

## EFFECTS OF PROCESSED CAJANUS CAJAN (PIGEON PEA) SEED ON LIPID PROFILE IN ALBINO WISTAR RATS

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### ABSTRACT

This study was designed to evaluate the nutritional effect of processed *Cajanus cajan* seeds on lipid profile of albino wistar rats. Sixty-four (64) (disease free stock) of albino rats were used. The rats were randomly assigned to eight (8) experimental groups A, B, C, D, E, F, G and H. Group H which served as the control group with eight rats received only water and normal rat feed. The *Cajanus cajan* fed rat groups were Group A (Boiled), Group B (Cooked), Group C (Fermentation with Decantation), Group D (Fermentation without Decantation), Group E (Raw), Group F (Roasted), Group G (Soaked). Each study group comprised of eight rats. Feeding went on for 30 days

during which the body weight indices of Groups A, B, C, D, F and G of the *Cajanus cajan* fed albino wistar rats were observed to be significantly increased ( $p < 0.05$ ) when compared to the control group and Group E was observed to be significantly decreased ( $P < 0.05$ ) when compared to the control group. The quantitative phytochemical analysis carried out showed the presence of alkaloids, saponin, tannin, phenolics, phytate, oxalate and flavonoids as well as the concentration at which they were present. The proximate nutrient composition showed the Percentile concentration of Carbohydrate, Protein, Fibre, Ash and Moisture in the samples. Lipid profile showed a significant decrease ( $P < 0.05$ ) in the total cholesterol, LDL and triglycerides in Groups A, B and F while the HDL levels showed a significant increase at ( $P < 0.05$ ) when compared to the control. LDL was significantly increased ( $P < 0.05$ ) in Groups C, D, E and G when compared to the control group. In conclusion, data obtained from this

study showed that boiled, cooked and roasted *Cajanus cajan* seeds have the best effect on Lipid profile of albino wistar rats.

**KEYWORD:** *Cajanus cajan*, phytochemicals analysis, proximate nutrient composition, Body weight indices. and lipid profile.

## INTRODUCTION

Throughout history plants have been used by human beings for medicinal purposes and in modern times have formed the basis of many pharmaceuticals in use. Plants produce a vast array of secondary metabolites as defense against environmental stress or other factors like pest attacks, wounds, and injuries. The complex secondary metabolites produced by plants have found various therapeutic uses in medicine from time immemorial (Pal *et al.*, 2011). The early history of modern medicine contains descriptions of plant-derived photochemical, many of which are still in use (Pal *et al.*, 2009). *Cajanus cajan* (L) Millsp. (In Sanskrit: Adhaki, Hindi: Arhar, English: Pigeon pea, Bengali: Tur) It is a perennial member of the family fabaceae. Other common names are red gram, Congo pea, gungo pea, and no-eye pea (Wu *et al.*, 2009). The cultivation of pigeon pea goes back at least 3000 years ago. The centre of origin is most likely Asia, from where it travelled to East Africa and by means of slave trade to the American continent. It is an erect, branched, hairy shrub, 1-2 meters high. Leaves are oblong-lanceolate to oblanceolate with three leaflets. Flowers are yellow, in sparse peduncled racemes, about 1.5-cm long. Pod is hairy, 4-7 cm long, 1 cm wide, containing two to seven seeds. India is a principal pigeon pea-growing country contributing nearly 90% of the total world production. Currently, it occupies an area of 3.85 million hectares with an annual production of 2.68 million tons (Kumar *et al.*, 2010). It is a multipurpose plant as it is extensively eaten as a dal. It is rich in proteins. In India its leaves are used for rearing silkworms; green pods are used as a vegetable; husk, green leaves and tops are used as fodder and also as green manure (Ambasta, 2004). Amongst its many medicinal uses, *Cajanus cajan* is indicated in the relief of pain in traditional Chinese medicine and as a sedative (Ahsan and Islam, 2009). In recent years it has also been explored for the treatment of ischemic necrosis of the caput femoris, aphtha, bedsore and wound healing. Chemical investigations have revealed the presence of two globulins, cajanin and concajanin (Ambasta, 2004). It has been used widely for many years for treating diabetes, sores, skin irritations, hepatitis, measles, jaundice, dysentery and many other illnesses; for expelling bladder stones and stabilizing menstrual period (Yuan-gang *et al.*, 2010).

