

## IMPACT OF LOCAL BEAN-BASED DIETS ON BIOCHEMICAL PARAMETERS OF WISTAR RATS

Ezeani, Ifeoma Grace Nkiru

Department of Biochemistry, Faculty of Natural Sciences, Chukwuemeka Odumegwu Ojukwu University, Anambra State- 431124, Nigeria.

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| ABSTRACT                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | KEYWORDS                                                                                                |
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| <p>This study investigated the chemical composition and effects of four locally consumed bean species on liver and kidney function in Wistar albino rats. The bean species evaluated were <i>Cajanus cajan</i>, <i>Vigna unguiculata</i> subsp. <i>sesquipedalis</i>, <i>Phaseolus vulgaris</i> L. 'Red kidney', and <i>Phaseolus vulgaris</i> 'Black turtle'. Proximate analysis showed that <i>P. vulgaris</i> 'Red kidney' (<math>18.54 \pm 0.01\%</math>) and 'Black turtle' (<math>18.36 \pm 0.01\%</math>) had the highest protein content, with no statistically significant difference (<math>P &lt; 0.05</math>). Phytochemical screening indicated the presence of beneficial compounds alongside anti-nutritional factors. Mineral content was highest in <i>V. unguiculata</i> subsp. <i>sesquipedalis</i> (<math>4.80 \pm 4.43</math> mg/100g), followed by <i>C. cajan</i> (<math>3.24 \pm 2.64</math> mg/100g).</p> <p>A total of 30 male Wistar rats were divided into five groups (<math>n = 6</math>). One group served as the control, while the remaining groups were fed diets containing 100g of the respective bean species alongside 50g of standard rat feed, administered based on body weight. Biochemical analysis of liver and kidney function markers showed no significant adverse effects in the test groups compared to the control.</p> <p>The findings suggest that these locally available beans are nutritionally rich and safe for consumption at the tested doses. However, the presence of certain anti-nutritional factors highlights the need for proper processing before consumption.</p> | <p><i>Phaseolus vulgaris</i>, <i>Cajanus cajan</i>, Red kidney bean, Liver enzymes, Kidney function</p> |

### 1. Introduction

The role of biodiversity in sustaining food, nutrition and health security with the growing world population and its resultant effect on the well-being of the people cannot be over emphasized. The need to balance the double burden malnutrition and health especially in developing countries calls for nutrient profiles of local plant food species and varieties that can bring about the achievement of the Millenium Development Goals (Ogunlade *et al.*, 2014). A bean is a seed that belongs to the family of the flowering plant Fabaceae. Currently, there are over 40,000 varieties of beans and only a small fraction of this are mass-produced for regular consumption (Laura and

Suszkiw, 2006; Sarah, 2015). Beans are rich in proteins and are used as meat substitutes. In addition to its nutritional qualities, they are inexpensive and widely distributed along different geographical locations. Due to its wide variability and distribution, they form an important part of delicacies of people around the world especially in the developing country like Nigeria. Some of the bean types includes *Phaseolus*, *Vigna*, *Cajanus*, *Mucuna*, *Vicia* etc.

*Phaseolus* is a genus in the family Fabaceae containing about 70 bean species, all native to the Americas, primarily Mesoamerica (Delgado-Salinas *et al.*, 2011). Varieties within this genus includes Common bean (*Phaseolus vulgaris*), French bean, black bean, kidney bean, pinto bean, green bean. Most prominent among these is the Common bean, which today is cultivated worldwide in tropical, semitropical, and temperate regions. In Nigeria, a specie in this class called “Ndudu brown” (Red kidney beans) and “Ndudu black” (Black turtle beans) are consumed locally and sold by food vendors.

The Red kidney bean is a variety of the Common bean, *P. vulgaris*. It is named for its visual resemblance in shape and colour to a kidney. Raw kidney beans contain relatively high amounts of phytohemagglutinin, and referred to as one of the most toxic bean varieties whose toxins are inactivated if pre-soaked and boiled (Rodhouse *et al.*, 1990).

The black turtle bean is a small, shiny variety of the Common beans. Varieties of black turtle beans exists. Samples of black turtle beans were reported in 2006 to contain total anthocyanins in their dried seed coats of 0–2.78 mg/g (Choung *et al.*, 2003).

