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RESEARCH ARTICLE

Nutriceutical effects of fermented *Parkia biglobosa* seeds on recovery of malnourished rats

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Abstract: *Parkia biglobosa* seeds or African locust beans (ALB) were evaluated in the recovery of malnourished rats. Thirty apparently healthy rats of weight range (120-150g) were experimented after dividing them into five groups, A to E. The animals in Group A which served as control were placed on a balanced diet; group B placed on protein deficient diet by the reducing the daily average diet with no supplements while the animals in groups C, D and E were placed on protein deficient diet by the decrease reducing the average daily required diet supplemented with 10, 20 and 30% ALB respectively. The ALB proximate analysis showed high percentage of total protein and crude lipid. It also consisted of vitamin A, B_1, B_2, B_3, C, D, E and lycopene. Other biochemical parameters analyzed were glucose, total protein, globulin, albumin, triglyceride, total cholesterol, bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine, urea and Na^{2+}, Cl^-, K^+ and HCO_3^- respectively. The results showed that rats in group D and E had significantly higher ($P<0.05$) total protein and globulin values when compared to the non-supplemented (control) B. The animals in group D and E also showed higher percentage weight gains ($P<0.05$) when compared to the group B (control). The phytochemical analysis of fermented ALB showed the presence of alkaloids, cardenolides and saponins. The proximate and nutritional compositions of ALB could be the reason for its nutriceutical effects. Conclusively, the fermented *Parkia biglobosa* seeds when properly processed could serve as a supplement for treating malnutrition.

Keywords: Nutriceutical, *Parkia biglobosa* seeds, malnutrition.

INTRODUCTION

Protein-Energy-Malnutrition (PEM) is a problem confronting most nations in Sub-Saharan Africa (Hassan and Umar, (2005). PEM occurs due to insufficient intake of protein sources such as meat, fish, and poultry products which are beyond the reach of the population due to poverty, geometric increase in population, natural disasters such as flood and desert encroachment (Hassan and Umar, 2005). Growth is a major measurement for the assessment of health and nutrition status of children (Onis *et al.,* (1992). Children with malnutrition related syndromes could subsequently suffer from nutritional deficiencies that can lead to inadequate absorption from the small intestine due to the degenerative changes of absorptive area of villi and microvilli. This could be caused by intrinsic factors lacking in the gastrointestinal tract due to malnutrition (Onis *et al.,* (1992). This also leads to PEM which has a grievous consequences in children which include low immunity, stunted growth, poor cognitive due to reduced brain development and even permanent brain damage.

Plants provide over 60% of the world supply of food for humans and livestock (Pirman *et al.,* 2001). Among leguminous plants exploited in Sub-Tropical African region. (*Parkia biglobosa (Jacq) Benth* (ALB) also play a major role. In Yoruba, the ALB is called "igba" or "irugba", in Hausa it is called "dorowa", while in Igbo it is called "Origili". It is predominantly found in the Savannah and could reach a height of 20m (Ajaiyeoba, (2002).

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P biglobosa is reported to be rich in protein constituents (Cook *et al.,* 2000; Lockeett *et al.*, 2000).

The findings of Bolajoko *et al.,* (2016) showed that the proximate analysis of various fermented forms of *P. biglobosa* consisted of useful constituents that could be exploited in nutrition.Previous studies showed that it has relatively high protein ash content and low carbohydrates making it a veritable plant with neutriceutical and PEM ameliorating potentials. In the same study it was also found out that it has anti-diabetic tendencies and could be used in the management against diabetes insupidus (Bolajoko *et al*., 2016). Hassan and Umar, (2005) found out that the whole seed, dehulled seed and the pulp are rich in crude protein, sulphur amino acid, total aromatic amino acid which include essential amino acids necessary for growth and development.