Legumes are particularly rich in healthy fibers, such as resistant starch and soluble fibers. Resistant starch and soluble fibers have a few things in common. They pass undigested through the stomach and small intestine until they reach the colon, where they feed the friendly bacteria residing there. Unpleasant side effects often include gas and bloating, but it also leads to the formation of short-chain fatty acids, such as butyrate, which may improve colon health and reduce the risk of colon cancer (Hylla *et al.*, 1998). Both resistant starch and soluble fibers are also very satiating and may reduce food intake, which in the long run can lead to weight loss (Clark *et al.*, 2013). Additionally, they are very effective at moderating blood sugar levels after meal and may improve insulin sensitivity (Robertson *et al.*, 2005). Legumes have an impressive nutritional profile, and are one of the best plant-based sources of protein. However, like many other plant foods, they also contain anti-nutrients, which may impair their nutritional value. Kidney beans may be toxic when raw but many processing methods can be used to neutralize these anti-nutrients. Throughout the ages, traditional methods like soaking, sprouting, and boiling, have been used to improve the nutritional level. At the end of the processing period, properly processed legumes are very healthy when consumed as part of a balanced, real food based diet (Messina, 1999).

## **MATERIALS AND METHODS**

### **Experimental animals**

Sixty-four female albino rats between 250-262g were purchased from Ladoko Akintola University of Technology, Ogbomosho, Nigeria and used for the study. The rats were randomly assigned on the basis of their body weight into eight (8) study groups of eight (8) rats each. Normal feeds and tap water were given to the rats, *ad-libitum* and food and water intakes were noted. They were kept in secure wooden cages of 8 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 treatment commenced.

### **Chemicals**

Chloroform and other chemicals of analytical grade were obtained from Fam-lab Nigeria Limited. Distilled water was also used during the experiment.

**Identification and authentication**

*Cajanus cajan* seeds were obtained from Ilesa central market (Oja-Oba) Osun State. The samples were taken to the department of Botany, Obafemi Awolowo University (OAU).

**Preparation of raw materials**

The Pigeon peas seeds were purchased from Ilesa central market (Oja-oba) Osun State. The research project was carried out at the Biochemistry Research Laboratory, Department of Chemical Sciences (Biochemistry Unit), Joseph Ayo Babalola University, Ikeji-Arakeji, Osun state Nigeria, from April-May, 2017. Seven (7 kg) of the pigeon pea seeds were cleaned by sorting to remove extraneous materials and were weighed and shared into seven desired groups of 1kg each. Each was subjected to toasting, cooking, boiling, soaking, raw, fermentation with decantation or fermentation without decantation.

**Raw Pigeon peas:** The Pigeon pea seeds were rinsed with clean water and dried under the sun. When fully dried, it was then pulverised to powdery form and then sieved so as to remove the seed coating.

**Soaking:** The Pigeon pea seeds were soaked by pouring 1kg of the pigeon pea seeds into 8 litres of water, allowing it to soak for 12 hours after which the water was sieved out and sun dried for about 3-4 days before pulverized into fine powder.

**Boiling:** This was carried out by pouring 1kg of the cleaned pigeon pea seeds into a cooking pot containing 8 litres of water, which was set to boiled at 100°C using an electrical hotplate. This was boiled for 1 hour. The water was sieved out and the boiled pigeon pea seeds were allowed to cool, then sun dried for 3-4 days before pulverized into fine powder.

**Cooking:** This was carried out by pouring 1kg of the cleaned pigeon pea seeds into a cooking pot containing 8 litres of water, which is set to boil at 100°C using an electrical hotplate. This was boiled until cooked. The water was sieved out and the cooked pigeon pea seeds were allowed to cool, then sun dried for 3-4 days before been pulverized into fine powder.

**Fermentation without decantation:** This was carried out by pouring 1kg of the pigeon pea seeds into 8 litres of water in an air-tightened container and soaked for 12 hours. The fermentation process began steadily immediately after 12 hours while still in the water. The water was sieved out of the container after 48 hours and was sun dried for about 3-4 days before pulverising into fine powder.

**Fermentation with decantation:** This method was similar to the soaking process done previously, 1 kg of the pigeon pea seed was poured into 8 litres of water in an air-tightened container and soaked for 12 hours. The water was sieved out and another 8 litres of water was poured into the seed and it was well covered allowing fermentation process to occur after sieving. The water was then sieved out after 48 hours and sun-dried for about 3-4days before pulverising into fine powder.