*Cajanus Cajan* (L.) Millsp. (Leguminosae) is a perennial legume which is a native of India but now found across major continents of the world such as Asia, Africa and America. In Nigeria, *C. cajan* is called “fiofio” among the Igbo ethnic group and “otili” in Yoruba language (Aiyeloja and Bello, 2006; Ogbunugafor *et al.*, 2013).

Black beans is locally called “Akidi” in Igbo language and scientifically referred to as *Vigna unguiculata* sub spp. *Sesquipedalis*. It is cultivated to be eaten as green pods and are best for vegetable use if picked before they reach full maturity. They differ from the Common beans. This study investigates the nutritional compositions, phytochemical compositions of four local species of beans (*C. Cajan*, *V. unguiculata* subsp. *sesquipedalis*, *P. vulgaris* L ‘Red kidney’, and *P. vulgaris* ‘Black turtle’) and their effects on the liver and kidney function of albino rats.

## **2. Materials and Methods**

### **2.1 Materials**

#### **2.1.1 Plant Materials**

The four species of beans, *C. Cajan*, *V. unguiculata* subsp. *sesquipedalis*, *P. vulgaris* L ‘Red kidney’, and *P. vulgaris* ‘Black turtle’ was obtained from a local market in Ihiala Local Government Area of Anambra State, Nigeria and were identified.

#### **2.1.2 Animals**

Thirty (30) Male Wistar albino rats weighing between 150-200g was used for the study. The rats was obtained from the Animal House of the Department of Zoology and Environmental Biology, University of Nigeria, Nsukka. The rats was fed with rat pellets and water *ad libitum*.

Ethical clearance was obtained from the designated ethical committee.

#### **2.1.3 Equipment**

The equipment used are those of the Department of Biochemistry, Chukwuemeka Odumegwu Ojukwu University, Uli; Professor John I.

Ihedioha Foundation for Education and Research on Health (FERH) Laboratory and Spring Board Research Laboratory, Awka, Nigeria. They were calibrated and was in a good working state.

#### **2.1.4 Reagents**

The chemicals and reagents used are of analytical grade and are products of British Drug House (BDH), England, Germany, Dermstadt, May and Baker, England, Sigma Aldrich, USA, and Quimica Clinica Applicada (QCA) HDL test kit (QCA, S.A. Spain).

### **2.2 Methods**

#### **2.2.1 Preparation of Bean Feed**

Bean samples was sieved to remove stones and debris after which it was washed, dried and ground to flour. This was sieved and kept in an airtight container.

#### **2.2.2 Proximate Composition Determination**

The proximate composition of the samples was determined using the methods of the AOAC (1990); WHO (1973); Onyeike and Osuji (2003); Nwinuka *et al.* (2005); ASEAN (2011).

#### **2.2.3 Phytochemical Studies**

Phytochemical analysis was qualitatively and quantitatively determined following standard procedures as outlined by Harborne (1973), Trease and Evans (1989) and Sofowora (1993).

#### **2.2.4 Trace Mineral Analysis**

The trace mineral element concentration in the various beans samples was determined by the Atomic Absorption Spectrophotometric method.

#### **2.2.5 Experimental Design**

Thirty (30) male Wistar albino rats was used for the study. They were acclimatized for seven days with free access to feed and water. After acclimatization, they were randomly distributed into five (5) groups of 6 rats each and were fed for 3 weeks (21 days) in which analyses was done on the last day. The experimental design are illustrated below.

**Group 1** (control group) was fed with 150 g of normal feed kg/body weight (b.w) each day

**Group 2** was fed 50g of Normal rat feed + 100g of *P. vulgaris* L 'Red kidney' kg/b.w.

**Group 3** was fed 50g of Normal rat feed + 100g of *P. vulgaris* 'Black turtle' kg/b.w.

**Group 4** was fed 50g of Normal rat feed + 100g of *C. Cajan* kg/b.w.

**Group 5** was fed 50g of Normal rat feed + 100g of *V. u. subsp. Sesquipedalis* kg/b.w.

#### **2.2.6 Liver Function Test**

##### **2.2.6.1 Determination of Liver Enzymes (ALT, AST, and ALP)**

Serum ALT and AST was determined by the Reitman-Frankel colorimetric method (Reitman and Frankel, 1957). Phenolphthalein monophosphate method was used for the determination of alkaline phosphatase in serum (Klein *et al.*, 1960; Babson *et al.*, 1966).

