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Water Hyacinth leaf protein concentrate maintains the levels of selected serum metabolites of rats

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Edible form of water hyacinth leaf protein concentrate (WHLPC) was extracted and the LD_{50} evaluated. The 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 tissues were collected biochemical and toxicological analyses. Biochemical metabolites studied are urea, creatinine, bilirubin, globulin and albumin of serum of experimental rats. Beginning from the 10th week through to the 20th week, the serum bilirubin levels (total and direct) of rats in group V were found to be significantly higher ($p<0.05$) than those of the remaining groups while the albumin and globulin levels of the same group of rats were observed to be significantly lower than those of the other groups of rats ($p<0.05$).

Keywords: Water Hyacinth, protein concentrate, serum, metabolite, urea, creatinine.

INTRODUCTION

Water hyacinths (*Eichhornia crassipes*) are vigorous growers known to double their population in two weeks (Simpson and Sanderson, 2002). The origin of water hyacinth has been traced to South America but the first surge of the weed in Nigeria was noticed in September, 1984 along the Badagry Creek in Lagos State where the weed formed a 'mat' over the water surface. By January 1985, it had spread to the creeks and lagoons in Lagos and its environs. By 1986, the weed had crossed the Lagos lagoon and has since covered most of the intricate system of waterways made up of rivers, lagoons and creeks in Lagos, Ogun, Ondo, Edo, Delta states and beyond. The weed is spreading fast along the coastal states of Nigeria.

Water hyacinth has invaded freshwater systems in over 50 countries on five continents; it is especially pervasive throughout Southeast Asia, the southeastern United States, central and western Africa, and Central America (Olivares and Colonnello, 2000). It is prevalent in tropical and subtropical water bodies where nutrient levels are often high due to agricultural runoff and insufficient wastewater treatment. There is not a clear record of how, why, and when water hyacinth was introduced to water bodies outside of its native range, but many populations of water hyacinth are well established and persistent despite control efforts. Its success as an invader is attributed to its ability to outcompete native vegetation and phytoplankton for light and its release from organisms that feed on it (*Neochetina eichhorniae* and *N. bruchi*) found within its native range. Changes in water hyacinth density have the potential to affect other ecological and human communities in areas where it is established; these changes may be

perceived as positive or negative depending on the designated or beneficial uses of the water body (Ghabbour et al., 2004).

The water hyacinth tragedy is closely connected with its exotic, hyacinth-like flowers. This insidious plant is commonly marketed as an ornamental and it is astonishing that in most tropical areas, until the present day, strict regulations have not been enforced to prevent further dispersion. In spite of the fatal invasion of the southern states of the USA, water hyacinth was spread into Africa, South East Asia and Australia in the early 1900s, mainly via introductions into botanical gardens. Thereafter it gradually started its dramatic advance in tropical and sub-tropical regions all over the world (Pieterse, 1978).

In 1985 the Nigerian Government established a National Committee on Water hyacinth, based at the National Agency for Science and Engineering Infrastructure (NASENI). This Committee coordinates activities through five sub-committees covering surveillance, biological, mechanical and chemical control, and practical uses of the weed. In light of the recent infestation of the Niger River, the Nigerian Government spent the bulk of its total budget for aquatic weed control of two million naira in 1994, to mechanically clear the Niger upstream of the Kainji reservoir. At present, it is part of NASENI's program to purchase a large number of small units of mechanical harvesters, and to distribute these to local governments of the Niger River areas.

Our previous investigation described the use of water hyacinth for the preparation of leaf protein concentrate and determines its value as food for humans (Adeyemi and Osubor, 2016). We have evaluated the response of haematological properties as well as body and organ weights of rats fed with water hyacinth leaf protein concentrate over a period of 20 weeks (Adeyemi and Osubor, 2017). The present study is another attempt to find practical uses for the water hyacinth plant and to explore further the health benefits inherent in it. Here, we investigated the effect of water hyacinth leaf protein concentrate (LPC) on serum metabolites of rats. The biochemical approach employed in this study could be used to design an attractive model for toxicology studies.

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 5.54^0E and 5.7^0W and latitude 5.31^0N and 5.6^0S in Warri, Delta State, Nigeria. More detailed information is as reported (Adeyemi and Osubor, 2016).

Sample Collection and Extraction

The Eichhonia *crassipes* (Mart.) Solms samples were collected and the water hyacinth leaf protein concentrate (WHLPC) was extracted based on the method described in our previous study (Adeyemi and Osubor, 2016; 2017).