**Roasting:** In this method, the pigeon pea seeds were toasted by pouring 1kg of the seeds into a frying pan containing fine sand set over fire. The stirring of the seed while on fire was continuous until it turned brown. Also the seeds were stirred to avoid excessive burning which could lead to complete destruction of the essential amino acids. The toasted pea was then pulverized into fine powder.

### **Experimental design**

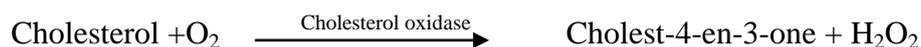
The grouping and feeding of processed *Cajanus cajan* given to the rats were as follows; Group A: Designated as boiled pigeon pea seeds group rats administered a portion boiled pigeon pea seeds and water *ad-libitum*. Group B: Designated as cooked pigeon pea seeds group rats administered a portion of cooked pigeon pea seeds and water *ad-libitum* Group C: Designated as fermentation with decantation pigeon pea seeds group rats administered a portion of decanted fermented pigeon pea seeds and water *ad-libitum*. Group D: Designated as fermentation without decantation pigeon pea seeds group rats administered a portion of undecanted fermented pigeon pea seeds and water *ad-libitum*. Group E: Designated as raw pigeon pea seeds group rats administered a portion of as raw pigeon pea seeds and water *ad-libitum*. Group F: Designated roasted pigeon pea seeds group rats administered a portion of roasted pigeon pea seeds and water *ad-libitum*. Group G: Designated as soaked pigeon pea rats administered a portion of soaked pigeon pea and water *ad-libitum*. Group H: Designated as control group rats administered a portion of normal rat feed and water *ad-libitum*.

### **Sacrifice of the animals**

At the end of the experimental period, rats in each study group were fasted overnight and sacrificed under anesthesia by cardiac puncture.

**Blood collection**

After the rats have been sacrificed, 3-4ml of blood was collected from each rat and placed in specific sterile bottles of lithium heparin bottles for lipid analysis and EDTA bottles for hematological indices for further analysis.

**Cholesterol estimation****Principle**

H<sub>2</sub>O<sub>2</sub>, one of the reaction products, is measured in a peroxidase catalyzed reaction that forms a colored dye

**Procedure**

1ml of working reagent was pipetted into test tube labeled blank, standard, and test. Then 10µl of standard and test sample was added to corresponding tube, mixed and allowed to stand at 25°C for 15 minutes the absorbance of standard and test was read against blank at 505nm.

$$\text{Calculate from; } \frac{\text{O D test} \times \text{concentration standard}}{\text{O D standard}}$$

**High density cholesterol (phosphotungstic/mgcl precipitation)****Principle**

All other fractions save HDL cholesterol were precipitated out of solution.

Cholesterol level in the supernatant after spinning down gives the HDL fraction.

**Procedure**

Into a clean centrifuge tube, 0.2ml of the sample was pipetted into 0.5ml precipitant. Mixed very well and allowed to stand for 5minutes, then Centrifuged at 3,000g for 10minutes. 50µl of clear the supernatant was pipetted in a test tube and 1ml cholesterol reagent was added.

For the standard tube 20µl standard was added into 1ml reagent mixed well and stand for 15minutes. The absorbances of test and standard was read against blank at 505nm

$$\text{Calculate from } = \frac{\text{O D test}}{\text{O D standard}} \times \text{Concentration Standard}$$

**Low density cholesterol (heparin precipitation)****Principle**

LDL fraction of cholesterol is precipitated out of sample and cholesterol assay in supernatant.  
Total cholesterol – cholesterol in supernatant = LDL cholesterol

**Procedure**

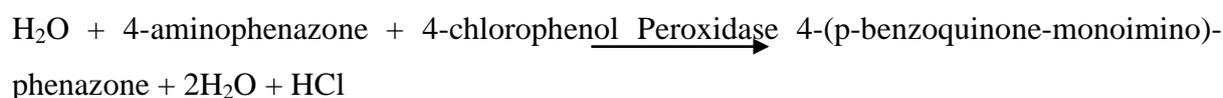
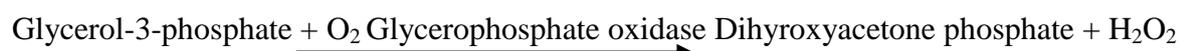
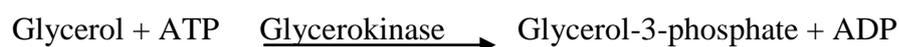
200µl of sample was pipetted into 800µl of precipitant was mixed vigorously and stand for 10 minutes. Centrifuged at 3,000g for 10 minutes and to 1ml cholesterol reagent, 10µl standard or 100µl of supernatant was added mixed and stand for 15 minutes.

