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Effect of African Yam Bean (*Sphenostylis stenocarpa*) on Serum Calcium, Inorganic Phosphate, Uric Acid, and Alkaline Phosphatase Concentration of Male Albino Rats

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Abstract

The effect of African yam bean (*Sphenostylis stenocarpa*) on serum calcium, inorganic phosphate, alkaline phosphatase and uric acid concentration was investigated. Eighteen male Wister albino rats were used for the experiment. The rats were divided into three groups of six rats each viz: the Baseline, Test and Control. The test group was fed with a diet prepared with 16% African yam bean, 50% maize flour, 23% groundnut cake and 10% fishmeal. The Control group received a diet without Africa yam bean but containing other components. The Baseline group was sacrificed at the onset of the study to ascertain the initial conditions. The study lasted for twenty eight days after which the serum calcium, inorganic phosphate, alkaline phosphatase and uric acid levels were estimated. The inorganic phosphate and alkaline phosphatase of the test group showed a significant ($p < 0.05$) increase with the values; 25.154 ± 4.329 and 506.00 ± 51.594 respectively compared to those of the Baseline and Control groups. Also there were significant ($p < 0.05$) reductions of the serum concentration of calcium and uric acid of the test group compared to those of the Baseline and Control groups. There were no significant ($p > 0.05$) differences in the serum levels of calcium, alkaline phosphatase, inorganic phosphate and uric acid of the Baseline and Control groups. These observed effects of African yam bean has gone a long way to provide an insight into the pharmacological potentials of this legume especially in the management of gout and arthritis in addition to the already known nutritional properties.

Keywords: yam bean, Wister albino rats, serum calcium, inorganic phosphate, uric acid, alkaline phosphatase

1. Introduction

Plant foods have remained the ultimate source of nutrients for a larger population of the world. They are irreplaceable food sources for humans. Plant foods enjoy a comparative advantage over other food sources in that they are available, affordable and acceptable. In Nigeria, most of these plant food sources have been neglected and underutilized. The African yam bean is one of such neglected and under utilized foods. The African yam bean is a tropical leguminous plant cultivated mainly in West African countries (Porter, 1992). It is mainly distributed in the southern part of Nigeria. The AYB is cultivated both for the seeds and tubers. The tuber which is found beneath the ground has resemblance with sweet potatoes or Irish potatoes. Above the ground, it produces a good yield of edible seeds. It could be found in forests, open wooded grasslands, rocky fields, and marshy grounds as weed and cultivated crops. It grows on wide range of soils including acid and highly leached sandy soils at altitudes from sea level to 1950m (Amoatey et al., 2000). The African yam bean like other tuberous legumes is a member of the Fabaceae family. It is cultivated mainly for home consumption and only about 30% of the dry grain produced is sold (Osuagwu & Nwofia, 2014). The crop is highly underexploited due to the fact that little is known about it. Another reason for its underutilization is due to its relatively low farm yield and long cooking time which is about 140 minutes (Nwokolo, 1987).

However, these factors are insignificant when compared to the nutritional composition of the crop. According to Fasoyiro et al. (2006), African yam bean is a good source of protein, fibre and carbohydrate. It is also rich in minerals such as phosphorous, Iron, potassium and contains some anti-nutrients such as trypsin inhibitor, phytate, tannin and other alkaloids. The nutritional analysis of the seed of African yam bean shows that it contains; 350 calories, 19.2 g protein, 1.1 g fat, 67 g carbohydrate, 5.2 g fibre, 55 mg calcium, 398 mg phosphorous, 9 ml of water and 0.6 mg thiamine per 100 g of the edible protein. The protein of AYB is made up of over 32% essential amino acids, with lysine and leucine being predominant (Onyenekwe, 2000).

Because of the anti-nutritional materials found in AYB, a lot of time is invested in cooking and processing it. It is usually cooked and eaten either alone or with yam, maize and rice. It can be used to replace cowpea in food preparation especially during the lean period when food is scarce among rural farmers (Akande, 2009). It is one of the ideal sources of protein supplementation of starchy foods. This will encourage the use of this lesser known and underutilized legume in a number of food preparations especially in the developing countries. Supplementation equally has resulted in products with high nutritional values. In line with the above, the effect of incorporation of AYB on the pasting, proximate and sensory attributes of cassava was studied by Nwokeke et al., 2013). Incorporation of AYB into cassava gives the product referred to as African yam bean fufu flour. The feasibility of using the legume (AYB) and guinea corn to produce a weaning food formulation has also been investigated by (Umerie et al., 2009).