Physicochemical Analysis of WHLPC

The physicochemical properties of WHLPC were done according to standard methods described and reported in our previous article (Adeyemi and Osubor, 2016; 2017).

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 reported (Adeyemi and Osubor, 2017).

Hundred Albino rats (*Rattus novergicus*) 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

Determination of LD_{50}

Rats prepared for the experiments included 100 rats weighing 17-20g. They were randomized into 5 groups. Each group contained 20 rats

respectively. After stopping to supply the normal feedstuff for 12 h, four groups of the rats were positively fed with 3.25, 8.37, 17.51 and 21.50 g/kg.bw Water Hyacinth Leaf Protein Concentrate by using an intra-gastric syringe, respectively. For the dosage of ≥17.51 g/kg.bw, the tested material was prepared by mixing 1 Water Hyacinth Leaf Protein Concentrate with 2 salad oil and the rats were fed twice in 2 h by using an intra-gastric syringe. Observation was undertaken to check the number of the rats poisoned to death by Water Hyacinth Leaf Protein Concentrate within 24 hours. Then, the normal feedstuff sticks and water were fed *ad libitum* to rats throughout the experimentation (Dixon, 1965; 1991).

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 levels of the serum metabolite to be monitored. This was repeated at week 5, 10, 15 while the remaining rats were sacrificed at the 20th week. After the sacrifice, a portion of the blood was collected into non EDTA sample bottles for serum biochemical analyses. The rats were anaesthetized by placing them in a jar containing cotton wool soaked with chloroform before being sacrificed by jugular puncture.

Serum Metabolites

Serum Urea Concentration

The serum urea concentration of the experimental rats was determined following the method described by Marsh et al., (1965).

When urea is heated in strongly acidic conditions with substances such as diacetyl monoxime, yellow condensation compounds are formed. The reaction is intensified by the presence of polyvalent ions such as ferric ions to form a red coloured complex.

Serum Creatinine Concentration

The serum creatinine concentration of the experimenta rats was determined following the method reported by Brod and Sirota (1948). Creatinine gives a red colour with alkaline solution of picric acid (Jaffe's reaction).

Serum bilirubin

The bilirubin in the serum is coupled with diazotized sulphanilic acid to form azobilirubin. The intensity of the purple colour that is formed is measured spectrophometrically at 520nm (Tietz, 1990).

Serum albumin

Albumin reacts with the dye-Bromocresol green in an acididc medium to produce a greenish coloured complex the intensity of which is proportional to the amount of albumin present (Tietz, 1990).

Serum globulin

Globulin is calculated based on the measured total protein and albumin.

Total Protein Concentration

The protein concentration in the tissue of experimental rats was determined following the method reported by Gornal et al., (1949).

Cupric ions in alkaline solution form a purple coloured complex with any compound containing repeated-CONH-links such as proteins. The purple colouration is due to the coordination between the cupric ions and the unshared electron pair of peptide nitrogen and the oxygen of water to form the colour coordination complex.

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.

RESULTS

Table 1 presents results of the LD_{50} test of WHLPC. The dose of WHLPC was varied from 3.25, 8.37, 17.51 and 21.50. It was observed that all the animals survived both at 24h and 12 days. The animals did not show any sign of weakness or loss of physical strength. The physical appearance of the animals did not reveal any noticeable changes. All the doses of WHLPC were well tolerated by the animals.

Tables 2-6 present results of serum concentrations of creatinine, urea, bilirubin (direct and total), albumin and globulin of experimental rats over a period of twenty weeks. The levels of serum metabolites of rats in the various groups are statistically the same over a period of five weeks. However, at the 10th week, the level of serum albumin and globulin of rats in group V were found to be significantly lower than those of

the remaining four groups. Beginning from the 10th week through to the 20th week, the serum bilirubin levels (total and direct) of rats in group V were found to be significantly higher ($p<0.05$) than those of the remaining groups while the albumin and globulin levels of the same group of rats were observed to be significantly lower than those of the other groups of rats ($p<0.05$).

Table 1: Results of LD_{50} test of Water hyacinth leaf protein concentrate

Rat ID	Dose (g/kg.bw)	Short-term result (48 h)	Long-term result (12 days)
A	3.25	Survival	survival
B	8.37	Survival	survival
C	17.51	Survival	survival
D	21.50	Survival	survival

Each value represents mean ± SEM of duplicate determinations from four different animals

Table 2: Selected serum metabolites of rats to be placed on feed formulated with water hyacinth leaf protein concentrate (WHLPC) over a period of 20 weeks.