The aim of this study is to evaluate the ameliorative or neutriceutical effect of *P. biglobosa* (commonly known as African locust beans) on some biochemical parameters which include: blood glucose, total protein, albumin, globulin, K^+, Na^+, creatinine, urea, bilirubin, triglyceride and total cholesterol and liver enzymes namely, AST, ALT and ALP. The wistar rats were used as animal models to simulate malnutritional syndrome and were subjected to lower daily average feed intake for induction of malnutrition and PEM

MATERIALS AND METHODS

Welfare and ethics of animal subjects

Ethical approval was sought from the Ethical Review Committee of the University of Ilorin. The study proposal was also presented at the University of Ibadan, where part of the research was conducted.Thirty apparently healthy weanling, grower Wistar rats, weighing between 120-150g were used in this study. The animals were acclimatized for two weeks and were fed on standard pelletized rat feed and water *ad libitum.* The animals housed in standard cage for rats and were monitored to ensure standard in welfare. The conditions in rat cages were maintained at their optimum levels. Each compartment had six rats.

Experimental design

Weanling rats were randomly separated into five groups with six rats per group. Group A: were fed on standard feed *ad libitum* using standard daily requirement of 58 g daily per rat. This group is considered as a control. The animals in Group B were fed on 29 g per animal daily; Group C; 29 g per per animal daily with 10% supplementation of fermented *P. biglobosa.* The animals of Group D were fed on 29 g daily with 20% supplementation of *P. biglobosa* while *Group* E animals were fed on 29 g daily with 30% supplementation of *P. biglobosa*. The rats were weighed using a digital weighing balance after acclimatization (approximately three weeks after the experiment).

Induction of PEM

Induction of malnutrition was carried out by considering the average-daily feed intake. For animals in test groups B to E, the average 58 g daily feed intake per rat was used as recommended by Council for International organizations of Medical Science; CIOMS (1985). The laboratory animal feed include fat/oil 6%, crude fibre 5%, calcium 1%, available phosphorus 0.4%, lysine 0.85%, methionine 0.35%, salt 0.3%, crude protein 18%, metabolisable energy 2900 Kcal.kg-1, (TOPFEEDS®, Lagos, Nigeria) as adopted by Biobaku and Shamaki, (2010). The test animals were given half the feed intake (29 g per rat)to decrease the average daily calorie intake and to induce PEM. The animals were subjected to different treatments for 21 days. The rats were fed with these diets with no precise time on each day to mimic the situation of PEM among children.

Procurement, processing of ALB and proximate and nutritional analysis

ALB seeds were processed by depulping, dehulling, hydrating before fermenting by methods previously adopted by Ikenebomeh and Kok, (1984) and Esenwah and Ikenebomeh (2008). ALB seeds of 1.5 Kg were soaked in 10 L of distilled water for 24 hours. The pulp was subsequently removed by friction created by rubbing using palms. The pulp of the seeds of *Pakia* was air dried at 25 ^{O}C in a laboratory for 3 days. The dehulling was carried out to free the cotelydon from the testae. The beans were then boiled in water, and rubbed to remove the testa. The seeds were hydrated by boiling for an hour.

This processed seeds are known as dehulled bean seeds by Esenwah and Ikenebomeh (2008) and synonymously termed processed substrates Esenwah and Ikenebomeh (2008).Following the fermentation, the colour of ALB is dark brown with a characteristic cheesy aroma due to the bacterial activity at the fermentation. The proximate and nutritional analysis were conducted using the method of AOAC (Association of Official Analytical Chemists) (2000).

Blood collection and biochemical parameters analysis

The experimental rats were then anaesthetized using diethyl ether of 2% solution Sigma–Aldrich, (Germany), in a jar and blood samples were collected by venous puncture of the median eye canthus. The blood was collected in sterile lithium heparinized sample tubes using a standard procedure as previously adopted by Ola-Davies *et al.* (2017). Plasma and serum was centrifuged at 4000 rpm for 10 minutes. Serum was collected and analyzed for glucose, total protein, albumin, globulin, K^+, Na^+, creatinine, urea, bilirubin, triglyceride and total cholesterol using Randox® (UK) Commercial. The liver enzymes were also assessed (AST, ALT, and ALP) using Randox® (UK) Commercial test kits and standard spectrophotometric method.

