Original Research Article

Heat treated African kudzu (*pueraria phaseoloides,roxb.benth.*) flour influenced lipid peroxidation, liver enzymes and organ histology of wistar rats

Absract

Scarcity of protein-rich foods has been the bane of developing countries with its attendant nutritional problems. Research efforts have been directed towards finding solutions to this problem. This include investigation into the nutritional potentials of some lesser known legumes and grains. This paper looked at the effect of processed *kudzu* flours on the liver enzymes,alkaline phosphotase (ALP), aspartate amino transferase (AST) and alanine amino transferase (ALT); lipid peroxidation of the liver and pancreatic tissues as well as the liver and pancreatic architecture of wistar rats. Raw, cooked and time-controlled autoclaved (20mins,40mins, 60mins) kudzu seeds were made into flours and compounded into 8 diets. Diet 1contained the raw kudzu flour,diet 2 had the cooked kudzu flour and diets 3-5 had time-controlled autoclaved (20mins,40mins, 60mins) *kudzu* flour respectively. Diet 6 was the negative control diet while diet 8 was the positive control.Diet 8 had lower ALP value (51.6±6.5iu) than the other groups though not statistically ($p \leq 0.05$) different. Diet 3had the highest AST(51.0 ± 0.iu) which was statistically ($p \leq 0.05$) different from the other groups and ALT (48.0 ± 0.1 iu) that was statistically ($p \leq 0.05$) not different with only group 4. The level of malondialdehyde in the liver was lowest in Diet 2 ($2.75 \times 10^{-5} \pm 0.07 \times 10^{-5}$MDA unit) followed by Diets 6 and 8.While the level in the pancreatic tissues was lowest in Diet 8 ($83.25 \times 10^{-5} \pm 0.07 \times 10^{-5}$ MDA unit). .However,the variations in the level of MDA in both organs in all the groups were not statistically ($p \leq 0.05$) different. Histopathological examination of the liver tissues showed various degrees of abnormalities in Diets (1, 3,4,5 and 6) while Diets 2,7 and 8 had normal cell architecture. The pancreatic tissues showed normal architecture. The results therefore suggest that kudzu when processed poses no toxicological danger to animal and by extension man.

Key words:African *kudzu*,liver enzymes,lipid peroxidation,organ histology.

Introduction

The worsening food insecurity globally,exacerbated by climate change[1], economic downturn of many nations,artificial and man-made disasters, various forms of regional and territorial armed conflicts[2,3] and recently a global disease pandemic[4] has necessitated the quest for multidimensional approach in dealing with the situation. Several approaches have been adopted while some are been worked out such as probing into the nutritional potentials of some food items and so encourage their exploitation for immediate and future food security, the others aim to make food production more environmentally friendly and more resilient to climate shocks [5] while others concentrate on improving the food environment and advocacy for policy briefs in food and nutrition issues.

Probing into the nutritional or functional properties of food items has been a common practice right from the early times. Our Ancestors through ‘crude methods’ or intuitions were able to decipher what food was ‘good’ for them. Indeed,the process was the basis for identifying edible and isolating the inedible food items long before the advent of civilizations. Subsequently, scientific technologies were developed and the investigations continued, providing more reliable information and data. Therefore, several authors have provided nutritional, phytochemical, therapeutic and toxicological information on different foodstuff including vegetables[6,7,8,9,10,11];fruits [12,13,14,15]; cereals[16,17,18];legumes [19,20 21]; roots and tubers [22,23 ,24], nuts and seeds[25,26,27] etc. The immediate and remote implications of foodinsecurity are burdensome. They include high food prices and attendant lack of access to food, food insecurity predisposes individuals to increased risk to a variety of diseases and other health challenges 28,29,30,31,32, 33] . Food insecurity is a major factor in the cause of different forms of crime and criminality. Food security index of a country depicts the country’s poverty and consequently her development index. Thus, food security is central for the achievement of the some of the Sustainable development goals (SDGs) particularly goals 1-3.Therefore,any contribution towards alleviating food insecurity will help in the development agenda of any nation. This study looked at the effect of some processing methods on selected parameters of wistar rats fed with diets formulated from an unutilized legume-*kudzu*. The broad objective is to popularize its use either for human or animal nutrition.