##### **2.2.6.2 Determination of Serum Total Bilirubin**

Jendrassik-Grof method was adopted for the determination of total bilirubin in serum (Doumas *et al.*, 1973).

##### **2.2.6.3 Serum Protein determination (Total protein, Albumin, and Globulin)**

The direct biuret method for the determination of total protein in serum was adopted (Lubran, 1978). Serum albumin was determined by the bromocresol green method (Doumas *et al.*, 1971; Doumas and Peters, 1997). Globulin levels was obtained by subtracting the quantity of albumins from that of total proteins.

**2.2.7 Kidney function test (Urea and Creatinine Test)** Modified method of Berthelot-Searcy was used for the determination of urea in serum (Fawcett and Scott, 1960; Searcy *et al.*, 1967), while the modified Jaffe method for adopted for the determination of creatinine in serum, plasma or urine (Blass *et al.*, 1974).



|                    |   |    |    |    |
|--------------------|---|----|----|----|
| Flavonoids         | + | ++ | ++ | +  |
| Alkaloids          | + | +  | +  | +  |
| Saponins           | + | +  | +  | ++ |
| Phenols            | + | ++ | +  | +  |
| Tannins            | + | +  | +  | ++ |
| Cardiac glycosides | + | +  | ++ | ++ |

Key: + = Slightly present; ++ = Moderately present; - = Absent.

### 3.3 The Quantitative Phytochemical Analysis of the Four Species of Beans

Results obtained from the quantitative phytochemical analysis of the four beans species were presented in Table 3. The results revealed that *P. vulgaris* L 'Red kidney' had the highest flavonoids ( $4.44 \pm 0.11^a$  %), Alkaloids ( $4.85 \pm 0.01^a$  %), Cardiac glycosides ( $10.41 \pm 0.93^a$  %) and Haemagglutinin ( $0.78 \pm 0.00^a$  mg/l). The *P. vulgaris* 'Black turtle' beans had the highest oxalate ( $0.54 \pm 0.00^a$  mg/l). *C. cajan* had the highest saponins ( $5.12 \pm 0.11^a$  %), with no significance difference in its phenol content ( $1.42 \pm 0.01^a$  %) and that of *V. u. subsp. Sesquipedalis* ( $1.47 \pm 0.00^a$  %). From Table 3, *V. u. subsp. Sesquipedalis* retained the lowest level of phytochemicals.

**Table 3:** The phytochemical composition of four different species of beans.

| Phytochemicals (%)                  | <i>C. cajan</i>   | <i>V. u. subsp. Sesquipedalis</i> | <i>P. vulgaris</i> L 'Red kidney' | <i>P. vulgaris</i> 'Black turtle' |
|-------------------------------------|-------------------|-----------------------------------|-----------------------------------|-----------------------------------|
| Flavonoids (%)                      | $3.21 \pm 0.02^c$ | $2.24 \pm 0.05^d$                 | $4.44 \pm 0.11^a$                 | $4.03 \pm 0.06^b$                 |
| Alkaloids (%)                       | $1.61 \pm 0.01^c$ | $2.79 \pm 0.01^b$                 | $4.85 \pm 0.01^a$                 | $2.42 \pm 0.02^b$                 |
| Saponins (%)                        | $5.12 \pm 0.11^a$ | $2.17 \pm 0.01^c$                 | $3.24 \pm 0.06^b$                 | $2.28 \pm 0.23^c$                 |
| Phenols (%)                         | $1.42 \pm 0.01^a$ | $1.47 \pm 0.00^a$                 | $1.17 \pm 0.01^b$                 | $1.29 \pm 0.23^b$                 |
| Tannins (%)                         | $1.49 \pm 0.01^b$ | $2.21 \pm 0.01^a$                 | $2.08 \pm 0.02^a$                 | $2.23 \pm 0.02^a$                 |
| Cardiac glycosides (%) <sup>c</sup> | $5.06 \pm 0.05$   | $6.11 \pm 0.12^{b,c}$             | $10.41 \pm 0.93^a$                | $7.08 \pm 0.07^b$                 |
| Haemagglutinin (mg/l) <sup>b</sup>  | $0.48 \pm 0.01$   | $0.33 \pm 0.01^b$                 | $0.78 \pm 0.00^a$                 | $0.51 \pm 0.02^c$                 |
| Phytate (%)                         | $0.39 \pm 0.01^a$ | $0.17 \pm 0.01^{b,c}$             | $0.14 \pm 0.00^c$                 | $0.22 \pm 0.01^{a,b}$             |
| Oxalate (mg/l)                      | $0.22 \pm 0.01^c$ | $0.27 \pm 0.01^c$                 | $0.37 \pm 0.01^b$                 | $0.54 \pm 0.00^a$                 |