The absorbance of standard, test was read against blank at 505nm

Calculate from =  $\frac{O D \text{ test}}{O D \text{ standard}} \times \text{concentration standard}$

**Triacylglycerol (TAG) determination**

Triacylglycerols are measured enzymatically in serum or plasma using a series of coupled reactions in which triglycerides are hydrolysed to produce glycerol. Glycerol is then oxidized using glycerol oxidase and H<sub>2</sub>O<sub>2</sub>, one of the reaction products, is measured as described above for cholesterol. Absorbance is measured at 500nm. The reaction sequence goes thus;

**Statistical analysis**

Data obtained were statistically analyzed using students' t-test and values expressed as Mean ± SEM at p<0.05 probability level.

RESULTS

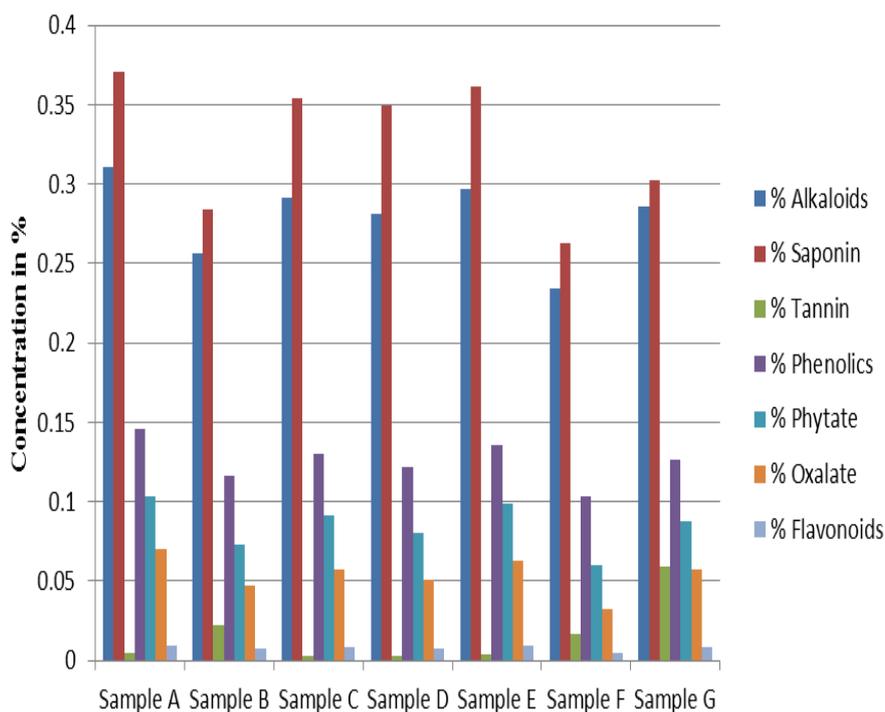


Figure 1.0: The results of the quantitative phytochemical analysis of processed *Cajanus cajan*.

