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EFFECTS OF PROCESSED VIGNA SUBTERRANEA (BAMBARA GROUNDNUTS) SEEDS ON HEPATIC SERUM ENZYMES IN ALBINO WISTAR RAT

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ABSTRACT

Seventy Wistar albino rats were used in a 28 days feed trial to determine the impact of different processed seeds of *Vigna subterranea* on the levels of serum enzymes. There were seven (7) experimental groups each made up of ten rats, allocated to the experimental diets. In this study it was observed that rats fed with the different processed samples of *Vigna subterranea* significantly increased in weights of the rats at $P<0.05$. Haematological tests were also conducted and subsequent data revealed an increase ($P<0.05$) in the Packed cell volume (PCV), haemoglobin, Mean Corpuscular haemoglobin concentration (MCHC), and total red blood cell of the rats fed with different processed seeds of *Vigna subterranea*. This was accompanied with a significant increase in the total white blood cell of the rats fed with roasted and raw samples of *Vigna subterranea* as against other experimental groups when compared with the control group. Serum transaminases; aspartate transaminase and alanine transaminases (AST and ALT) and serum alkaline phosphatase (ALP) showed significant ($p<0.05$) increase in activity in the serum of rats fed with roasted and fermented samples of *Vigna subterranea* as against other experimental groups when compared with the control group.

KEYWORD: *Body weight indices, Hepatic serum enzymes and Heamatological indices.*

INTRODUCTION

Food legumes have a major role to play in the fight against malnutrition, it is therefore necessary that their levels of consumption, which are already too low in a number of

developing countries, should be increased (Borget, 1992). Legumes serve as a source of protein to a large proportion of the population in the poor countries of the world by being the least expensive, easily stored and transported non-processed protein source for rural and urban dwellers (Rachie and Silvester, 1977). The high carbohydrate (65%) and relatively high protein 18% content of Bambara groundnut make it a complete food (Doku, 1995). Bambara groundnut is probably the most drought–resistant of the grain legumes and may be found growing successfully where annual rainfall is below 500 mm and optimum between 900–1000 mm per year (Ocran *et al.*, 1998). The plant can be grown under dry climatic conditions where the rainfall during the rainy season would be adequate to enable them to accomplish their vegetative cycle (Borget, 1992). An evenly distributed rainfall in the range 600–1000 mm encourages optimum growth but satisfactory yields can be obtained in areas with a pronounced dry season since the crop is relatively drought resistant (Messiaen, 1992) Bambara groundnut is resistant to high temperatures and can be grown on poor marginal soils not suitable for other leguminous crops (Yamaguchi, 1998). Bambara groundnut is not attacked by disease and pests in any of its production regions. However, in damp conditions, it may be susceptible to various fungal diseases (Baudoin and Mergeai, 2001). It has a very low insect pest and disease susceptibility (Tweneboah, 2000).

In West Africa bambara groundnut (*Vigna subterranea)* was for a long time at par with, or slightly ahead of cowpea (*Vigna unguiculata)* in terms of production (market availability) and utilization. In Ghana, over 40,000 cans (various sizes) of Bambara groundnut were produced annually throughout the 1960's and early 1970's.The canned product was very popular throughout West Africa and competed favorably with Heinz baked beans. The status of the nut however, started to decline from 1970's with introduction of high yielding varieties of groundnut (*Arachis hypogaea)* and pest control methods for cowpea (Doku, 1996).The protein of Bambara groundnut is of good quality and has surplus lysine which complements cereals in the diet (Ocran *et al.*, 1998). The composition of the seeds, from the point of view for human nutrition is very well balanced, as they contain 20% soluble carbohydrates and 8% fats (Messiaen, 1992). It is high in protein but unlike ordinary groundnuts contains very little oil (Tweneboah, 2000). Bambara groundnut has been ranked as the third most important grain legume, after groundnut *(Arachis hypogaea L.)* and cowpea *(Vigna unguiculata)* in semi-arid Africa, but has not been accorded due attention in research (Rachie and Silvester, 1977). Little research has been done to date to improve Bambara groundnut. The work done on the crop has been limited to mass selection of a few local varieties, followed by a

purification phase for the main agronomic characteristics. International Institute of Tropical Agriculture (IITA) has recently evaluated a large collection of Bambara groundnut comprising more than 1000 introductions collected from all over Africa (Baudoin and Mergeai, 2001).