Values are mean  $\pm$  standard deviations (n=3). Values within the same row bearing the same superscript letters are not significantly different at  $P < 0.05$ .

**3.4 The Trace Mineral Composition of the Four Species of Beans** The four species of beans analyzed for the trace mineral compositions revealed varying concentrations of antioxidant minerals (Table 4). *V. u. subsp. Sesquipedalis* had the highest Fe content ( $2.73 \pm 0.11^a$  mg/100g), Zn content ( $12.06 \pm 0.06^a$  mg/100g), Mn content ( $1.48 \pm 0.02^a$  mg/100g) and Se content ( $7.17 \pm 0.01^a$  mg/100g), while *C. cajan* had the highest Cu content ( $1.04 \pm 0.03^a$  mg/100g).

**Table 4:** The trace mineral composition of four different species of beans.

| Mineral | <i>C. cajan</i> | <i>V. u. subsp.</i> | <i>P. vulgaris</i> L | <i>P. vulgaris</i> |
|---------|-----------------|---------------------|----------------------|--------------------|
|---------|-----------------|---------------------|----------------------|--------------------|

|             |                        |                         |                        |                          |
|-------------|------------------------|-------------------------|------------------------|--------------------------|
| Fe          | 2.03±0.02 <sup>b</sup> | 2.73±0.11 <sup>a</sup>  | 1.65±0.02 <sup>c</sup> | 1.85±0.04 <sup>b,c</sup> |
| Zn          | 6.03±0.02 <sup>b</sup> | 12.06±0.06 <sup>a</sup> | 3.78±0.01 <sup>c</sup> | 3.77±0.02 <sup>c</sup>   |
| Cu          | 1.04±0.03 <sup>a</sup> | 0.56±0.02 <sup>b</sup>  | 0.23±0.01 <sup>c</sup> | 0.38±0.01 <sup>b,c</sup> |
| Mn          | 0.52±0.02 <sup>b</sup> | 1.48±0.02 <sup>a</sup>  | 0.22±0.01 <sup>b</sup> | 0.34±0.02 <sup>b</sup>   |
| Se          | 6.58±0.01 <sup>b</sup> | 7.17±0.01 <sup>a</sup>  | 6.22±0.03 <sup>b</sup> | 5.63±0.34 <sup>c</sup>   |
| Σ Mean      | 3.24±2.64 <sup>b</sup> | 4.80±4.43 <sup>a</sup>  | 2.42±2.38 <sup>b</sup> | 2.39±2.22 <sup>b</sup>   |
| Composition | <i>Sesquipedalis</i>   | 'Red kidney'            | 'Black                 |                          |
| (mg/100g)   | turtle'                |                         |                        |                          |

Values are mean ± standard deviations (n=3). Values within the same row bearing the same superscript letters are not significantly different at  $P < 0.05$ .

### 3.5 Liver Function Test Results

The effects of *P. vulgaris* L 'Red kidney', *P. vulgaris* 'Black turtle', *C. Cajan*, and *V. u. subsp. Sesquipedalis* on the liver functions (ALT, AST, ALP, Bilirubin, Serum total proteins, Albumin, Globulin) of Wistar albino rats were presented in Figures 1-7.