Group of Rats	Creatinine (mmol/L)	Urea (mmol/L)	Bilirubin		Albumin (g/dL)	Globulin (g/dL)
			Direct (mg/dL)	Total (mg/dL)		
Basal Level						
I	0.20±0.02 [a]	5.6±0.3 [a]	1.05±0.02 [a]	5.01±0.20 [a]	12.98±2.50 [a]	8.45±0.22 [a]
II	0.20±0.03 [a]	5.6±0.4 [a]	1.06±0.02 [a]	5.06±0.20 [a]	13.11±2.53 [a]	8.53±0.22 [a]
III	0.20±0.03 [a]	5.8±0.3 [a]	1.05±0.02 [a]	5.03±0.20 [a]	13.02±2.51 [a]	8.48±0.22 [a]
IV	0.20±0.02 [a]	5.7±0.3 [a]	1.07±0.02 [a]	5.11±0.20 [a]	13.24±2.55 [a]	8.62±0.22 [a]
V	0.21±0.03 [a]	5.9±0.4 [a]	1.06±0.02 [a]	5.05±0.20 [a]	13.08±2.52 [a]	8.52±0.22 [a]

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

Table 3: Selected serum metabolites of rats placed on feed formulated with water hyacinth leaf protein concentrate (WHLPC) over a period of 5 weeks.

Group of Rats	Creatinine (mmol/L)	Urea (mmol/L)	Bilirubin		Albumin (g/dL)	Globulin (g/dL)
			Direct (mg/dL)	Total (mg/dL)		
I	0.20±0.03 [a]	5.7±0.30 [a]	1.26±0.02 [a]	6.01±0.24 [a]	18.17±3.50 [a]	11.83±0.31 [a]
II	0.20±0.02 [a]	5.7±0.40 [a]	1.27±0.02 [a]	6.07±0.24 [a]	18.35±3.54 [a]	11.95±0.31 [a]
III	0.21±0.02 [a]	5.9±0.30 [a]	1.26±0.02 [a]	6.03±0.24 [a]	18.23±3.51 [a]	11.87±0.31 [a]
IV	0.20±0.03 [a]	5.8±0.30 [a]	1.29±0.02 [a]	6.13±0.24 [a]	18.54±3.57 [a]	12.07±0.31 [a]
V	0.21±0.02 [a]	6.0±0.40 [a]	1.27±0.02 [a]	6.06±0.24 [a]	17.01±3.28 [a]	11.07±0.29 [a]

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

Table 4: Selected serum metabolites of rats placed on feed formulated with water hyacinth leaf protein concentrate (WHLPC) over a period of 10 weeks.

Group of Rats	Creatinine (mmol/L)	Urea (mmol/L)	Bilirubin		Albumin (g/dL)	Globulin (g/dL)
			Direct (mg/dL)	Total (mg/dL)		
I	0.20±0.01[a]	5.6±0.30[a]	1.16±0.02[a]	5.51±0.22[a]	17.39±3.35[a]	11.32±0.29[a]
II	0.20±0.01[a]	5.6±0.40[a]	1.17±0.02[a]	5.57±0.22[a]	17.57±3.38[a]	11.44±0.30[a]
III	0.20±0.01[a]	5.8±0.30[a]	1.16±0.02[a]	5.53±0.22[a]	17.45±3.36[a]	11.36±0.30[a]
IV	0.20±0.01[a]	5.7±0.30[a]	1.18±0.02[a]	5.62±0.22[a]	17.74±3.42[a]	11.55±0.30[a]
V	0.21±0.01[a]	6.0±0.40[a]	1.16±0.02[a]	5.56±0.22[a]	16.09±3.10[b]	10.48±0.27[b]

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

Table 5: Selected serum metabolites of rats placed on feed formulated with water hyacinth leaf protein concentrate (WHLPC) over a period of 15 weeks.

Group of Rats	Creatinine (mmol/L)	Urea (mmol/L)	Bilirubin		Albumin (g/dL)	Globulin (g/dL)
			Direct (mg/dL)	Total (mg/dL)		
I	0.20±0.01[a]	5.7±0.30[a]	1.00±0.02[a]	4.76±0.19[a]	17.52±3.38[a]	11.41±0.30[a]
II	0.20±0.02[a]	5.7±0.41[a]	1.01±0.02[a]	4.81±0.19[a]	17.70±3.41[a]	11.52±0.30[a]
III	0.21±0.02[a]	5.9±0.30[a]	1.00±0.02[a]	4.77±0.19[a]	17.58±3.39[a]	11.44±0.30[a]
IV	0.20±0.03[a]	5.8±0.30[a]	1.02±0.02[a]	4.85±0.19[a]	18.45±3.55[a]	12.01±0.31[a]
V	0.23±0.01[a]	6.5±0.41[a]	1.27±0.02[b]	6.06±0.24[b]	15.61±3.01[b]	10.16±0.26[b]

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

Table 6: Selected serum metabolites of rats placed on feed formulated with water hyacinth leaf protein concentrate (WHLPC) over a period of 20 weeks.

