



***Cajanus cajan*: Potentials as Functional Food**

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Abstract

Cajanus cajan seeds constitute a major part of the diet in most parts of Eastern Nigeria. In this study, the seeds were evaluated on some indices of good health in normal male albino rats. Two groups of six rats each were used for the experiment. The *C. cajan* seeds were compounded (1:1) with commercial rat pellet and fed to the test rats while the control was fed commercial pellets for 30 days. The effects on animal weight, serum proteins, electrolytes and hematological parameters, were determined. In addition, antioxidant enzymes which include superoxide dismutase (SOD), catalase (CAT) and Glutathione peroxidase (GPx) and lipid peroxidation as biomarkers of oxidative stress and lipoproteins profile of the animals were also evaluated. Results showed lower weight gain ($p < 0.05$) in *C. cajan*-fed compared to control rats. Total proteins, and hematological indices – PCV, Hb, RBC and MCHC were increased ($p < 0.05$), while serum electrolytes were unchanged ($p > 0.05$). Activities of SOD and GPx were lowered ($p > 0.05$), however, catalase activity was elevated ($p < 0.05$). MDA level was unchanged. There was decrease ($p < 0.05$) in TC, VLDL, TAG, while HDL was increased ($p < 0.05$). These results indicate positive effect on indices of good health, and parameters that predispose a subject to development of chronic diseases such as obesity, diabetes and cardiovascular disease.

Key words: *Cajanus cajan*, hematological indices, antioxidant enzymes, lipids

Introduction

Cajanus Cajan (L.) Millsp. (Leguminosae) known as “*fio fio*” in Igbo, *otiili* in Yoruba and pigeon pea in English (Aiyelaja and Bello, 2006) is native to India which is the world’s largest producer. It is also grown in Africa and the Americas, and has been suggested to be one of Africa’s drought-tolerant crops referred to as ‘orphan crop’ because it falls into the group of least researched crops world-wide (Odeny, 2007). It serves both as a food and forage crop. *C. cajan* can be combined with cereal to make a well-balanced human food. It is used in combination with soya bean to produce one of the popular fermented, flavouring product soy sauce (Muangthai *et al.*, 2009).

The chemical composition of this valuable food plant seed has been evaluated. The plant is rich in protein (21.5%), while the fiber content is about 2.5% (Akande *et al.*, 2010). This makes it a very important protein source particularly in a region

where 41% of the populace is still chronically malnourished (Anuonye *et al.*, 2012). It has appreciable amount of the essential amino acids; phenylalanine – which has been reported to possess anti-sickling properties (Ekeke and Shode, 1990); tryptophan which is involved in the treatment of hypertension (Ajaiyeoba *et al.*; 2004); methionine and lysine, the latter, among other properties help in effective absorption of calcium (Ahmad *et al.*, 2008).

C. cajan contains minerals like potassium, magnesium, calcium and is significantly low in sodium. The low sodium content might be one of the reasons it is employed in ethno-medicine for the treatment of hypertension (Lawal, 2012). It also has vitamins such as vitamin A, niacin and small amount of thiamin, riboflavin, folate and pantothenic acid (Makkar and Becker, 1996; Akande *et al.*, 2010).

Ethno-medical reports have it that it is used in treatment of diseases such as bladder stone, jaundice, cardiac diseases such as hypertension, genetic diseases such as sickle cell, and that smoke from the burnt leaves is inhaled to the relief cough and asthma (Lawal, 2012).

Maintaining healthy living particularly protection from chronic diseases such as hypertension, diabetes, cardiovascular diseases and cancer through the use of plant foods, fruits, vegetables and herbs is growing amongst scientists and health givers (Ka'kho'nen *et al.*, 1999).

Recently in Nigeria, the incidences of chronic diseases such as diabetes, hypertension and other cardiovascular diseases have been on the increase, in contrast to its near absence in the past (Ononogbu, 2002). This is probably due to several factors which include change in diet particularly in the methods of their preparation, lifestyle, and greater consumption of processed foods and drinks. This rise has presented a critical need to find a way to their treatment and management using diet.

Therefore, the effect of consumption of *C. cajan* seeds compounded in equal ratio (1:1) with commercial rat pellet on some indices of good health in normal male adult Swiss albino rats was evaluated. The effects on weight, serum proteins, hematological parameters, serum electrolyte were investigated. Furthermore, indices of chronic diseases such as the effect on levels of antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase), oxidative stress marker (lipid peroxidation), and lipid profile were also examined.