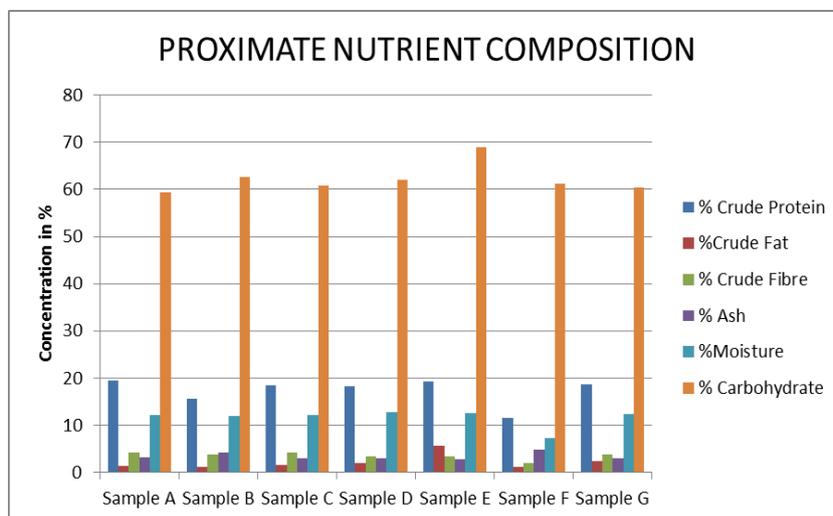
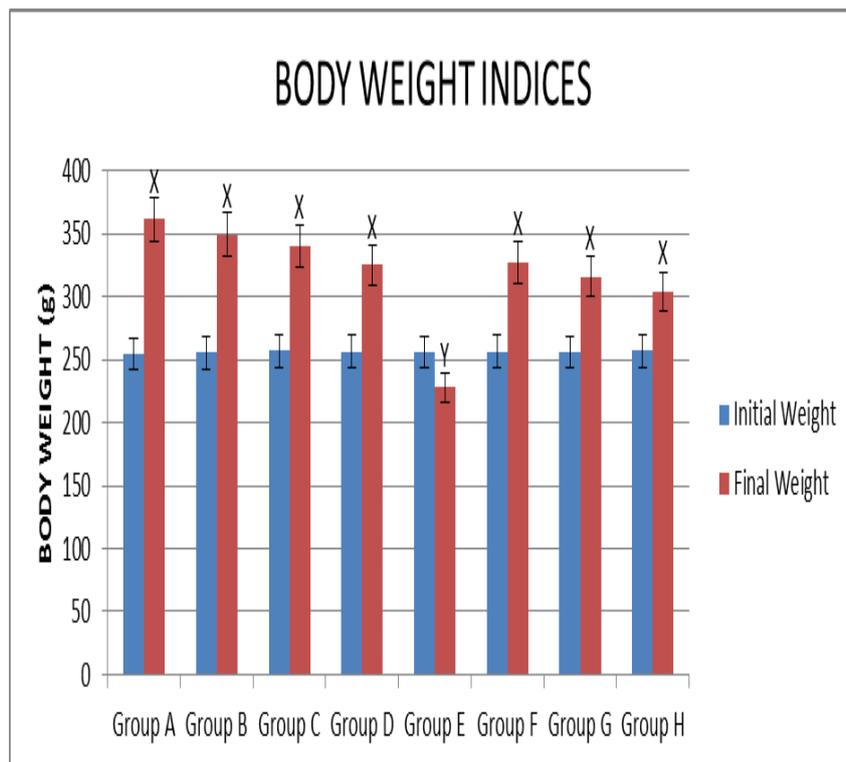
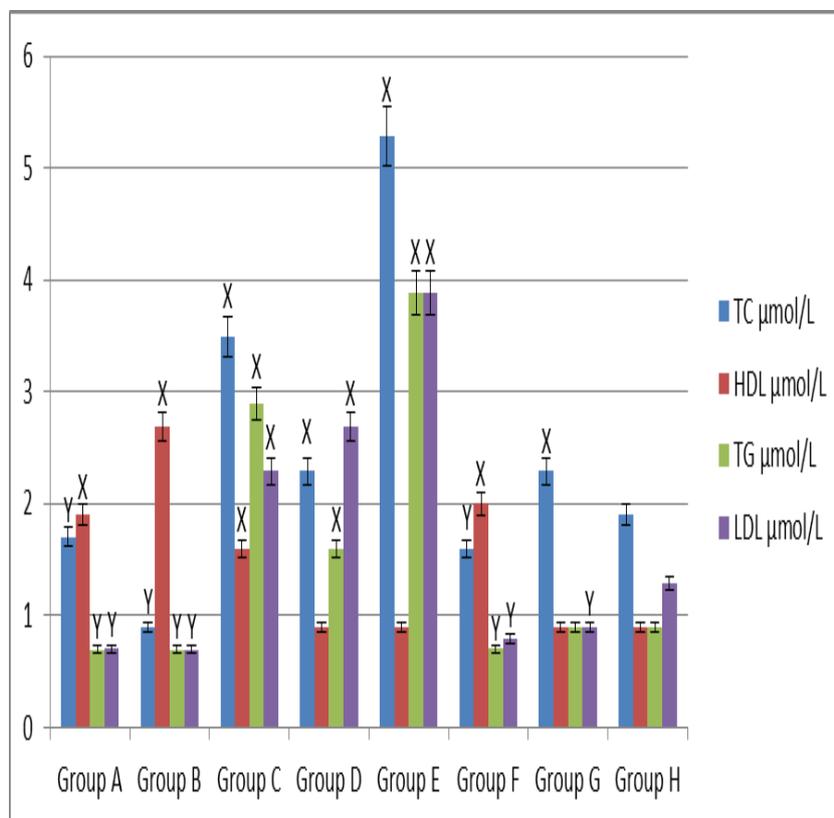


Figure 2.0: The results of Proximate Nutrient Composition of Processed *Cajanuscajan*.