**3.5.1 Effects of the Bean Species on Serum ALT Activity** The effects of the beans species on the serum ALT level revealed a decrease in serum ALT levels (IU/L) when compared to the control at  $P < 0.05$  (Fig. 1). Similar response pattern was observed within the groups. The ALT levels of the control was found to be  $11.82 \pm 1.30$  IU/L while that of *P. vulgaris* L 'Red kidney', *P. vulgaris* 'Black turtle', *C. Cajan*, and *V. u. subsp. Sesquipedalis* was found to be  $7.15 \pm 0.42$  IU/L,  $7.31 \pm 1.20$  IU/L,  $8.05 \pm 0.63$  IU/L, and  $7.75 \pm 0.67$  IU/L respectively.

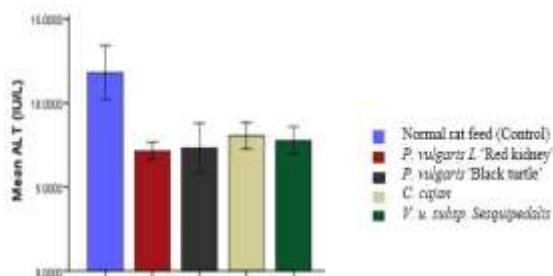


Figure 1: Effects of the beans species on the serum ALT level in Wistar albino rats.

**3.5.2 Effects of the Bean Species on Serum AST Activity** The effects of *P. vulgaris* L 'Red kidney', *P. vulgaris* 'Black turtle', *C. Cajan*, and *V. u. subsp. Sesquipedalis* on the serum AST levels of Wistar albino rats were presented in Figure 2. The result revealed that the control group fed normal rat feed had the highest level of AST ( $57.96 \pm 1.82$  IU/L). This was followed by the group fed *V. u. subsp. Sesquipedalis* ( $55.11 \pm 3.08$  IU/L), while group fed *C. Cajan* had  $52.85 \pm 2.73$  IU/L, *P. vulgaris* L 'Red kidney' group had  $46.94 \pm 1.88$  IU/L and the least AST activity was found in group fed *P. vulgaris* 'Black turtle' ( $45.31 \pm 2.73$  IU/L).

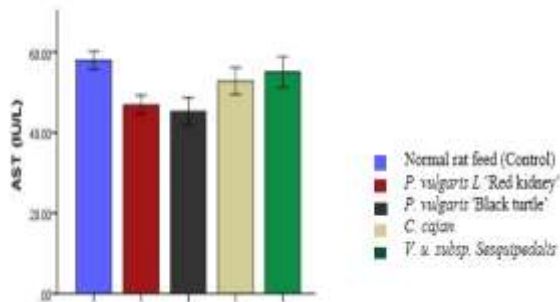


Figure 2: Effects of the beans species on the AST level in Wistar albino rats.

### 3.5.3 Effects of the Bean Species on Serum ALP Activity

The results for the effects of *P. vulgaris* L 'Red kidney', *P. vulgaris* 'Black turtle', *C. Cajan*, and *V. u. subsp. Sesquipedalis* on serum ALP level (IU/L) of Wistar albino rats were presented in Figure 3. It was observed that the group fed *V. u. subsp. Sesquipedalis* had the highest ALP activity ( $174.30 \pm 10.48$  IU/L) which was followed by group fed *P. vulgaris* L 'Red kidney' ( $135.78 \pm 12.27$  IU/L). The lowest levels of ALP activity was observed in group fed *C. Cajan* ( $96.72 \pm 5.58$  IU/L) while no significance difference existed between the control group ( $125.43 \pm 5.34$  IU/L) and group fed *P. vulgaris* 'Black turtle' ( $118.14 \pm 8.21$  IU/L).

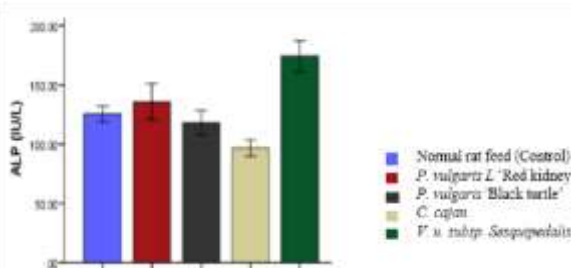


Figure 3: Effects of the beans species on the serum ALP level in Wistar albino rats.

