	138.96±5.90 ^b	73.40±4.78 ^a	15.48±0.89 ^a	0.83±0.04 ^a	1.18±0.02 ^a	11.66±2.45 ^a	1.20±0.04 ^a	1.23±0.04 ^a

Each value represents mean ± SEM of two determinations of liver 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 liver of rats placed on feed formulated with water hyacinth leaf protein concentrate (WHLPC) over a period of 20 weeks.

Group of Rats	AST	ALP	ACP	GGT	LDH	CAT	SOD	MDA (nmol/mg)	GSH (µg/mg)	GST	
I	26.38±1.26 ^a	16.70±2.14 ^a	6.70±0.51 ^a	156.04±6.25 ^a	81.37±4.62 ^a	17.23±1.11 ^a	0.87±0.04 ^a	1.17±0.02 ^a	13.30±2.23 ^a	1.30±0.05 ^a	1.32±0.02 ^a
II	26.59±2.35 ^a	17.13±1.88 ^a	8.40±1.17 ^a	149.45±6.39 ^a	78.07±4.37 ^a	16.81±0.95 ^a	0.84±0.03 ^a	1.17±0.02 ^a	11.49±2.55 ^a	1.27±0.06 ^a	1.30±0.04 ^a
III	26.81±1.43 ^a	16.38±2.05 ^a	7.98±1.15 ^a	152.53±5.18 ^a	80.31±4.23 ^a	17.02±1.07 ^a	0.90±0.05 ^a	1.38±0.02 ^a	12.34±1.91 ^a	1.28±0.02 ^a	1.31±0.03 ^a
IV	26.27±2.16 ^a	17.34±1.67 ^a	7.23±0.91 ^a	155.83±5.58 ^a	77.44±4.51 ^a	16.70±0.94 ^a	0.86±0.04 ^a	1.28±0.03 ^a	12.87±2.23 ^a	1.31±0.05 ^a	1.34±0.02 ^a
V	24.11±1.61 ^a	15.27±1.81 ^a	6.24±0.85 ^a	136.18±5.78 ^b	71.93±4.69 ^a	15.17±0.87 ^a	0.82±0.04 ^a	1.15±0.02 ^a	11.43±2.40 ^a	1.17±0.04 ^a	1.20±0.04 ^a

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

Histology studies revealed no abnormality in the hepatocyte architecture, the cells were found to be well arranged and distinct (Figures 1-5).

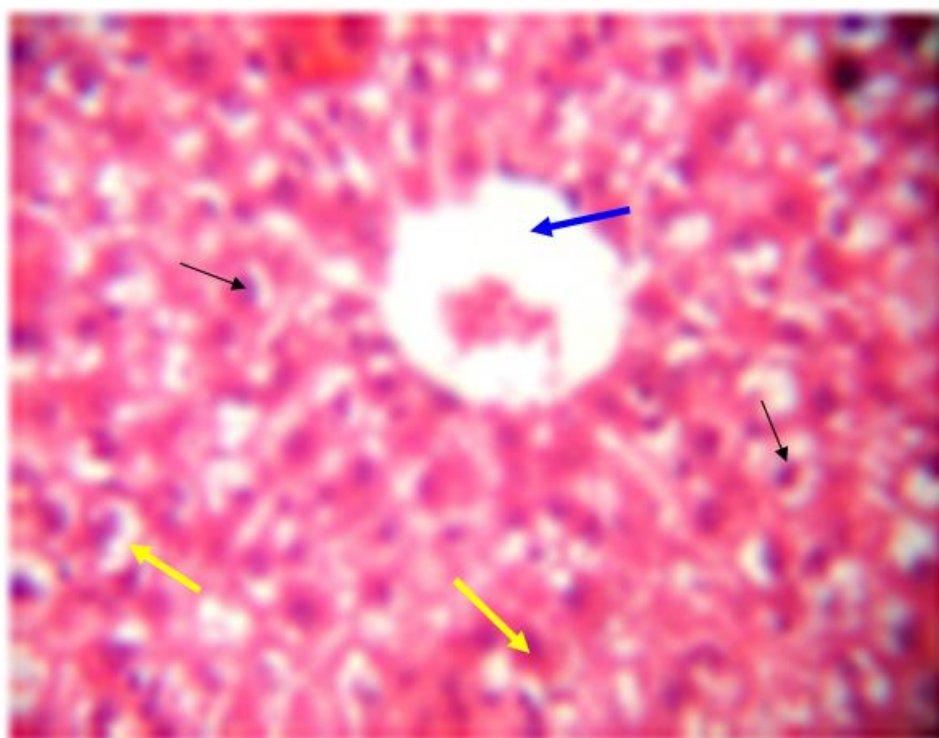


Figure 1: Photomicrograph (H&E, x40) of liver of Control rats. Prominent are the nuclei (black arrows), central vein (blue arrow) and the distinct hepatocytes which are well veneserated by sinusoidal space (yellow arrow).