**Figure 3.0: Effect of processed *Cajanus cajan* on body weight indices of experimental Rats.**



**Figure 4.0: Effect of processed *Cajanus cajan* on the Lipid Profile of experimental rats.**

## DISCUSSION

Legumes are staple foods for many people in different parts of the world (Dahl *et al.*, 2012). Legumes are one of the most sustainable sources of protein in the world (Zenter *et al.*, 2004). This study was designed to evaluate the nutritional effects of processed *Cajanus cajan* seeds on albino wistar rats. Phytochemicals are non-nutritive plant chemicals that have protective or disease preventative properties. Figure 2 shows the results of the quantitative analysis of processed *Cajanus cajan*. The quantitative phytochemical analysis carried out showed the presence of alkaloids, saponin, tannin, phenolics, phytate, oxalate and flavonoids and the concentration at which they were present in all the samples. The proximate nutrient composition showed the percentile concentration of Carbohydrate, Protein, Fibre, Ash and Moisture in the samples.

The body weight indices of the experimental groups, at the commencement of the experiment compared to its weight at the end of the experiment demonstrated a significant increase in all the study groups except the raw group (Group E). This increase was however significant ( $p < 0.05$ ) in Group A (boiled) and Group B (cooked) compared to the control group (Group H). There was also a significant decrease ( $p < 0.05$ ) in the raw group (Group E) compared to the control group (Group H). The decrease in body weight is due to the presence of anti-nutrients because they interfere with the absorption and digestion of other nutrients and therefore impede growth. The increase in body weight is due to the fact that the processing methods have helped to neutralize these anti-nutrients and improved the nutritional levels (Messina, 1999).

The Lipid Profile of the experimental rats was also assayed consisting of the HDL (High Density Lipoprotein), LDL (Low Density Lipoprotein), TG (Triacylglycerols) and Total Cholesterol as shown in figure 7 above. HDL, also known as good cholesterol is a lipoprotein that can transport fat molecules out of artery walls, reduce macrophage accumulation. There was significant increase ( $p < 0.05$ ) in the HDL of groups A (boiled), B (cooked) and F (roasted) when compared to the control (group H) with the peak increase in Group B (Cooked). There was no significant increase or decrease ( $p < 0.05$ ) in Group E (Raw) because the anti-nutrients are still present. The increase in HDL of the said groups is because anti-nutrients present were reduced during the processing methods (Cooking, Boiling and Roasting). LDL (Bad cholesterol) is a lipoprotein that can transport their content of Lipid molecules into artery walls and attract macrophages. There was a significant decrease

( $p < 0.05$ ) in the LDL of groups A, B and F compared to the control (group H) with the peak decrease observed in Groups A and B. This is due to the absence of anti-nutrients in these groups. A significant increase ( $p < 0.05$ ) was observed in groups C, D and E with group E having the peak increase as a result of the presence of anti-nutrients in the sample. TC which refers to the sum of HDL, LDL and triglycerides in the blood showed a significant decrease ( $p < 0.05$ ) in study groups A, B and F with group B being the lowest. A significant increase ( $p < 0.05$ ) was also observed in Groups C, D, E and G with group E having the peak increase. Triglycerides, also known as triacylglycerol which is the major form of fat stored in the body showed a significant decrease ( $p < 0.05$ ) in Groups A, B, F and G compared to the control and a significant increase ( $p < 0.05$ ) in Groups C, D, E and F with group E having the peak increase. The boiled, cooked and roasted groups proves to be the most active of all the groups because the anti-nutrients present were significantly reduced during boiling, cooking and roasting. One common thing observed with these processing methods was that they all involved the application of heat. These processing methods (boiling, cooking and roasting) helped to reduce the anti-nutrients content and also improved protein and starch digestibility. This is in accordance with the findings of Zia-ur *et al* (2005).

## CONCLUSION

Thus, the most nutritious form of *Cajanus cajan* on lipid profile level of albino wistar rats are the cooked, boiled and roasted forms. This is due to low levels of LDL (bad cholesterol) and High levels of HDL (Good cholesterol) and increase in the protein and starch digestibility and reduction in anti-nutrient levels. It is therefore concluded from the data that cooked, boiled and roasted *Cajanus cajan* seeds has the best effect on lipid profile.

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