### 3.5.4 Effects of the Bean Species on Bilirubin level

The results obtained from the analysis of serum bilirubin levels were presented in Figure 4. The result indicates that there was no significant difference at  $P < 0.05$  compared to the control and Wistar albino rats fed four different species of beans (*P. vulgaris* L 'Red kidney', *P. vulgaris* 'Black turtle', *C. Cajan*, and *V. u. subsp. Sesquipedalis*). The levels of bilirubin are found in the following order: *V. u. subsp. Sesquipedalis* ( $6.36 \pm 0.57$   $\mu\text{mol/L}$ ), *P. vulgaris* L 'Red kidney' ( $6.25 \pm 0.15$   $\mu\text{mol/L}$ ), *C. Cajan* ( $6.22 \pm 0.57$   $\mu\text{mol/L}$ ), *P. vulgaris* 'Black turtle' ( $6.08 \pm 0.15$   $\mu\text{mol/L}$ ) while the control group bilirubin levels was found to be  $5.95 \pm 0.33$   $\mu\text{mol/L}$ .

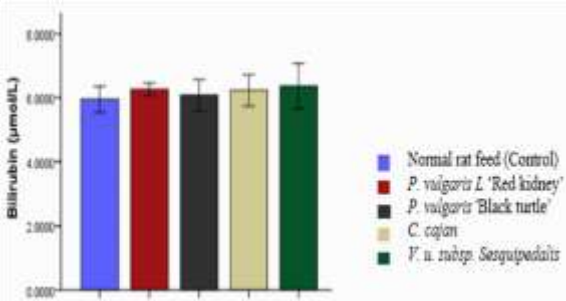


Figure 4: Effects of the beans species on the Bilirubin level in Wistar albino rats.

**3.5.5 Effects of the Bean Species on the Total Protein Level** The results obtained from the serum total protein level (g/L) analysis were presented in Figure 5. The result shows that there was no significant difference at  $P < 0.05$  when compared to the control. The control group has the total serum protein of  $73.88 \pm 4.26$  g/L. This was

closely followed by the group fed *P. vulgaris* L 'Red kidney' ( $72.82 \pm 5.30$  g/L). Other groups had the following concentrations of serum total protein; *C. Cajan* ( $72.68 \pm 3.76$  g/L), and *P. vulgaris* 'Black turtle' ( $70.82 \pm 3.92$  g/L).

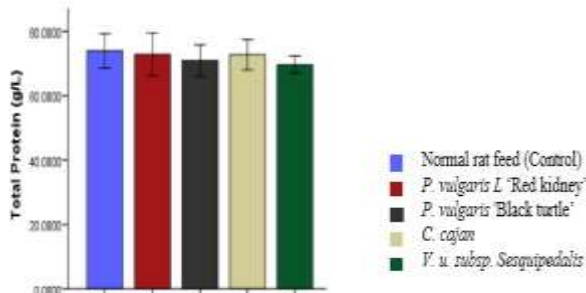


Figure 5: Effects of the beans species on the serum total protein in Wistar albino rats.

**3.5.6 Effects of the Bean Species on the Albumin Level** The albumin level (g/dl) observed for the various groups of Wistar albino rat different species of beans (*P. vulgaris* L 'Red kidney', *P. vulgaris* 'Black turtle', *C. Cajan*, and *V. u. subsp. Sesquipedalis*) were presented in Figure 6. The result indicates that there was no significant difference at  $P < 0.05$  compared to the control. The least levels was found in group fed *V. u. subsp. Sesquipedalis* ( $35.00 \pm 1.89$  g/L). Other groups was found to exhibit different concentration of albumin in the following order: *P.*

*vulgaris* 'Black turtle' ( $38.58 \pm 2.90$  g/L), control group ( $38.16 \pm 2.93$  g/L), *P. vulgaris* L 'Red kidney' ( $37.42 \pm 2.37$  g/L), and *C. Cajan* ( $37.20 \pm 3.09$ ).

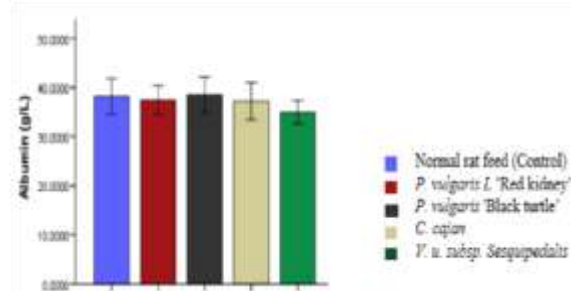


Figure 6: Effects of the beans species on the albumin level in Wistar albino rats.