Bambara groundnut is eaten in several ways and at different stages of maturation. The young fresh seeds may be boiled and eaten as a snack in a manner similar to boiled peanuts and could be made into pudding locally called Moi Moi or Okpa (bean porridge) in the some parts of Nigeria. It has been reported that in Zambia, Bambara groundnut is used for bread making (Brough *et al.*, 1993) while (Poulter and Caygill, 2006) also reported that it could be used formilk making. (Wazael, 2004) described the great genetic diversity potential of Bambara groundnut. Although different studies have reported the nutritional values of Bambara groundnut, there is inadequate research report on the different processing methods of Bambara groundnut in rats. In view of this, this investigation will determine the nutritional values of different processing methods of Bambara groundnut. It is our hope that the outcome of this investigation will create awareness on the nutritional buoyancy of Bambara groundnut in animal model. Bambara groundnut is a legume crop native to Africa commonly grown for its seeds by subsistence farmers. It is grown in many parts of Africa, in parts of Asia, especially Indonesia, and South America (Linnemann and Azam-Ali, 1993; Basu *et al*., 2007). Major producers of Bambara groundnut are Nigeria, Niger, Ghana and Cote d'Ivoire; but it is widely grown in Eastern Africa and Madagascar. The crop was taken to Asia, particularly India, Indonesia, Malaysia, Philippines, Thailand and Sri Lanka. It is also found in south and Central America as well as in northern Australia (Linnemann and Azam-Ali, 1993).

Bambara groundnut (*Vigna subterranea* is considered an underutilized plant species as it has been inadequately characterized and, until recently, neglected by research and conservation. Among the vast repository of underutilized crops, Bambara groundnut has enough potential to warrant various sorts of investment towards its improvement. It has outstanding traits such as drought tolerance, nitrogen fixation, commendable nutritional composition and an ability to produce yields in marginal soils among others.

MATERIALS AND METHODS

Experimental animals: Seventy female albino rats weighing between 146-189g were purchased from the animal house of Ladoke Akintola University of Technology, Osogbo,

Nigeria and used for the study. The rats were randomly assigned on the basis of their body weight into six (7) study groups of ten (10) rats each. Normal feeds and tap water were given to the rats *ad-libitum* and food and water intake were noted. They were kept in secure wooden cages of 10 rats per cage placed in a well-ventilated animal room of Joseph Ayo Babalola University at normal temperature of 30-35°C. The cages were cleaned daily and the rats were treated according to the international guidelines for the care and use of laboratory animals (NIH, 2008). The animals were allowed for two weeks of acclimatization and their weights were measured before treated commenced.

Chemicals and equipments: Chloroform and other chemicals were obtained from Fam-lab Nigeria Limited. Alanine transaminase (ALT), aspartate transaminase (AST), and Alkaline phosphatase (ALP) were obtained from Randox Laboratory Limited, UK. Distilled water was also used during the experiment. **A**pparatus; mettler balance, evaporating dish, desiccator, crucible, soxhlet extractor apparatus, distilled water, absolute ethanol, muffle furnace, micro kjedhal digestion flask, beakers, conical flasks, burette and pipette.

Materials and identification: *Vigna subterranean* seeds were obtained from Akure Central Market (Oja-Oba) Akure Ondo State. The samples were taken to the Department of Botany, Obafemi Awolowo University (OAU). The research project was carried out in the Department of Chemical Sciences (Biochemistry Unit), Joseph Ayo Babalola University, Ikeji-Arakeji, Osun State Nigeria, from April-May, 2016.