Phytochemical analysis of **ALB**

The ALB was properly pulverized and dissolved to prepare a test sample then treated with dilute hydrochloric acid which was later filtered. The filtrate was then treated with Mayer's reagent (Potassium mercuric iodide). A yellow colour precipitate was observed which confirmed the presence of alkaloids. The filtrate was also treated with Dragendroffs reagent (which is a solution of potassium bismuth iodide). A red colour precipitate was observed further indicating the presence of alkaloids in ALB. The remaining filtrate was also treated with Hager's (which is a saturated picric acid solution) and the test was also positive with an appearance of a yellow coloured precipitate .

A 0.5g of ALB was pulverized and filtered; the filtrate was subsequently treated with glacial acetic acid solution then one drop of ferric chloride solution was added afterwards. A 1 ml concentrated H_2SO_4 was added to the test sample. A brown ring was observed indicating the presence of deoxysugars which is classical of cardenolides (Keller-Killian test). The Kedde test in the study was also positive when assessing presence of cardiac glycosides. The filtrate of ALB was boiled with 10 ml of H_2SO_4 and filtered before cooling. The filtrate was properly shaken with 5 ml of a 10 percent solution of chloroform ;the chloroform layer was then pipette into a test tube then 1 ml of dilute ammonia was added.No change in colour indicating the absence of anthraquinones. Test for saponins was conducted by shaking a sample in a test tube. The froth created was mixed with three drops of olive oil. The mixture was then homogenised well to observe an emulsion indicating the presence of saponins in ALB. The test for the presence of tannins was also conducted using acetic acid and a 1 % solution of ferric chloride reagent. There were no colour changes indicating that ALB was negative for tannins.

Analysis was conducted at Department of Chemistry, Laboratory, University of Ibadan using the methods given in Trease and Evans (1983).

Assessment of weights of Experimental animal

Digital top loading balance was used to weigh each rat prior to the experiment, after induction of PEM and after the treatments introduced.

Statistical analysis

The data generated were analyzed using SPSS, (2006). Analysis of variance was used to compare means of various treatments group, while Duncan's test was used as the mean separation method and the level of significance considered was ($P<0.05$).

RESULTS AND DISCUSSION

The proximate analysis of fermented seeds of *P.biglobosa* showed percentages of constituents as thus: 30.49±0.20 of total protein, crude lipid of 0.91±0.02 ,carbohydrates of 24.21±4.03, crude fibre composition 5.94±0.01, while the calorific value composition was 947.65 ±30.02 KJ/100g. The ash and moisture components of ALB were 4.55±0.02 and 33.90±0.05, respectively as given in Table 1.The ALB was also found to be nutritionally endowed with an array of vitamins. The table 2 showed the nutritional compositions of vitamins A, B_1, B_2, B_3, C, D and lycopene. The findings showed higher protein levels which was in agreement with previous studies of Aiyelaagbe, (1996a), in their previous study