Calcium is a very important mineral in the body. It plays a key role in the bone formation process. Disorders of Calcium metabolism may result in hypocalcaemia or hypercalcaemia. Some causes of hypocalcaemia include Vitamin D deficiency, rickets, osteomalacia, drugs (Furosemide), acute pancreatitis, sepsis and high calcitonin levels. Hypercalcaemia associated with high plasma concentrations of calcium may result from; parathyroid hormone abnormalities, high bone turnover (as seen in thyrotoxicosis and Paget's disease), high level of Vitamin D, drugs (like thiazides), and HIV infection. Clinical effects of hypercalcaemia include renal damage, depression of neuromuscular excitability in both voluntary and involuntary muscles and bone and joint pains (Crook, 2012).

Phosphate is an important intracellular buffer and is also essential for buffering hydrogen ions in urine. In addition, it has structural role as a component of phospholipids, nucleoproteins and nucleic acids. Approximately, 80% of phosphate is bound in the bony skeleton, while the other 20% is distributed in the soft tissues and muscles (Kaplan & Pesce, 2010).

The alkaline phosphatases are a group of enzymes that hydrolyze organic phosphates at high pH. They are present in most tissues, but are in particularly high concentrations in the osteoblasts of bones and the cells of the hepatobilary tract, intestinal wall, renal tubules and placenta (Crook, 2012). The primary importance of measuring alkaline phosphatase is to check the possibility of bone disease or liver disease. Since the mucosal cells that line the bile system of the liver are the source of alkaline phosphatase, the free flow of bile through the liver and down into the biliary tract and gall bladder are responsible for maintaining the proper level of this enzyme in the blood. When the liver, bile ducts or gall bladder system are not functioning properly or blocked, this enzyme is not excreted through the bile and alkaline phosphatase is released into the bloodstream. Thus, the serum alkaline phosphatase is a measure of the hepatobiliary system and the flow of bile into the small intestine (Kaslow, 2015).

Uric acid is an end product of purine metabolism. Crystallization of uric acid in joints, especially those of the feet produce the classic picture of gout. Uric acid precipitation may also occur in subcutaneous tissues. Such deposits are called gouti tophi. Factors that may contribute to high plasma uric acid concentration include: increased synthesis of purines, increased uptake of purines, increased turnover of nucleic acids, increased rate of urate formation and reduced rate of urate excretion. Gout is a chronic disorder of uric acid metabolism. Croft (2005) describes gout as a group of conditions which may be characterized by; an elevated serum uric acid, bone and joint destruction in some cases, aggregates of uric acid crystals (tophi) in and around joints, soft tissues, and various organs, tophus in bone leading to erosions in some cases and kidney disease and stones.

The various bioactive compounds present in AYB suggests its potential in health management. Plant bioactive compounds have been shown to have pharmacological and beneficial effects in disease management (Soetan, 2008). This study therefore was aimed at providing information on the health benefits associated with the consumption of African yam bean. This work assessed the effects of the seeds of AYB on serum calcium, uric acid, alkaline phosphatase and inorganic phosphate levels of male albino rats.

2. Materials and Methods

2.1 Collection of Plant Material

The African yam bean (*Sphenostylis stenocarpa*) used for the study was bought from New Market, Enugu, Enugu State, Nigeria. It was identified at the Laboratory of the Department of Botany, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria. The fishmeal, groundnut cake and maize flour used were purchased from *Ose* market, Onitsha, Anambra State, Nigeria. The African yam bean was prepared by soaking it in cold water for about five minutes after which it was roasted and dehulled.

2.2 Animal Preparation

A total of eighteen (18) male Wister albino rats were used for the study. The rats were purchased from the Department of Veterinary, Medicine, University of Nigeria, Nsukka. They were allowed to acclimatize at the animal house of the Department of Applied Biochemistry, Nnamdi Azikiwe University, Awka, Anambra State for seven days. At the end of the acclimatization period, the animals were weighed and randomly divided into three groups (Baseline, Test, and Control). The animals were housed in aluminum cages kept in a room maintained between 28 ^{o}C-32 ^{o}C. The light cycle was 12 hours light and 12 hours dark. Food and water were given *ad libitum*.

2.3 Feed Preparation

The feeding regime was based on the method of Ononogbu and Emole (1978).