Processing methods: *Vigna subterranea* seeds (6 kg) were cleaned by sorting to remove extraneous materials and were weighed and shared into six lots desired. Processing method employed in this study includes toasting, boiling, soaking, fermentation with decantation and fermentation without decantation. Boiling: 1kg of *Vigna subterranea seeds* was suspended into a cooking pot containing 8 litres of water at 100^0C set over fire. This was boiled for 1hour until it was well cooked. The water was sieved out and the nuts were sun dried for 3-4days before milling. Soaking: 1kg of *Vigna subterranea seeds* were soaked by pouring 1kg of the seed into 8 litres of water for 12hours and the water was sieved out and the nuts were sun dried for 3-4days before milling into powdery form. Without decantation: 1kg of *Vigna subterranea* seeds was put into 8litres of water in an air-tightened container and soaked for 12hrs. The fermentation process started immediately after 12hrs without sieving out the water. The water was sieved out after 48hrs and was allowed to dry under the sun for 3-4days before milling into powdery form. Fermentation with decantation: 1kg of *Vigna subterranea*

seeds was put into 8litres of water in an air-tightened container and soaked for 12hrs as similar to the soaking process. The water was sieved out and another 8litres of water was poured into it and it was well covered and the fermentation process started immediately after sieving. The water was then sieved out after 48hrs and sun-dried for 3-4days before milling into powdery form. Toasting: 1kg of *Vigna subterranea* seeds was put into a frying pan containing fine sand set over fire. The seed was stirred to avoid excessive burning. The seeds were stirred on fire continuously until it turned brownish. Excessive burning was avoided so as not to completely destroy the essential amino acids. The toasted nut was then milled. Raw Bambara groundnut: 1kg of *Vigna subterranea* seeds was rinsed with clean water and dried under the sun. It was then milled and sieved so as to remove the seed coat.

Proximate analysis of processed *Vigna subterranea*

Moisture Content Determination: This was done by the gravimetric method according to AOAC (1990). The weight of the moisture was calculated and expressed as a percentage of weight of the sample analyzed. This was given by the expression below:

$$\% \text{ moisture content} = \frac{W2-W3}{W2-W1} \times \frac{100}{1}$$

Where; W1= weight of empty evaporating dish

W2= weight of sample +evaporating dish

W3= weight of sample + evaporating dish after drying at 105°C

Ash content determination

This was done by the gravimetric method according to AOAC (1990). Weight of a previously washed and dried empty crucible was determined using a mettle balance as (W1). 5g of the sample was weighed into the crucible (W2). The crucible and sample were then placed in muffle furnace set at 550°C and was ashed for 4 hours. After ashing, the crucible and sample was then placed in the desiccator to cool to room temperatures after which it was then weighed (W3). The percentage ash content was then calculated thus:

$$\% \text{ Ash} = \frac{W3-W1}{W2-W1} \times \frac{100}{1}$$

Where; W 1 =weight of empty crucible

W2=weight of sample +crucible

W3=weight of sample + crucible after ashing at 35°C

Fat content determination

The crude fat content was then calculated thus

$$\% \text{ Fat} = \frac{W2-W3}{W2-W1} \times \frac{100}{1}$$

Where; W1= weight of empty extraction thimble

W2= weight of sample + extraction thimble

W3 = dried weight of defatted sample + extraction thimble

Crude fibre determination

This analysis was done using the AOAC (1990) method.. The loss of weight on incineration was mass of crude fibre expressed thus

$$\% \text{ Crude fibre;} \quad \frac{W2-W3}{W1} \times \frac{100}{1}$$

Where; W1=weight of defatted sample

W2= weight of sample at 105°C

W3= weight of sample at 550°C

Crude protein determination

The protein content of the sample was determined by the micro-kjedahl method.

$$V2 \times W$$

Where; N.F =Nitrogen factor (0.014)

M= Morality of HCl (0.014)

V1=Final volume of digest (50ml)

V2=Volume of digest used (10ml)

T = Titre volume of distillate

W=Weight of sample used

PF=Protein multiplication factor (6.25)

Carbohydrate Determination

The total carbohydrate content of each sample was estimated by "difference''. The sum of the percentage concentrations of each parameter of the other proximate compositions were subtracted from 100, i.e.,

Total carbohydrate =100-(% moisture + % ash +%fat +%protein +%crude fibre)

PHYTOCHEMICAL SCREENING OF PROCESSED BAMBARA NUT: Qualitative analysis was carried on each of the test samples using diverse methods. The phytochemicals: flavonoids, tannins, saponins, phenolics, phytate and oxalate were tested for, using the methods of Trease and Evans, (1996); as modify by Harbone (1996) and Sofowora (1993).