however observed high glutamic acid content. Glutamic acid could be the part of amino acids which could be among the factors responsible for the recovery from malnutrition. Table 3 showed there was an insignificant increases in the levels of total cholesterol in animals from groups C, D and E. The increased total cholesterol levels in rats could possibly be due to the improvement of caloric intake and lesser extent of lipolysis that accompanies glycogenolysis at starvation and malnutrition. The ALB crude fats might have also supplemented the body reserves. The results of crude fats in fermented ALB was in agreement with the findings of Aiyelaagbe (1996b),which also reaffirms the presence of arachidic acid in fermented ALB. This plays a major part of the nervous system and may prevent neuronal degenerative changes due to the PEM. The other fatty acids could maintain the membrane integrity of cellular components and enhance energy recovery of malnourished rats. This coupled with the vitamins that act can as co-enzymes, improve metabolic intermediary pathways, physiologic functions, immunity and neuroendocrine functions.Vitamin C and lycopene were also present in ALB which would added nutritional value to its ameliorative effect. These nutritional components possess dietary antioxidant property that could aid in the mitigation of malnutritional stress. This could be the explanation for biochemical parameters and weights of grower Wistar rats that were subjected to induce PEM. When the average daily meal requirement decreased, it induced the PEM in test groups. The decreased average daily required quatity was supplemented with10%, 20% and 30% ALB. The spplementations of higher percentages have helped the animals to ameliorate the PEM during the course of the experiment.Table 3 showed ameliorative effect of ALB supplementation on some biochemical parameters in rats those subjected to induced malnutrition for three weeks. There was a significant increase ($P< 0.05$) in total plasma protein in D and E test groups in which the rats were supplemented with 20% and 30% ALB compared to group B with no added supplementation. However, group C supplemented with 10% of ALB was not significantly improved. This observation can be explained pharmacologically that the ameliorative therapeutic effect of ALB is efficacious only at higher percentages such as 20% and 30%. The other biochemical parameters were not significantly influenced by the supplementation of ALB. This study suggests that leguminous seeds of ALB improved the protein balance in rats. Hassan and Umar, (2005) observed number of essential amino acids in *P. biglobosa* bean seeds which include glycine phenlyalanine, serine, threonine, and tyrosine. All these amino acids may have ameliorated the PEM in the test groups given with higher percentages of ALB.This may have also improved the metabolism in animals induced with PEM. The outcomes were reflected on the significant weight increases shown by test animals ($P<0.05$) in groups D and E (Table 4).The amino acids present in ALB have improved the nutritional value of the diet given to test animals. Other parameters showed no significant influence on the liver enzymes (ALT, AST, ALP) and renal function tests (Table 3). This study also showed that ALB is not nephrotoxic and hepatotoxic. ALB also may contained cytoprotective property on the hepatocytes and other parenchyma when predisposed to stress due to malnutrition. This finding was in line with the previous findings of Patrick-Inwanyau *et al.*,(2010), where they found the hepatoprotective property of *Pakia clappertoniania* and *Xylopia aethiopica* in rats. In this study, *Pakia* was combined with *Xylopia aethiopica* and this combination has elicited its effect by protecting the hepatocytes and prevented an increase in the levels of liver enzymes in the serum due to the toxic effect of CCl_4. In both studies, the animals were subjected to stress and *Pakia* supplementations played a role in the maintenance of the cell integrity of parenchymal cells. Another study by Tolulope *et al.*,(2013) also had a similar trend with the biochemical parameters when methanolic extracts of leaf of *Pakia biglobosa* was administered, showing that various parts of this plant are most likely possess antioxidants constituents.This study also gave credence to the nutriceutical potentials of ALB. The incorporation of ALB in the usual feedis considered standard in conformity with the values of FAO/WHO/UNU, (1991) given in previous studies. The phytochemical constituents of ALB given in table 5 shows the presence of cardenolides, alkaloids and saponins and also the absence of anthraquinones and tannins. One or more of these phythochemical constituents may have enhanced metabolic activities of the experimental animals. The antioxidants in ALB could also play a role in improving the health

and wellbeing of the experimental rats. The active compounds including cardenolides and saponins are speculated to increase gastrointestinal functions and assessory glands of such as exocrine portion of the pancreas. This can improve appetite loss that can be resulted due to adverse effects of malnutrition. Therefore, the restoration of appetite may also be considered as an important imapct towards the recovery from PEM-induced experimental rats.

Table 1: Proximate composition of ALB Mean± SD.

Parameters	**Value In**	**Composition**
Total protein (%)		30.5±0.20
Crude lipid (%)		0.91±0.02
Moisture (%)		33.9 ±0.05
Ash (%)		4.55±0.02
Crude fibre (%)		5.94±0.01
Carbohydrates (%)		24.2 ± 4.03
Calorific Value (KJ/100g)		947.6±30.2

Table 2 : Nutritional composition of ALB Mean± SD.