Group of Rats	Creatinine (mmol/L)	Urea (mmol/L)	Bilirubin		Albumin (g/dL)	Globulin (g/dL)
			Direct (mg/dL)	Total (mg/dL)		
I	0.20±0.03[a]	5.7±0.30[a]	1.01±0.02[a]	4.81±0.19[a]	17.70±3.41[a]	11.52±0.30[a]
II	0.20±0.02[a]	5.7±0.40[a]	1.02±0.02[a]	4.86±0.19[a]	17.88±3.44[a]	11.64±0.30[a]
III	0.21±0.02[a]	5.9±0.30[a]	1.01±0.02[a]	4.82±0.19[a]	17.75±3.42[a]	11.56±0.30[a]
IV	0.20±0.02[a]	5.8±0.30[a]	1.03±0.02[a]	4.90±0.20[a]	18.64±3.59[a]	12.13±0.32[a]
V	0.21±0.02[a]	6.0±0.40[a]	1.28±0.02[b]	6.12±0.24[b]	15.77±3.04[b]	10.26±0.27[b]

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

DISCUSSION

The results of the determination of LD_{50} indicated that all of the rats tested were alive at the doses of 3.25, 8.37, 17.51 and 21.50 g/kg.bw of WHLPC, respectively (Table 1). This means that the LD_{50} of WHLPC is more than 21.50 g/kg.bw. According to WHO/FAO standard, if the LD_{50} of a chemical compound is 5 - 15 g/kg.bw, it should be considered to be acutely non-toxic (WHO, 1989). Therefore, it is suggested that WHLPC is not acutely toxic. However, an investigation of the long-term toxic effect of WHLPC through animal feeding test, as in the present study, is worth embarking upon.

Glomerular filtration rate is the best estimate of number of functioning nephrons and functional renal mass. Accurate measurement of glomerular filtration rate is time-consuming and expensive, but a number of filtered substances may be measured to estimate glomerular filtration rate, including Blood Urea and Serum Creatinine (Amin-ul-Haq et al., 2010). Urea is produced as a breakdown product of protein. Creatinine is a metabolic by-product of muscle metabolism. Urea and creatinine are filtered and excreted by the kidney. No significant difference ($p>0.05$) was observed in the serum levels of urea and creatinine among the various group of rats, the levels of urea and creatinine are within the normal range values (Table 2). WHPLC has probably not obstructed glomerular filtration rate. This observation lends credence to the fact that WHLPC is a suitable substitute to soybean as a dietary protein source.

Adeyemi et al., (2010) demonstrated that serum bilirubin, albumin and globulin concentrations are some of the biochemical indices for monitoring liver function in the blood. Abnormal levels of these proteins have been reported to be associated with haemolysis or increased breakdown of RBC and/ or liver damage (Islam et al., 2004). Increased total bilirubin causes jaundice and can signal a number of problems. Studies had shown that if direct (that is, conjugated) bilirubin is normal, then the problem is an excess of unconjugated bilirubin and the location of the problem is upstream of bilirubin excretion. Anemia, viral hepatitis, or cirrhosis can be suspected. If direct bilirubin is elevated, then the liver is conjugating bilirubin normally, but is not able to excrete it. Bile duct obstruction by gallstones or cancer should be suspected. Bilirubin is a breakdown product of heme (a part of haemoglobin in red blood cells). The liver is responsible for clearing the blood of bilirubin by taking it up into hepatocytes,

conjugated (modified to make it water soluble) and secreted into the bile, which is excreted into the intestine. Although the bilirubin values are within the normal range, the cause of the significantly higher ($p<0.05$) levels of both direct and total bilirubin observed from the 15th week in rats (Group V) placed on feed formulated with 30.96% WHLPC is not clear (Tables 2-6). However, data on growth of rats suggested that adjusted level of WHLPC beyond 15.46% may not be well tolerated.