**3.5.7 Effects of the Bean Species on the Globulin Level** The globulin level (g/L) observed from the various groups of Wistar albino rat fed four different species of beans were presented in Figure 7. The result indicates that there was no significant difference at  $P < 0.05$  compared to the control. The least globulin levels was found in group fed

*P. vulgaris* 'Black turtle' ( $32.34 \pm 2.50$  g/L), followed by the group fed *C. Cajan* ( $35.34 \pm 1.91$  g/L), while group fed *V. u. subsp. Sesquipedalis* has the serum globulin levels of  $33.58 \pm 1.07$  g/L. There was no significance difference between the globulin levels of the control group ( $35.72 \pm 3.72$  g/L), and group fed *P. vulgaris* L 'Red kidney' ( $35.72 \pm 1.42$  g/L).

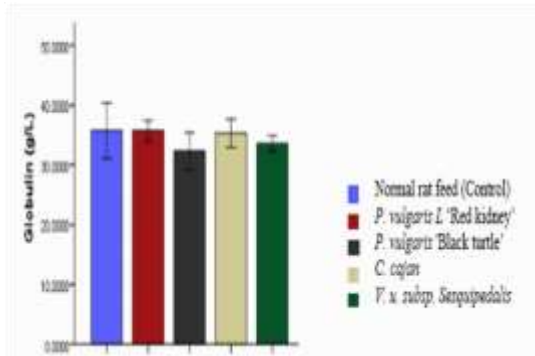


Figure 7: Effects of the beans species on the Globulin level in Wistar albino rats.

### 3.6 Kidney Function Test Results

The effects of *P. vulgaris* L 'Red kidney', *P. vulgaris* 'Black turtle', *C. Cajan*, and *V. u. subsp. Sesquipedalis* on the kidney functions (Urea and Creatinine) of Wistar albino rats were presented in Figure 13 and 14.

#### 3.6.1 Effects of the Beans Species on Urea Level

The effects of the bean species on the serum urea level of Wistar albino rats was presented in Figure 13. The Urea level (mmol/L) showed slight decrease which is not significant when compared to the control at  $P < 0.05$ . The control group had the serum urea levels of  $9.25 \pm 0.56$  mmol/L. This was followed by the group fed *C. cajan* ( $8.69 \pm 0.54$  mmol/L). The group fed *P. vulgaris* 'Black turtle' had the serum urea level of  $8.14 \pm 0.27$  mmol/L. This is followed by the group fed *V. u. subsp. Sesquipedalis* ( $7.99 \pm 0.53$  mmol/L) while the least urea levels was found in the group fed *P. vulgaris* L 'Red kidney' ( $7.36 \pm 0.14$  mmol/L).

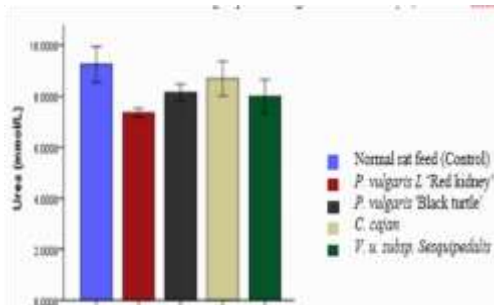


Figure 13: Effects of the beans species on the Urea level in Wistar albino rats.

**3.6.2 Effects of the Beans Species on the Creatinine Level** The effects of *P. vulgaris* L 'Red kidney', *P. vulgaris* 'Black turtle', *C. Cajan*, and *V. u. subsp. Sesquipedalis* on the serum creatinine level ( $\mu\text{mol/L}$ ) was presented in Figure 14. The creatinine level showed slight decrease which is not significant compared to the control at  $P < 0.05$ . The control group had the highest serum creatinine levels of  $65.78 \pm 4.03$   $\mu\text{mol/L}$ . This was followed by the group fed *P. vulgaris* 'Black turtle' ( $57.30 \pm 5.81$   $\mu\text{mol/L}$ ), *C. Cajan* ( $55.88 \pm 4.61$   $\mu\text{mol/L}$ ), *V. u. subsp. Sesquipedalis* ( $53.05 \pm 4.33$   $\mu\text{mol/L}$ ), and the least as *P. vulgaris* L 'Red kidney' ( $51.64 \pm 1.48$   $\mu\text{mol/L}$ ).