Table 1. Percentage feed composition

	Test (%)	Control (%)
Fish meal	10	10
Groundnut cake	24	23
Maize Flour	50	67
African yam bean	16	-

A total of 3 kg each of test and control feed weights were prepared according to the percentages by weight as shown in Table 1. Compounding was done by thoroughly mixing the components while adding an appropriate amount of water. The feed was then pelleted using a pelleting machine. Drying was in an oven at a temperature of 50 ^{o}C-55 ^{o}C.

2.4 Experimental Design

The albino rats were divided into three groups of six rats each making up the Baseline, Test and Control. The Baseline group was sacrificed at the onset of the experiment to determine initial parameters. The test and control groups were fed with the compounded test and control feed respectively for twenty eight days. At the end of the experiment, the animals were sacrificed by cervical dislocation and blood collected by cardiac puncture for further studies.

2.5 Determination of Parameters

The assays on alkaline phosphatase, uric acid, calcium and inorganic phosphate were all spectrophotometric using kits supplied by Randox Laboratories, UK.

2.6 Uric Acid Estimation

Uric acid is converted by uricase to allantion and hydrogen peroxide, which under the catalytic influence of peroxidase, oxidizes 3,5-Dichloro 2-hydroxybenzenesulfonic acid and 4-aminophenazone to form a red-violet quinonemine compound which is measured spectrophotometrically at 520 nm.

2.7 Alkaline Phosphatase Estimation – Spectrophotometric Method of King and Kind (1954)

Alkaline phosphatase catalyzes the transphosphorylation of p-nitrophenylphosphate (NPP) to p-nitrophenol (p-NP) in the presence of the transphosphorylating buffer, 2-amino-2-methyl-1-propanol (AMP). The reaction is enhanced through the use of magnesium and zinc ions and measured at a wavelength of 405 nm.

p-nitrophenylphosphate + H_2O $\xrightarrow{ALP + Mg^{++}}$ p-nitrophenol + inorganic phosphate

2.8 Serum Calcium Estimation

This is based on the fact that calcium ions form a violet complex with o – cresolphthalein complexone in an alkaline medium. This is measured spectrophotometrically at a wavelength of 570 nm.

2.9 Serum Inorganic Phosphate Estimation – Tietz (1990)

Inorganic phosphorous reacts with ammonium molybdate in the presence of sulphuric acid to form a phosphomolybdate complex which is measured at 340 nm.

2.10 Statistical Analysis

The results obtained from the different estimations were analyzed using the students' T-test. $P < 0.05$ was considered significant.

3. Results and Discussions

Table 2. The Effect of African yam bean on the average weight (grams) of animals

	Initial weight	Week 1	Week 2	Week 3	Week 4
Control group	45.10 ± 2.64	72.40 ± 3.28	87.80 ± 3.36	120.20 ± 4.98	141.20 ± 8.39
Test group	57.60 ± 4.87	88.00 ± 5.21	104.20 ± 5.20	120.10 ± 5.57	127.10 ± 4.78

Note. *Results expressed as Mean ± S.E.M.

Table 3. Effect of African Yam Bean (*Sphenostylis stenocarpa*) on serum calcium, inorganic phosphate, uric acid, and alkaline phosphatase concentration of male albino rats

	Calcium (mgldl)	Inorganic phosphate (mg/dl)	Uric acid (mg/dl)	Alkaline phosphatase (Iu/L)
Baseline	8.522 ± 0.181a	4.738 ± 0.097a	4.939 ± 0.569a	199.1 ± 6.125a
Control	8.220 ± 0.142a	4.877 ± 0.156a	5.324 ± 0.652a	205.7 ± 4.232a
Test	5.329 ± 0.241b	25.154 ± 4.392b	1.327 ± 0.142b	506.0 ± 51.594b

Note. *Results expressed as Mean ± S.E.M.

*Means with different superscripts (a, b) in a column are statistically significant ($p < 0.05$).

The serum uric acid level of the test animals was reduced and the reduction is significant ($p < 0.05$) when compared to those of the Baseline and Control groups. Though there was an increase in serum uric acid concentration of the control group compared to that of the Baseline, this was not significant ($p > 0.05$). The result of the serum uric acid level is similar to that of serum calcium level except that there was a reduction in calcium level of the Control compared to that of the Baseline but this was also not significant ($p > 0.05$). The result of the serum alkaline phosphatase and Inorganic phosphate levels followed a similar pattern. There was a significant ($p < 0.05$) increase in the levels of these parameters in the test group compared to their levels in the Baseline and Control.