MATERIALS AND METHODS

Chemicals and Reagent

Epinephrine, glutathione, hydrogen peroxide, thiobabitoric acid (TBA), kits for determination of lipoprotein concentrations were supplied by Sigma-Aldrich chemical company, Germany. All other chemicals and reagents including solvents were of analytical grade.

Preparation of feed

The *C. cajan* seeds and the rat pellet (3kg each) were ground into powder using an electric miller. A ratio (1:1) of the powder and commercial rat pellet was mixed into a dough using 5L of water,

and the mixture was pelleted. The pellets were allowed to dry on the laboratory bench.

Experimental design

Twelve adult male Swiss albino rats were purchased from the animal house of Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Agulu. They were allowed to acclimatize for a period of two weeks. The animals were weighed and the weights recorded before commencement of experiment. The rats were sorted and grouped into two - control and test groups of 6 rats each. The test group was fed the formulated feed while the control group was fed rat pellet only at 125g per day for 30 days with water *ad libitum*. The animals were handled in accordance with the guidelines of Institute for Laboratory Animal Research (ILAR)

Blood Collection

The animals were fasted overnight and sacrificed by severing the jugular vein with a surgical blade. Blood was allowed to flow freely and was collected in plain bottles. The blood was allowed to clot and then centrifuged at 1500 x g for 5 min after which the clear supernatant (serum) was separated from pellet and used for enzyme assay.

Weight assessment

The weight of each rat was recorded on day 0 and 30 of the study period using a top loading weighing balance AFP- 800L, ADAM.

Protein estimation

The serum protein concentration of the animal was estimated using autoanalyser (Biochemistry Autoanalyzer (BS 290 Mindray Canada).

Serum electrolyte determination

The serum electrolyte concentration was determined using Biochemistry autoanalyser (BS-200, Mindray Canada).

Heamatological analysis

Heamatological parameters – Packed Cell Volume (PCV), Hemoglobin concentration (Hb), Red Blood Cells count (RBC), White Blood Cells count (WBC) and its differential, Mean Cell Heamoglobin (MCHC) and platelets count were

analyzed using haematology autoanalyzer (BC 6800 Mindray, Canada).

Antioxidant enzymes assay

Superoxide dismutase

The method of Sun and Zigma as described by Ogbunugafor *et al.*, (2010 a) was adopted. The reaction mixture approximately 3ml contained 2.95ml of sodium carbonate buffer (0.05mM and pH 10.2) and 0.02ml of serum sample. 0.03ml Epinephrine (3 M) in 0.005 N HCl was used to initiate the reaction, the reference cuvette contained 2.95ml buffer, 0.02 ml of water and 0.03ml of epinephrine. The enzyme activity was determined by measuring the change in absorbance at 480nm for 3-5minutes. An extinction coefficient of $4020 \text{ m}^{-1}\text{cm}^{-1}$ was used to calculate enzyme activity and expressed in unit of mg protein^{-1} .

Catalase:

Serum catalase activity was determined according to the method of Beers and Seizer with some modification as described by Usoh *et al.*, (2005). The reaction mixture containing (2.2 ml) contained 0.1ml of serum, 0.1ml H_2O_2 and 2 ml of phosphate buffer (50 mM, PH 7.0). The test reading was read against blank devoid of serum. Decrease in absorbance due to decomposition of H_2O_2 was measured in UV spectrophotometer at 30 seconds interval for 3minutes. An extinction coefficient for H_2O_2 , at 240nm of $40.0 \text{ m}^{-1}\text{cm}^{-1}$ was used for the enzyme activity calculation and expressed in unit mg protein^{-1} .

Glutathione peroxidase

Glutathione peroxide was determined by the method of Beutler and Kelly as adapted by Anthony *et al.*, (2003). The reaction mixture contained 1ml of 0.3M phosphate buffer (pH 7.4), 0.3ml 10mM glutathione, 0.3ml 15mM freshly prepared H_2O_2 , and 1.37 ml distilled water. The serum (0.1ml) was added to the mixture in the cuvette, shaken and absorbance was read at 340nm. Extinction coefficient of $1.622 \times 10^{-3} \text{ m}^{-1}\text{cm}^{-1}$ was used to calculate enzyme activity and expressed in unit mg protein^{-1} .