Haematological analysis

Packed Cell Volume: was determined using microhaematocrit method (Baker and Silverton, 2003). Haemoglobin Estimation::was determined using Cyanmethaemoglobin method (Baker and Silverton, 2003). Total white blood count: was measured using the Bulk dilution method (Ochei and Kolhatkar, 2007) Mean corpuscular haemoglobin concentration: It was estimated by dividing the hemoglobin by the hematocrit. Reference ranges for blood tests are 32 to 36 g/dL, or between 19.9 and 22.3 mmol/L. It is thus a mass or molar concentration.

Determination of l-alanine aminotransferase (ec.2.6.1.2) activity: L-alanine aminotransferase activity (ALT) was estimated by the method of Reitman and Frankel (1957).

Determination of l-aspartate aminotransferase (ec.2.6.1.2 activity: L-spartate aminotransferase (AST) activity was estimated using the method of Reitman and Frankel (1957).

Determination of alkaline phosphatase activity: Alkaline phosphatase activity was assayed according to the method described by Bassey *et al*., (1946) and modified by Wright and Plummer (1974).

Statistical analysis: The mean of ten data samples in each group were expressed as Mean Value + S.E.M (Standard error of mean) and subsequently analyzed using student's single t-test. Thereafter the values were considered to be statistically significant at probability level of $p<0.05$.

RESULTS

Table 1.0: Proximate composition (g/100gDM) of the different processed *Vigna subterranean* seed meal (Bambara Nut).

Analysis (%)	TBG	BBG	SBG	FBG (wo)	FBG (w)	RBG Raw
Crude protein	22.64	22.73	22.72	22.73	28.59	22.58
Dry matter	90.74	92.52	93.20	92.45	91.59	93.45
Crude fibre	8.71	10.17	9.84	9 .61	8.79	8.13
Ash	2.44	2.18	2.46	2.19	2.40	2.24
Ether extract	7.65	7.82	7.79	7.85	7.59	7.9

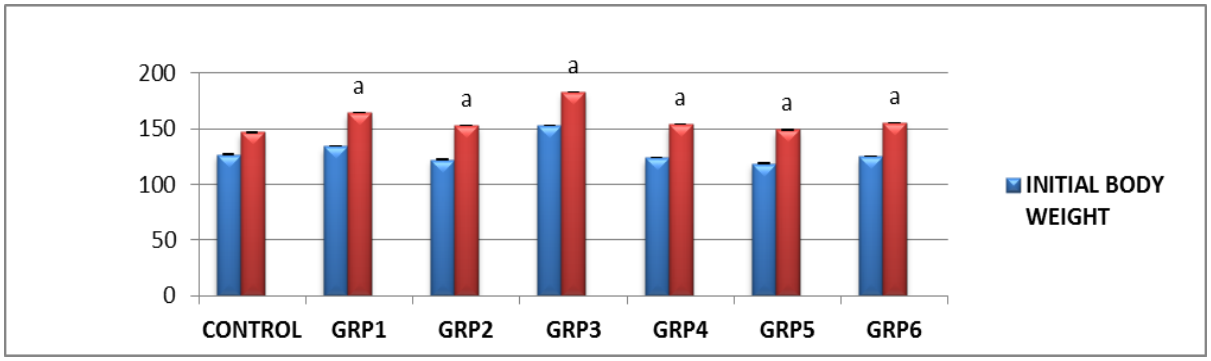
BBG= Boiled Bambara groundnut, SBG= Soaked Bambara groundnut, FBG (wo) = Fermentation without decantation, FBG(w)= Fermentation with decantation, RBG= Roasted Bambara Groundnut, NFE= Nitrogen Free Energy.

Table 2.0: Phytochemical Screening of processed *Vigna subterranea* seeds.

	Processed *Vigna subterranea* seeds (Bambara nuts)						
S/ No	**Phytochemicals**	**Toasted**	**Soaked**	**Raw**	**Fermentation with decantation**	**Fermentation without decantation**	**Boiled**
1	Alkaloids	-	+	-	+	+	_
2	Tannins	+	+	+	+	+	+
3	Antraquinone	-	-	-	-	-	+
4	Saponins	-	-	+	+	+	+
5	Flavonoids	+	-	-	-	-	-
6	Cardiac glycosides	_	_	+	_	_	_
7	Phenolics	_	+	_	_	_	+
8	Steriods	_	_	_	+	_	+
9	Triterpenes	+	_	_	_	_	_
10	Cardenolides and dienolides	+	+	+	+	+	+

+ = PRESENT

- = ABSENT



Values are expressed in MEAN + S.E.M of 10 determinations.