Materials and Methods

Kudzu seeds were obtained from International Institute for Tropical Agriculture, (IITA) Onne, Rivers State while maize *(Zea mays)* grains and red palm oil were purchased from the Mile 3 Market, Port Harcourt, Rivers State.Granulated pure cane sugar (sucrose) and nutrend were purchased from Sunrise Supermarket, University of Port Harcourt.Vitamins and mineral mixtures were bought at Raf - Veterinary Store, Rumuigbo Port Harcourt, Rivers State.One kilogram (1kg) of the seeds was not given any heat treatment while same quantity was cooked; autoclaved at 121^0C (15psi) for 20 minutes, 40 minutes and 60 minutes respectively. They were later ground into fine powder (710mm sieve) using an electric mill and individually packed in air-tight bags and stored in the refrigerator at 4^0C until used for analysis.

Seven (8) diets were formulated using corn starch, kudzu flour (raw and processed), sucrose, non-nutritive cellulose, palm oil and vitamin/mineral mixture.Diet1 contained the raw kudzu, diet 2 cooked kudzu, diet 3 kudzu autoclaved at 121^0C (15psi) for 20 minutes, diet 4 kudzu autoclave at 121^0C 15psi for 40 minutes, diet (5) kudzu autoclave at 121^0C (15psi) for 60 minutes. The sixth diet (6) was the basal diet(protein free diet) while diet seven (7) was made up of 100% cooked kudzu and diet eight (8) was the reference diet (nutrend). The diets were formulated to provide 16% protein.

A total of 40 wistar rats aged between 24-28 days, obtained from the Biochemistry Department, University of Port-Harcourt were used for the study. All experimental procedures herein were carried out in accordance with the ethical guidelines for laboratory animals and complied with the guide for the care and use of laboratory animals (National Research Council, 2011). The animals were weighed and divided into eight groups of five (5) rats each on the basis of their body weights. They were singly housed in perforated Perspex wire bottom cages with facilities for food and water. The rats were acclimatized for seven days and fed with the experimental diet and water *adlibitum* for 28 days. They were then sacrificed by cervical dislocation on the 28th day. Their blood collected for foe estimation of liver enzyme.The animals were dissected, their liver and pancreas exercised, inspected for any pathological abnormalities and later histological procedure carried out on them according to Baker and Silverton [34] method. MDA estimation was done on the tissues using Gutteridge and Wilkins[35] method modified from Hunter et al., 36].

The results were analyzed statistically by the use of one way analysis of variance (ANOVA) to determine the differences between the mean values at $P < 0.05$ level [37]

Results and Discussion

The results of the lipid peroxidation of the liver and pancreatic tissues as well as the liver enzyme assay of the experimental animals are shown in Table 1.The values for malondialdehyde (MDA) in the liver tissues was highest among the animals on diet 1($32 \times 10^{-5} \pm 0.0$ MDA unit) and lowest in animals on diet 4($2.7 \times 10^{-5} \pm 0.14$ MDA unit).The values were however not statistically($P< 0.05$) different from the other groups.There were also no statistical difference in the MDA values for pancreatic tissues.MDA is a by-product of lipid peroxidation. Lipid peroxidation occurs in tissues when tissues are exposed to adverse environment including toxicants. It is an oxidative damage that affects cellular membranes, lipoproteins, and other molecules that contain lipids. Cellular membrane lipids represent most often substrates of oxidative attack [38]. Lipid peroxidation is a chain reaction and is created by free radicals influencing unsaturated fatty acids in cell membranes, leading to their damage. Free radicals are initiators and terminators of lipid peroxidation processes. Once activated, reaction continues autocatalytically; it has a progressive course, and its final result is structural and functional changes of substrate [39]; [40].Thus, high level of MDA has been implicated in several human diseases.For instance, corneas of patients suffering from keratoconus and bullous keratopathy have increased levels of malondialdehyde [41] and MDA also can be found in tissue sections of joints from patients with osteoarthritis.[42].