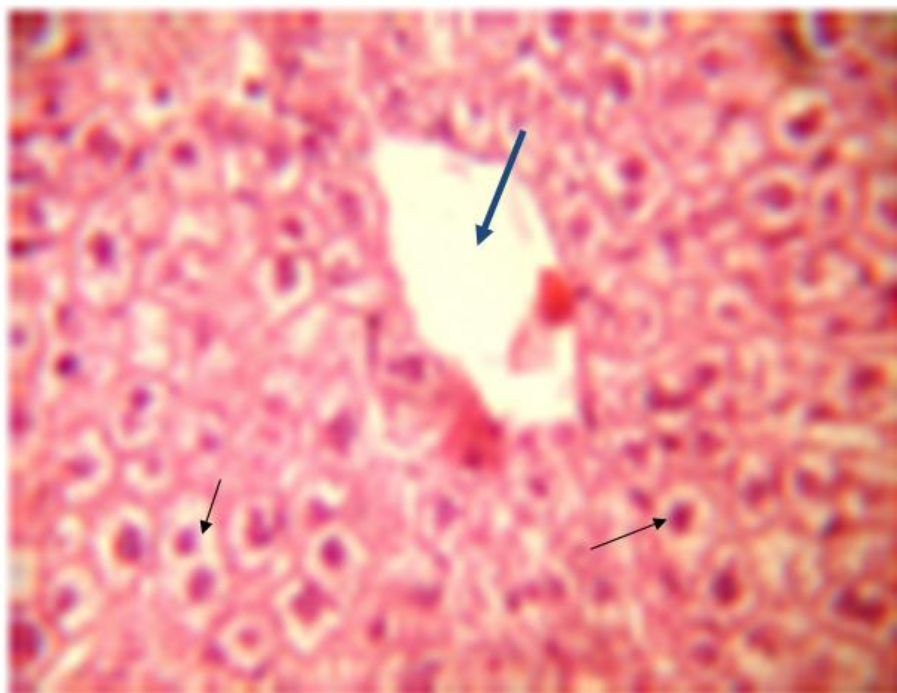


Figure 2: Photomicrograph (H&E, x40) of liver of rats placed on WHLPC1 over a period of 20 weeks. Prominent features include the central vein and the nucleus (blue arrow). The hepatocytes (black arrows) appear well arranged.