Figure 1.0: Effect of processed *Vigna subterranea* seeds on body weight indices in Albino Wistar rats.

Control: Rats fed with normal feed *ad-libitum.*

GRP1: Rats fed with toasted *Vigna subterranea* seeds *ad-libitum.*

GRP2: Rats fed with soaked *Vigna subterranea* seeds *ad-libitum.*

GRP3: Rats fed with fermented (without decantation) *Vigna subterranea* seeds *ad-libitum.*

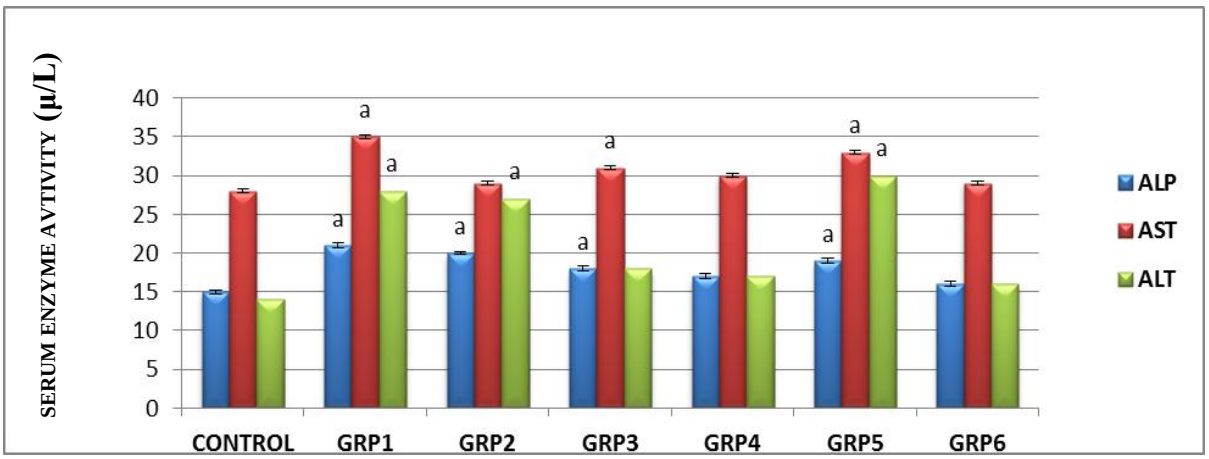
GRP4: Rats fed with fermented (with decantation) *Vigna subterranea* seeds *ad-libitum.*

GRP5: Rats fed with boiled *Vigna subterranea* seeds *ad-libitum.*

GRP6: Rats fed with raw *Vigna subterranea* seeds *ad-libitum.*

a= significant increase at (p<0.05)

b= significant increase at (p<0.05)



Values are expressed in MEAN + S.E.M of 10 determinations.

Figure 2.0: Effect of processed *Vigna subterranea* seeds on serum enzyme in Albino Wistar rats.

Control: Rats fed with normal feed *ad-libitum.*

GRP1: Rats fed with toasted *Vigna subterranean* seeds *ad-libitum.*

GRP2: Rats fed with soaked *Vigna subterranean* seeds *ad-libitum.*

GRP3: Rats fed with fermented (without decantation) *Vigna subterranean* seeds *ad-libitum.*

GRP4: Rats fed with fermented (with decantation) *Vigna subterranean* seeds *ad-libitum.*

GRP5: Rats fed with boiled *Vigna subterranean* seeds *ad-libitum.*

GRP6: Rats fed with raw *Vigna subterranean* seeds *ad-libitum.*

a= significant increase at ($p<0.05$)

b= significant increase at ($p<0.05$)