Determination of lipid peroxidation

The lipid peroxidation effect of the feed on the animals as a measure of malonylaldehyde (MDA) was determined according to method of Buege

and Aust as described by Choi and Hwang (2005). An aliquot of 0.4ml of the serum was collected into the tests tube, 1.6ml of 0.25N HCL was added together with 0.5ml of 15% trichloroacetic acid (TCA) and then mixed thoroughly. The reaction mixture was then placed in 80°C boiling water for 15mins, allowed to cool and centrifuged at 3000rpm for 10mins. The supernatant was collected and the optical density recorded at 532nm against reagent blank containing distilled water. The lipid peroxidation effect (MDA concentration) mM L^{-1} was calculated using the formula: Optical density / Time x Extinction coefficient/vol. of sample.

Lipid profile determination

The lipoproteins concentrations were determined using an assay kits supplied by Sigma-Aldrich Germany.

RESULTS AND DISCUSSION

Foods termed 'functional foods' are foods that have potential positive effect on health beyond basic nutrition. They satisfy basic nutritional needs as well as therapeutic needs. Thus, evaluation of food crops that constitute a major part of our diet in the past when the incidences of chronic diseases was much lower (Ononogbu, 2002); is a way to finding solution to the management of these diseases. This approach towards linking increased occurrence of certain diseases to change from traditional diet is the recent trend among scientist.

In the present study, *C. cajan*-fed rats gained less weight ($p < 0.05$) compared to commercial pellet-fed (control) rats. This finding is supported by Shar (1991) who reported that raw *C. cajans* did not support growth in rats. This could be due to low digestibility of raw *C. cajans* and/or the effect of anti-nutrients present in the seeds (Shar, 1991; Ilelaboye and Pikuda, 2009). However, this result may be seen in a positive light since excessive weight gain is a risk factor in chronic diseases, such as diabetes, arthritis, and hypertension. Moreover, the seeds are usually consumed cooked by humans, which according to Shar (1991) comparative studies that cooked *C. cajans* seeds supported growth. Furthermore, Igene *et al.*, (2012) revealed that addition of increasing proportions of par-boiled *C. cajans* in place of

soyabeans in poultry feed showed reduced weight gain in broilers.

Total proteins were significantly ($p < 0.05$) increased in test rats, an indication that the functions of plasma proteins such as their role in immunity, transport of substances, as enzymes and as buffering agents may be enhanced (Nduka, 1999). In addition, the test rats exhibited a significantly ($p < 0.05$) increased levels of haematological parameters – PCV, Hb, RBC and MCHC. This is in agreement with reports that the seeds of the food plant is rich in vitamin B₁₂, folate and proteins which are ingredients required in blood formation (Olaniyan and Adeleke, 2005). The result is therefore, suggestive of polycythemia and a positive erythropoetic effect (Okpuzor *et al.*, 2009). Thus, the *C. cajan*-fed rats may have had increased oxygen carrying capacity of the red cells. Moreover, haematology is a developed and well utilized parameter in assessing the health of both man and livestock (Svobodova, *et al.*, 1991). Thus, the neglect of this food plant in our diets; particularly in that of children is an undesirable development (Anuonye *et al.*, 2012).

However, some anti-nutritional factors present in *C. cajan* such as concavalin A, saponins, etc. have been reported to have negative effect on haematological parameters, affect palatability and intake of nutrients (Ilelaboye and Pikuda, 2009).

The serum electrolytes concentration was unchanged ($p > 0.05$) in *C. cajan*-fed rats compared to control. This result might indicate that the *C. cajan* feed did not disturb the electrolyte balance of the animals.

The total white blood (WBC) cells did not change between the *C. cajan*-fed rats and control. The implication of this finding might be that there was no stimulating effect from the *C. cajan* feed that could trigger production of white blood cells of the immune system of the animals. These cells such as monocytes and neutrophils belong to the family of defense cells and share similar mechanisms that consist of ingesting the foreign material (bacteria) through phagocytosis and killing infectious agents by producing reactive oxygen species (ROS) (Tsumbu *et al.*, 2011).