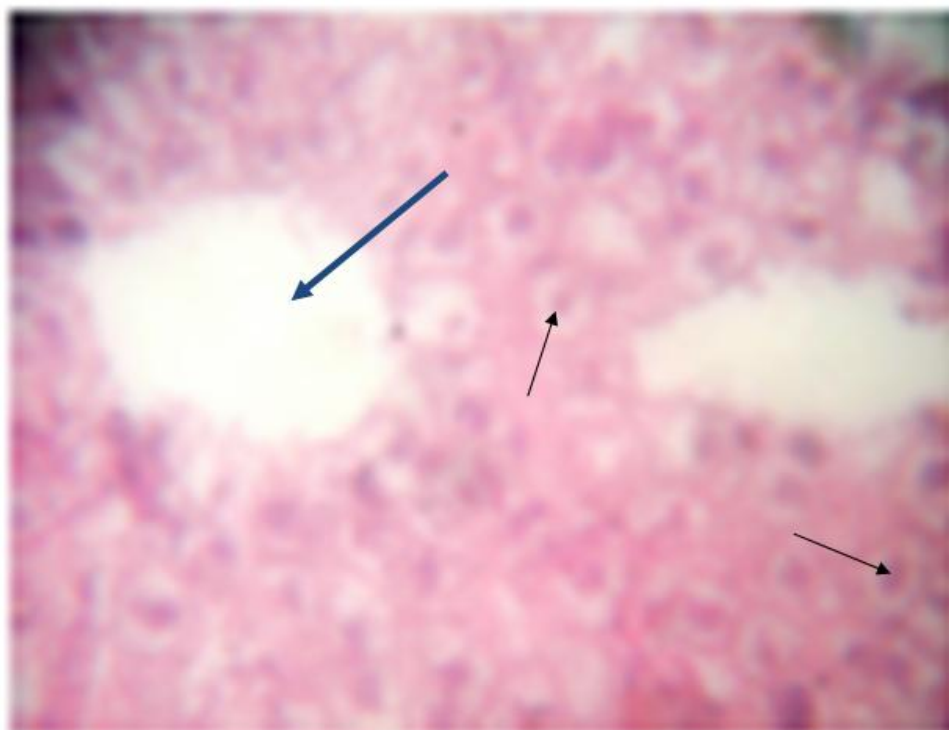


Figure 3: Photomicrograph (H&E, x40) of liver of rats placed on WHLPC2 over a period of 20 weeks. Prominent features include the central vein and the nucleus (blue arrow). The hepatocytes (black arrows) appear well arranged.

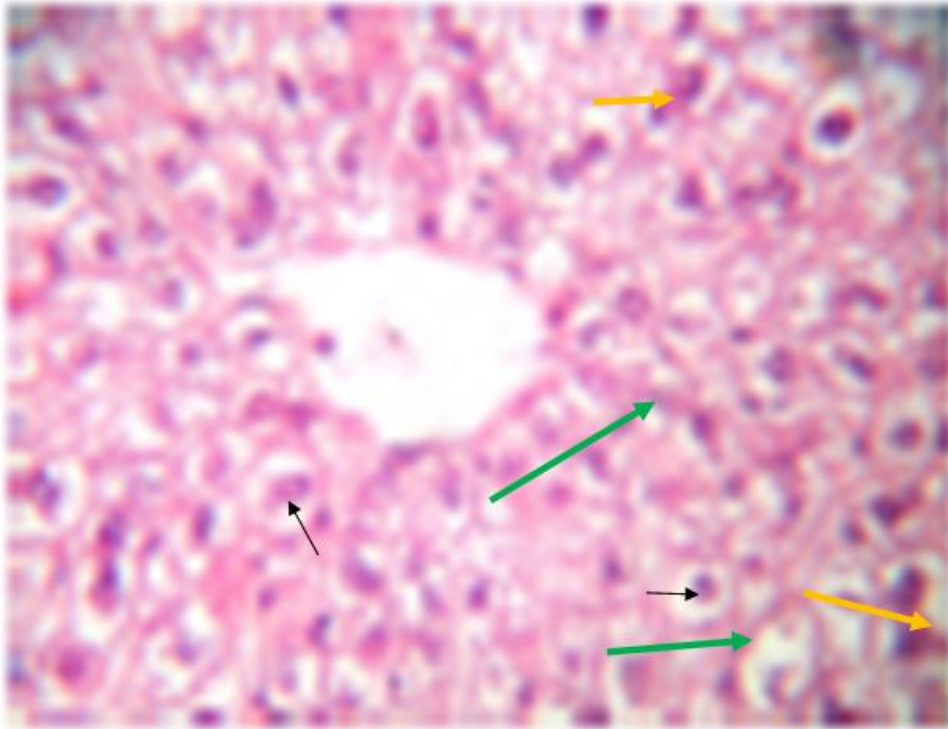


Figure 4: Photomicrograph (H&E, x40) of liver of rats placed on WHLPC3 over a period of 20 weeks. Distinct layers of hepatocytes (black arrows) lined by endothelium (green arrows). The hepatic venules are prominent and clear (yellow arrows).