There are immense nutritional potentials and possibilities in neglected and underutilized crop species. African yam bean (*Sphenostylis stenocarpa*), a tropical legume is one of such species with duo-food products (Adewale et al., 2013). Apart from the nutritional potentials of this crop, there lies a lot of other health benefits that can be derived from this plant.

The reduction in uric acid level is an indication that African yam bean consumption could be a good remedy for gout. The pathology of gout involves the deposition of urate crystals at the joints and tissues (Nsirim, 1999). Therefore, the reduction of uric acid level by African yam bean shows its potential in mopping up urate crystals. Again, some gouty conditions usually lead to arthritis (gouty arthritis). This also means that the African yam bean can be used as a local analgesic under this condition. The reduction in serum uric acid concentration of Africa yam bean compares well with that of soyabean. Soyaben consumption was found to significantly increase urate clearance (Garrel et al., 1991) and subsequently reduce uric acid concentration (Wurzner, 2001).

The increase in inorganic phosphate level is a good indicator for the antiarthritic potential of the African yam bean. The anti-arthritic drug; K-phos increases the serum phosphate level upon administration and this is in line with the observed result with African yam bean.

However, the increase in serum alkaline phosphatase level could be attributed to necrosis of some organs. Necrosis is as a result of intrahepatic cholestasis in which bile secretion from the hepatocytes into the canaliculi is impaired due to toxins. When this happens, there is an increased synthesis of alkaline phosphatase in the affected duct and this increases the level of the enzyme in the plasma (Mayne, 1998). African yam bean has been shown to have some antiunutritional factors which may be responsible for these observations. These antinutritional factors can be reduced by using efficient processing techniques and proper cooking (Adewale et al., 2013). Good processing ensures safe consumption of African yam bean meals by human and livestock. The result obtained by Anosike et al. (2008) showed a decrease in alkaline phoshatase activity of Wister albino rats fed with soybean supplemented diet.

Disorders of Calcium metabolism are common and may result in hypocalcaemia or hypercalcaemia as well as bone abnormalities. Closely associated with calcium disorders are disorders involving phosphate. The total body calcium depends upon the calcium absorbed from dietary intake and that lost from the body. Ninety eight percent of body calcium is found in the skeleton. The remaining amount is essential because of its effect on neuromuscular excitability and cardiac muscles. In healthy subjects, the mean plasma calcium concentration is tightly regulated at around 2.15-2.55 mmol/L. Calcium is mainly present as either bound to proteins (mainly albumin) or as free ionized calcium (Ca^{2+}). Changes in plasma protein concentration, particularly of albumin alter the concentration of plasma total calcium; both not that of the free ionized fraction.

The decrease in serum calcium concentration observed in the test animals can be as a result of the low calcium to protein content of the seed of African yam bean which is 55 mg to 19.2 g per 100 g edible portion of the seed (Tindall, 1986). According to Roughead (2003), an increase in dietary protein has a negative effect on bone calcium level. The proposed mechanism is that increased protein intake (especially proteins with sulfur containing amino acids) leads to an increased glomerular filtration rate, reduced renal reabsorption of calcium, hypercalciuria and thus leaching of calcium out of the bone (Kumar et al., 2013).

The result also showed an increase in weight for animals in both the control and the test groups. However, there was a significant ($p < 0.05$) increase in weight for the control group compared to the test group. This can be attributed to the antinutritional factors and low fat content of the bean. These factors interfere with nutrient intake and lower plasma cholesterol. This suggests that African yam bean can be used for weight management. Overweight and hypercholesterolaemia have been implicated in certain health conditions like cardiovascular diseases and diabetes. This result is in line with that of Onwuka et al. (2009) who studied the effect of germination on the performance characteristics of AYB seed meal on Albino rats. Onyeike et al. (1995) studied the influence of heat processing of AYB seed flour on the growth and organ weights of Wister rats. Body weight change, feed utilization and feed conversion ratio were improved by heat processing. This could be as a result of heat inactivation of toxic factors especially trypsin inhibitors.

From the foregoing, the African yam bean is an indispensable leguminous crop because of its nutritional and pharmacological potentials in the treatment and management of gout and arthritis. AYB will therefore find application as a component of specialty foods targeted towards the treatment and management of gout and arthritis. Efforts should be geared towards efficient processing techniques which will help to reduce its antinutritional factors and increase its acceptability and consumption.

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