DISCUSSION

This present study was undertaken to evaluate the effect of different processing methods of *Vigna subterranea* on the serum enzymes profile in Wistar albino rats fed with different processed seeds of *Vigna subterranea.* Although the rats were fed *ad-libitum*, our findings revealed that the rats increased significantly ($p<0.05$) in weight compared to the control group. Plant proteins provide nearly 65% of the world supply of proteins for humans from 45-50% cereals and 10-15% legumes (Mahe *et al.*, 1994), with legumes being a major source of proteins in tropical countries. *Vigna subterranea* is one of the leguminous crops that have been described as a complete food with sufficient amounts of nutrients. The crop is a major source of proteins, minerals and vitamins; proteins (16-25%), carbohydrates (42-60%), and fat (5-6%) and this may explain the significant increase in weight of the rats (Poulter and Caygill 1980; Linnemann 1987 and Arora, 1995).

Table 1 shows the proximate composition of toasted, fermented (with and without decantation), boiled, soaked and raw seeds of *Vigna subterranea.* The values of crude fibre significantly increased in fermented samples; with decantation and without decantation, and in the soaked sample with values 7.7342 + 0.3904, 7.711 + 0.3891, and 7.9821 + 0.4021 respectively. The crude protein content was highest in the fermented sample (with decantation) while carbohydrate content was increased in all the samples with toasted sample having the highest value with 32.679 + 1.650.

Table 2 shows the phytochemical composition of toasted, fermented (with and without decantation), boiled, soaked and raw seeds of *Vigna subterranea.* Phytochemical analysis is very useful in the evaluation of some biological components of medicinal plants. Of all the phytochemicals detected, steroids, tannins, and saponins have been implicated in therapeutic relevance. Steriods are substances that are naturally produced in the living organism. They help control many different functions such as in the way in which fats, protein, and carbohydrates are metabolized. They also help to reduce inflammation, regulate immune

system and the balance of salt and water in the cell. They can also be used to help reduce an allergic reaction to certain chemotherapeutic drugs, in low doses as an anti-sickening drug, or to improve appetite (Trease and Evans, 1989). Tannin is a class of polyphenolic compounds that exhibit antimicrobial action by precipitating microbial protein (Scalbert, 1991) while saponins is a class of a special class of glycosides that inhibit the growth of *S. aureus* Steroids was detected in the fermented (with decantation) and boiled sample while saponins was detected in the fermented sample (with decantation), raw and boiled samples and tannin was detected in all processed samples of *Vigna subterranea.* The therapeutic effect of these phytochemicals detected in *Vigna subterranea* may account for the increase in weights of the rats.

Figure 2.0 shows the specific activities of some enzymes in the serum of rats fed on the different experimental diets. Transaminases are the most commonly used indicators of cellular necrosis and high levels in serum may indicate liver malfunctioning (Rosenthal, 1977). They occupy a central position in amino acid metabolism; increase in their activities in the serum as herein observed could have a consequential effect on the amino acid metabolism in these tissues. Furthermore, it may indicate some sort of injury to the organs. Such damage may cause the enzymes to leak from the injured organs to the blood stream (Concepcion *et al*., 1993).In this study, serum enzymes activity increased in all the experimental groups. Alkaline phosphatase activity significantly increased ($p<0.05$) in all the experimental groups when compared to the control group but peaks were observed in toasted and fermented (with and without decantation) sample-fed rats respectively Aspartate transaminase is associated with the mitochondria and cytoplasm and alteration in its activity could imply alteration in the cytosolic content. The mitochondrion is regarded as the power house of the cell and exposure of this organelle to assault of any form could imply cell death. Similar trends were observed for AST activities at $p<0.05$ with peaks in toasted, fermented (with decantation) and soaked samples respectively while ALT activities were significantly increased ($P<0.05$) in toasted, fermented (without decantation) and soaked samples respectively. These observations could be as a result of the negative effect of anti-nutrients. Similar report had been made earlier by Alletor and Fetuga (1985) on the activities of these enzymes in blood of rats injected with legume anti-nutrients.

CONCLUSION

From the result obtained, it can be seen that processed Bambara groundnut by toasting, fermenting boiling, and soaking before its inclusion into rat diets was able to improve the haematological parameters in the experimental rats. It could also be observed that some anti-nutrients are present in Bambara groundnut which cannot be totally removed by processing techniques like toasting could subsequently inflate the activity of serum enzymes.

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