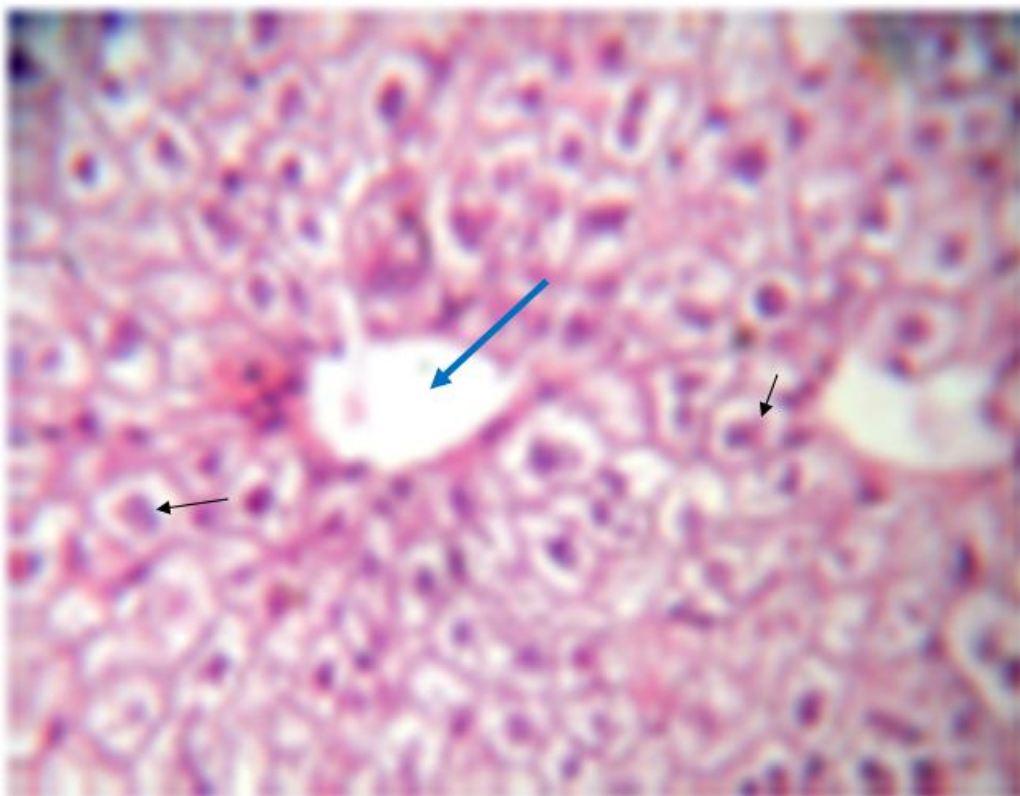


Figure 5: Photomicrograph (H&E, x40) of liver of rats placed on WHLPC4 over a period of 20 weeks. Prominent features include the central vein and the nucleus (blue arrow). The hepatocytes (black arrows) appear well arranged.

IV. Discussion

Nutritional factor, among other factors, has been identified to play a key role in liver diseases [19]. The liver plays host to a number of enzymes and antioxidants, a measure of the activity of these enzymes and determination of the levels of antioxidants give useful information about its state of health [20]. The result of activity of enzymes and levels of antioxidants of the liver of rats placed on feed formulated with WHLPC (Tables 1-5) observed in this study lend credence to the submission that WHLPC is not likely to be hepatotoxic. The enzymes and antioxidants of the liver of rats placed on feed formulated with WHLPC compared favourably with that of rats placed on feed formulated with soybean. It is worthy of note to report that the activity of the enzymes and levels of antioxidants of the liver of rats placed on feed formulated with WHLPC were maintained at normal level throughout the period of experiment. This evidence is compelling and may portend that WHLPC is hepatoprotective.

A great many diseases reveal themselves at the cellular level: many cancers, bone and connective tissue diseases, vascular diseases, liver diseases, kidney diseases and others can be definitively diagnosed using histological techniques. Photomicrographs of the liver (Figures 1-5) showed normal cellular architecture without lesion or inflammation, this observation revealed that consumption of WHLPC compared favourably with that of soybean. It also suggests that WHLPC is well tolerated by rats and extrapolation could be drawn to man. This finding lends credence to our earlier report that WHLPC is nutritionally adequate to maintain normal circulating level of plasma proteins²¹.

V. Conclusion

This study documents that water hyacinth leaf protein concentrate (WHLPC) (7.73, 15.46, 23.19, and 30.92) %w/w did not significantly affect the activity of (AST, ALT, ALP, ACP, GGT, LDH, CAT, SOD) enzymes of the liver. Histological evidence from this study also showed WHLPC did not cause any damage to the liver at the cellular level. This is novel in that we extracted WHLPC in a form that is not only digestible by rat but also safe to the liver. We, therefore submit that WHLPC is probably hepatoprotective.

Conflict of Interest

Authors declare no conflict of interest

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