Levels of malondialdehyde is also used to assess the membrane damage in spermatozoa; this is crucial because oxidative stress affects sperm function by altering membrane fluidity, permeability and impairing sperm functional competence.[43]. The comparable values of MDA in all the groups in this study may suggest that the toxic phytochemicals in the legume had no negative effect on the lipid-containing structural components of the experimental animals.

The values for the liver enzyme,alkaline phosphatase (ALP) showed that diet 2 had the lowest value of 49.67 ± 9.29 IU followed by diet 7 (50.7± 5.0IU) and diet 8(51.60± 5.5IU) but the values were not significantly($P< 0.05$) different from others.Aspartate amino transferase (AST) was least in diet 8(39.2± 6.83 IU) and statistically lower than only diet 3(51.0± 2.16 IU).Alanine amino transferase(ALT) had the lowest value in diet 7(36.0± 7.62 IU) followed by diet 8(37.0± 0.0 IU) and the values were statistically different from those of diet 3(48.0± 1.10 IU) and 4(46.67± 3.51 IU).Enzymes are membrane –bound and damage to the cells and membrane of the liver may result in their release with other intracellular constituents into the blood plasma[44,45].Increases in the activities of these enzymes following exposure to toxicants have been reported[46]. The serum levels of these enzymes reflect the physiological state of the liver. Since the values of these liver enzymes in the experimental animals fed with the cooked diets were always comparable with those of the reference diet,it can be inferred that the result was due to the lowering effect of wet heat on some toxic phytochemicals [47,48].

The microphotograghs of the liver and the pancreas are shown on plates 1-16.The pictures for the two organs in diets 2,7 and 8 were essentially normal while others showed varying degrees of abnormalities which may be attributed to the presence of toxic phytochemicals.However,since the MDA values for the organs across the groups were comparable as well as the liver enzyme activities,it may be inferred that the observed abnormalities in the organs may not be physiologically detrimental.

Table 1 Lipid Peroxidation (MDA unit) and Enzyme Assay (UI)

Diet	Liver	Pancreas	ALP	AST	ALT
1	3.2×10^{-5a}	99.35×10^{-5a}	52.25^{a}	47.25^{a}	43.25^{a}
	$\pm$ 0.0	$\pm 46\times10^{-5}$	$\pm$ 4.43	$\pm$ 3.3	$\pm$ 3.86
2	2.75×10^{-5a}	89.7×10^{-5a}	49.679^{a}	45.67^{a}	39.0^{a}
	$\pm 0.21\times10^{-5}$	$\pm 9.05\times10^{-5}$	$\pm$ 9.29	$\pm$ 6.11	$\pm$ 0.0
3	3.1×10^{-5a}	96.1×10^{-5a}	54.25^{a}	51.0^{b}	48.0^{b}
	$\pm$ 0.0	$\pm$ 0.0	$\pm$ 1.71	$\pm$ 2.16	$\pm$ 1.16
4	2.7×10^{-5a}	92.9×10^{-5a}	55.33^{a}	49.67^{a}	46.67^{b}
	$\pm 0.14\times10^{-5}$	$\pm 4.53\times10^{-5}$	$\pm$ 6.51	$\pm$ 4.16	$\pm$ 3.51
5	2.90×10^{-5a}	92.95×10^{-5a}	54.0^{a}	46.35^{a}	41.0^{a}
	$\pm 0.21\times10^{-a}$	$\pm 13.65\times10^{-5}$	$\pm$ 9.54	$\pm$ 14.36	$\pm$ 13.08
6	2.85×10^{-5a}	92.95×10^{-5a}	55.0^{a}	44.0^{a}	41.5^{a}
	$\pm 0.35\times10^{-5}$	$\pm 13.65\times10^{-5}$	$\pm$ 6.68	$\pm 9.5^{a}$	$\pm$ 10.34
7	3.05×10^{-5a}	89.7×10^{-5a}	50.5^{a}	41.75^{a}	36.0^{a}
	$\pm 0.21\times10^{-5}$	$\pm$ 0.00	$\pm$ 5.0	$\pm$ 7.5	$\pm$ 7.64
8	2.85×10^{-5a}	83.25×10^{-5a}	51.60^{a}	39.2^{a}	37.0^{a}
	$\pm 36\times10^{-5}$	$\pm 0.07\times10^{-5}$	$\pm$ 6.5	$\pm$ 6.83	$\pm$ 6.63

Values are mean± SD for 5 rats per diet group (n=5). Means in the same column with similar superscript letters are not significantly different at 5% level ($p<0.05$).