Parameters	**Value In Composition**
Vitamin A (µg/100g)	54.72±1.07
Vitamin B_1 (mg/100g)	0.205±0.00
Vitamin B_2 (µg/100g)	7.70±0.07
Vitamin B_3 (µg/100g)	13.26±0.33
Vitamin C (mg/100g)	1.66±0.02
Vitamin E (µg/100g)	0.38±0.01
Lycopene (µg/100g)	0.38±0.01

Table 3: Ameliorative effect of *P. biglobosa* supplementation on some Biochemical parameters in rats induced malnutrition for three weeks.

Parameters	**A**	**B**	**C**	**D**	**E**
Glucose (mg/dl)	107.14±8.64	95.84±8.00	87.86±4.13	61.70±32.34	82.93±5.00
Total Protein (g/l)	$56.54±1.74^{ab}$	$49.20±1.91^{b}$	$54.20±4.11^{ab}$	$60.00±0.70^{a}$	$52.48±3.10^{ab}$
Albumin (g/l)	$30.84±0.61^{ab}$	$30.00±1.02^{ab}$	$29.91±0.81^{ab}$	$32.07±0.82^{a}$	$29.90±0.80^{ab}$
Globulin (g/l)	$25.70±1.49^{ab}$	$18.40±1.00^{c}$	$24.24±3.22^{ab}$	$27.97±0.51^{a}$	$22.58±2.50^{ab}$
K^+(M/mol)	4.64±0.22	3.50±0.32	3.88±0.30	3.37±0.15	3.60±0.00
Na^+(M/mol)	140.60±1.32	139.29±2.29	139.33±2.45	137.22±1.77	139.5±4.07
Creatinine (mg/dl)	0.88±0.04	0.98±0.05	0.98±0.03	0.79±0.06	0.85±0.02
Bilirubin (mg/dl)	0.67±0.40	0.75±0.03	0.76±0.04	0.79±0.06	0.82±0.02
Triglyceride (mg/dl)	105.56±7.00	95.85±3.46	89.12±9.00	90.97±5.10	86.23±8.84
Total Cholesterol (mg/dl)	80.24±7.02	92.82±4.28	103.92±8.60	104.70±8.28	99.22±7.80
AST(IU/l)	198.80±19.20	206.00±8.30	165.80±12.82	180±6.00	190.00±12.94
ALT (IU/l)	56.00±8.32	48.00±6.00	47.65±12.91	47.75±8.00	50.71±9.10
ALP (IU/l)	170.40±15.20	170.00±21.10	176.40±5.80	170.67±16.00	175.53±1.30

Means abc with different superscript are considered significant ($P<0.05$)

Table 4: Effect of ALB supplementation on weight increase of Wistar rats.

Parameters	A	B	C	D	E
Weight (g) prior supplementation	150.35±6.3	120.34±7.3	126.33±8.0	130.34±3.3	123.78±5.2
Weight (g) after supplementation	198.32±3.2[a]	127.85±6.2[b]	130.87±7.3[b]	190.52±3.3[a]	191.54±7.5[a]
Percentage (%) weight increase	31.90[c]	6.24[d]	3.61[e]	46.17[b]	54.74[a]

Significant means with different superscript are considered significant at $P<0.05$.

Table 5: Photochemical analysis of ALB.

Tests conducted	Test	Test	Test
Alkaloid tests	Dragendroff's	Meyer's	Hager's
	+ve	**+ve**	**+ve**
Cardenolides	Keller Killani	Kedde	
	+ve	**-ve**	
Anthraquinones	Chloroform/Ammonia	$FeCl_3$	
	-ve		
Saponins	Frothing		
	+ve		
Tannins	Ferric chloride		
	-ve		

+ve indicates present; while –ve is considered absent.

In conclusion, P. *biglobosa* beans (ALB) when added to the feed had a nutritive value and could be used as a supplement to enhance recovery from malnutrition. It also improved growth during adversity of malnutrition. There were also some few antinutritional factors that would warrant proper processing before consumption to denature them to avoid any interaction or competition with valuable nutrients.

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