Hundreds of proteins are dissolved in the plasma. By measuring the concentration of these proteins, the researcher can obtain information regarding disease states in different organ systems. The measurement of protein is done on serum, which is the fluid that remains after plasma has clotted, thus removing fibrinogen and most of the clotting factors. The liver produces albumin at less than half of its capacity. The primary factors affecting albumin synthesis include protein and amino acid nutrition, colloidal osmotic pressure, the action of certain hormones, and disease states. Fasting or a protein-deficient diet causes a decrease in albumin synthesis as long as the deficiency state is maintained. The globulin fraction includes hundreds of serum proteins including carrier proteins, enzymes, complement, and immunoglobulins. Most of these are synthesized in the liver, although the immunoglobulins are synthesized by plasma cells. All the test diets, in this study, led to serum globulin and albumin levels within normal range indicating that they are nutritionally adequate to maintain normal circulating level of these proteins (Enitan, et al., 2012).

CONCLUSION

Water hyacinth leaf protein concentrate (WHLPC) is not acutely toxic, this was inferred from the LD_{50} result. Further conclusions drawn from experimental evidence in this study are;

1-Results of serum urea and creatinine suggests that WHPLC probably did not obstructed glomerular filtration rate

2-All the test diets, in this study, maintained serum globulin and albumin levels within normal range indicating that they are nutritionally adequate to maintain normal circulating level of these proteins

Over all, WHLPC is a good source of protein; however, adjusted level of WHLPC beyond 15.46% may not be well tolerated.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

OCC and AO conceived and designed the research. AO conducted the experiments. OCC analyzed the data. AO wrote the manuscript. OCC reviewed the manuscript. All authors read and approved the final version.

REFERENCES

Adeyemi O, Oloyede OB, Oladiji AT, 2010. Biochemical evaluation of leachate-contaminated groundwater on the kidney of Albino rats. Exptal Toxicol Path 62:483–488.

Adeyemi O, Osubor CC, 2016. Assessment of nutritional quality of water hyacinth leaf protein concentrate. Egyptian Journal of Aquatic Research 42:269–272.

Adeyemi O, Osubor CC, 2017. Effects of water hyacinth leaf protein concentrate on growth and haematological properties of rats. European Journal of Biomedical and Pharmaceutical Sciences 4(6):116-123.

Amin-ul-Haq, RM, Zahoor A, Jamil-ur-Rehman GJ, 2010. Association of serum uric acid with blood urea and serum creatinine. Pak J Physiol 6(2):46-49.

Brod J, Sirota JM, 1948. Serum creatinine determination. Clin Invest 27:645.

Dixon WJ, 1965. The Up-and-Down method for small samples. Journal of Amer Statist Assoc 60:967-978.

Dixon WJ, 1991. Staircase Bioassay: The Up-and-

Down method. Neurosci Biobehav Revised 15:47-50.

Enitan SS, Ajeigbe KO, Josiah SJ, Ehiaghe FA, 2012. Haematological and Hepatotoxic Potential of Onion (Allium cepa) and Garlic (Allium sativum) Extracts in Rats. Eur J Med Plts 2(4):290-307.

Ghabbour EA, Davies D, Lam YY, Vozzella ME, 2004. Metal binding by humic acids isolated from water hyacinth plants (Eichhornia crassipes [Mart.] Solm-Laubach: Pontedericeae) in the Nile Delta, Egypt. Envtal Pollution 131:445-451.

Gornal AG, Bardawill JC, David MM, 1949. Determination of serum proteins by means of biuret reaction. J Biol Chem 177:751-760

Islam MS, Lucky NS, Islam MR, Ahad A, Das BR, Raham MM, Siddiui MSI, 2004. Haematological parameters of Fayoumi, Assil and local chickens reared in sylhet region in Bangladesh. Int J Poult Sci 3(2):144-147.

Marsh WH, Fingerhurt B, Muller H, 1965. Automated and manual direct methods for the determination of blood urea. Clin Chem 11:624-625.

Olivares E, Colonnello G, 2000. Salinity gradient in the Manamo River, a dammed distributary of the Orinoco Delta, and its influence on the presence of Eichhornia crassipes and Paspalum repens. Interciencia 25:242-248.

Pieterse AH, 1978. The water hyacinth (Eichhornia crassipes) - a review. Abstracts on Trop Agric 4:9-42.

Simpson D, Sanderson H 2002. *Eichhornia crassipes*. Curtis's Bot Mag 19(1):28-34.

Tietz NW, 1990. In: Clinical guide to laboratory tests, 2nd ed. Philadelphia, USA: W.B. Saunders Company. pp. 554.

WHO 1989. Thirty-third report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report N0. 776. WHO, Geneva.