Legend:MDA-Malondiadehyde,ALP-Alkaline phosphotase,ALT-Alanine amino trananferase,AST-Aspartate amino transferase.

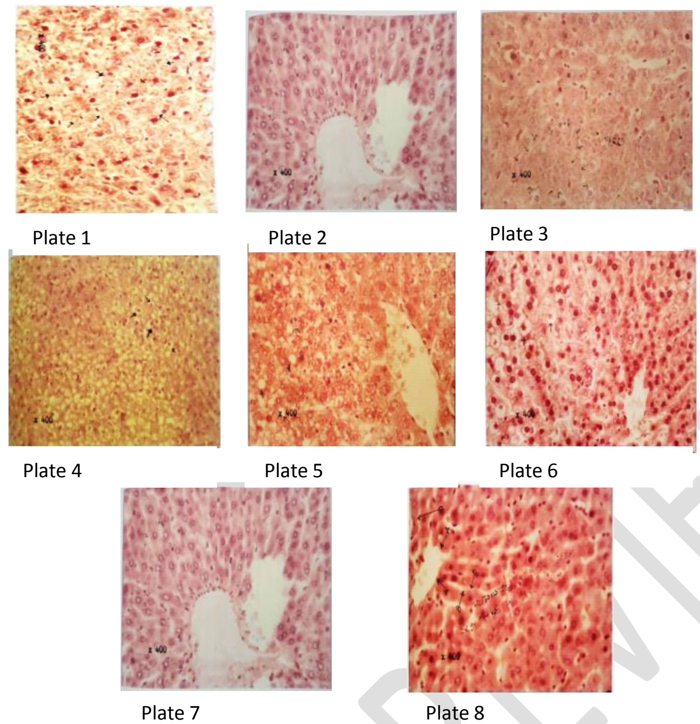

Figure 1: (Plates 1-8) Histological examination of the liver tissue magnification × 400

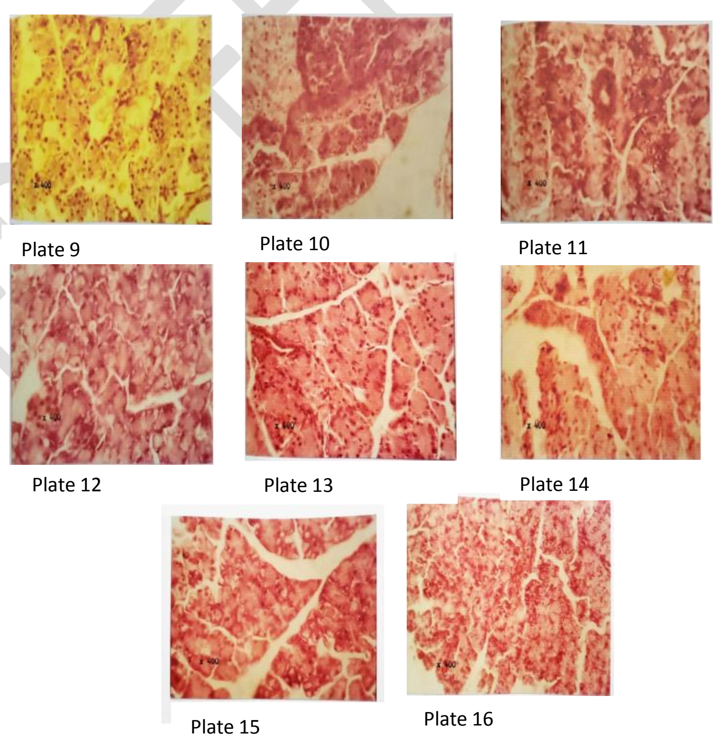

Figure 2: (Plates 9-16) Histological examination of the pancreas tissue magnification × 400

Conclusion

This study has revealed that, African *kudzu* when processed by cooking posed no obvious toxicologically threat to the experimental animals. The legume could there be used for either of human or animal feed.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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