However, an excessive ROS production can be deleterious for cells, since ROS can attack important bio-molecules, causing changes in the

structure and function of enzymes- proteins, and carbohydrates, lipids and nucleotides. It is well established that ROS are involved in the long term pathogenesis of various diseases like cancer, diabetes, rheumatoid arthritis, cell aging and cardiovascular diseases, including atherosclerosis, (Wang, *et al.*, 2010; Tsumbu *et al.*, 2011).

Therefore, the evaluation of the effects of our common foods on endogenous antioxidant enzymes is a good strategy. This might highlight the role these foods could have played in the lower incidence of chronic diseases such as hypertension in the past.

Results show that serum SOD and GPx activities declined ($p < 0.05$) in *C. cajan*-fed rats when compared to control. This result suggests that the enzymes might not have been induced in the animals' system. This finding might indicate that the feed could be playing the scavenging role of the enzymes, thus the enzymes were spared and their induction became unnecessary. This is in consonant with studies by Belquith-Hadriche *et al.*, (2010) that showed that the enzymes activities were lowered when ethyl acetate extract of fenugreek seed (*Trigonella foenum-graecum* (Fabaceae)) was administered to rat fed with cholesterol-rich diet. SOD is the enzyme that catalyzes the dismutation of oxygen free radicals, converting them to H₂O₂ which is further broken down by catalase to molecular oxygen and water (Ogbunugafor *et al.*, 2007 b). Glutathione peroxidase on the other hand, changes hydroperoxide group to the much less toxic hydroxyl moiety (Gurr and Harwood, 1987). The elevated activity ($p < 0.05$) of serum catalase in the rats suggests that the enzyme was induced, and might have increased the ability of the animals to scavenge the non-radical derivative of oxygen - H₂O₂. Akinloye *et al.*, (2011) is in agreement with this finding in their work that reported that *C. cajan* possess free radical scavenging property. Additionally, studies by Muagman *et al.*, (2011), reported that an isolated protein from *C. cajan* seeds possessed antioxidant effect on hydrogen peroxide induced cellular damage.

Lipid peroxidation (i.e. malonylaldehyde level) was not significantly ($p > 0.05$) increased in the

serum of the test rats. We infer that lipid peroxidation did not occur in the test rats. Unsaturated fatty acids are the major constituents of biomembranes, but they are particularly sensitive to oxidation. Oxidation of lipids (lipid peroxidation) is triggered by free radicals chain reaction occurring in membrane lipids; and has been found to be correlated with swelling and possible lysis of mitochondrion, microsomes, lysosomes and cells (Sevanian and Hochstein, 1985). Thus, the absence of peroxidation of lipids may be a positive result particularly in relation to chronic diseases.

Lipids and lipoproteins are known to play an important role in the genesis of atherosclerosis. Ononugbu (2002) reported that Nigerians have low serum cholesterol compared to their Caucasian counterparts; which was reflected in the lower incidence of coronary heart disease due to atherosclerosis among Nigerians in the early seventies. However, this trend is changing at an alarming rate in recent times, therefore the investigation of our diet in relation to the index of major chronic disease is important.

The concentrations of total cholesterol (TC), very low density lipoprotein (VLDL), and triacylglycerides (TAG) were significantly ($p < 0.05$) lowered in *C. cajan*-fed rats compared to control. Low density lipoprotein (LDL) was also lowered but this was not significant ($p > 0.05$), while high density lipoprotein (HDL) was elevated ($p < 0.05$). This is an important finding as total cholesterol particularly the LDL and VLDL fraction, is a major risk factor in atherosclerosis, hypertension and insulin dependent diabetes (Chattopadhyay and Bandyopadhyay 2005). Cholesterol build up forms plaques that hardens and narrows the arteries, increasing the risk of coronary heart diseases (Bagarad *et al.*, 2012). The combination of reduced cholesterol VLDL and LDL and elevated HDL is a pointer to effectiveness of the diet to act as a therapy for chronic disease. The lipid lowering activity of the seeds of this food plant therefore, suggests that the plant may protect against cardio-related chronic diseases (Ogbunugafor *et al.*, 2012 c). Furthermore, the lipid content of the seed as reported by Ononugbu, (2002) is low (1.79%) with the sterols' value as 8.08 mg/100 ml, which might have contributed to the depressed lipid levels in the rat's serum. Reports have shown that

are that plant sterols lower cholesterol by lowering LDL (Ostlund, 2002).

CONCLUSION

This study on *Cajanus cajan* has highlighted the potentials of this seed plant to act as functional food, a nutrient rich diet that can also prevent the development of the now common chronic diseases such as diabetes and hypertension. Therefore, consumption of *C. cajan* by the populace should be encouraged by creating awareness of its nutritional and medicinal benefits.

Table 1: Packed Cell Volume (PCV), Hemoglobin (Hb), Red blood cell (RBC) and Platelets in *C. cajans*-fed and control rats

Parameter	<i>C. cajans</i> -fed	
Control		
PCV (%)	45.48 ± 2.72 ^a	41.18 ± 2.10 ^b
HB (g/dL)	15.283 ± 0.82 ^a	13.62 ± 0.71 ^b
RBC (x10 ¹² /L)	8.582 ± 0.42	7.698 ± 0.41
PLT (x 10 ⁹ /L)	485.5 ± 42.7 ^a	450.7 ± 29.10 ^b

Values are mean ± SEM. Values with dissimilar letters (a,b) are significantly ($p < 0.05$) different from each other with respect to parameter investigated.

Table 2: Total proteins, albumin and globulin (µg/dL) in serum of *C. cajans*-fed and control rats

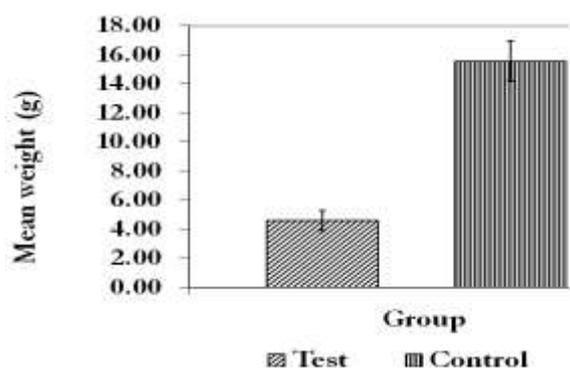
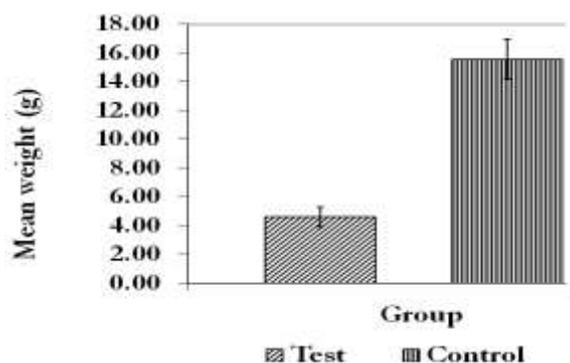
Parameter (µg/dL)	<i>C. cajans</i> -fed
Control	
Total proteins	6.52 ± 0.17 ^a
5.82 ± 0.07 ^b	
Albumin	3.12 ± 0.01
2.92 ± 0.03	
Globulin	3.20 ± 0.12
2.90 ± 0.05	

Values are mean ± SEM. Values with dissimilar letters (a,b) are significantly ($p < 0.05$) different from each other with respect to parameter investigated.

Table 3: Lipid Profile (mg/dL) of *C. cajan*-fed and control rats

Parameter (mg/dL)	<i>C. cajan</i> -fed	Control
TC 1.54 ^b	38.67 ± 2.85 ^a	60.17 ±
LDL 1.12 ^b	9.17 ± 1.85 ^a	11.33 ±
HDL 2.12 ^b	40.33 ± 2.32 ^a	30.17 ±
VLDL	9.17 ± 0.54 ^a	18.67 ± 2.11 ^b
TAG	44.8 ± 2.7 ^a	92.2 ± 10.3 ^b

Values are mean ± SEM. Values with dissimilar letters (a,b) are significantly ($p < 0.05$) different from each other with respect to parameter investigated. TC= total cholesterol, LDL= low density lipoprotein, HDL= high density lipoprotein, VLDL= very low density lipoprotein, TAG= triacylglyceride.

Figure 1: Mean weight of animals fed *C. cajan* and control groupFigure 2: Changes in Gluthathione peroxidase (GPx), Superoxide dismutase (SOD) and Catalase (CAT) activities in animals fed with *C. cajan* and

control group. SOD and CAT activities were expressed as IU x 10⁻⁴ and IU

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