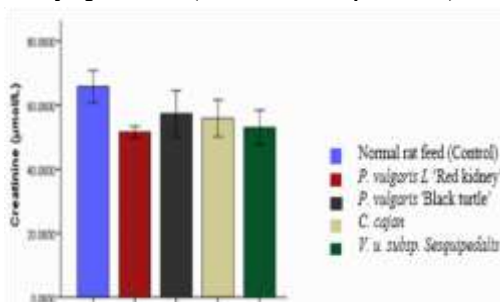


Figure 14: Effects of the beans species on the serum creatinine level in Wistar albino rats.

#### 4. Discussion

Beans is one of the common classes of food that are widely consumed worldwide due to its high protein and nutritional contents. Many varieties of beans exists with dearth of scientific information concerning their compositions and most importantly their effects in the body viz. the vital organs (liver and kidney). There are notably over 40,000 species of beans out of which only a small fraction has been studied. The result obtained from the proximate composition analysis revealed that the protein content of the bean species was highest in *P. vulgaris* L 'Red kidney' ( $18.54 \pm 0.01^a$  %), and *P. vulgaris* 'Black turtle' ( $18.36 \pm 0.01^a$  %) with no significance difference at  $P < 0.05$  between the two species (Table 1). *V. u. subsp. Sesquipedalis* ( $10.05 \pm 0.01^c$  %) had the least protein content while *C. cajan* had  $15.01 \pm 0.01^b$  %. The result for the fibre content revealed that *P. vulgaris* 'Red kidney' had the highest fibre content with no significant difference between the fibre content of *V. u. subsp. Sesquipedalis*. The highest level of moisture and fat was found in *V. u. subsp. Sesquipedalis*. There was no significance difference observed in the crude fat content of the bean species. *P. vulgaris* L 'Red kidney' had the highest ash content ( $8.43 \pm 0.03^a$  %), while *V. u. subsp. Sesquipedalis* had the highest carbohydrate content ( $75.79 \pm 0.01^a$  %). The estimation of the energy levels shows that *P. vulgaris* 'Black turtle' had the highest energy level  $360.69 \pm 0.57^a$  %, followed by *V. u. subsp. Sesquipedalis* ( $352.40 \pm 0.53^{a,b}$  %).

*P. vulgaris* L 'Red kidney' ( $338.39 \pm 0.53^b$  %), and *C. cajan* ( $316.53 \pm 0.51^b$  %). The results suggests that beans is a good source of nutrient which correlates with the nutritional benefits for beans as discussed by Taub-Dix (2014), in which he noted that beans provide myriad health benefits, and this made it fit into several different food groups.

The result obtained for the preliminary phytochemicals screening revealed the presence of flavonoids, alkaloids, saponins, phenols, tannins, and cardiac glycosides in all the four beans aqueous extract (Table 2). The presence of these phytochemicals is an indication that these local bean species can serve as a valuable source for the discovery of novel bioactive compounds for drug discovery (Egbuna and Ifemeje, 2015; Egbuna and Ifemeje, 2017; Arul *et al.*, 2018; Tijjani *et al.*, 2018; Ezzat *et al.*, 2019; Kavitha *et al.*, 2019; Nwosu *et al.*, 2019; Srivastav *et al.*, 2019). The quantitative analysis revealed that *P. vulgaris* L 'Red kidney' had the highest flavonoids ( $4.44 \pm 0.11^a$  %), alkaloids ( $4.85 \pm 0.01^a$  %), cardiac glycosides ( $10.41 \pm 0.93^a$  %) and haemagglutinin ( $0.78 \pm 0.00^a$  mg/l) (Table

3). The result suggests that *P. vulgaris* L 'Red kidney' although could be a potential source of antioxidant phytochemicals but care should be taken in its consumption due to the haemagglutinin content. Similarly, *P. vulgaris* 'Black turtle' beans had the highest oxalate ( $0.54 \pm 0.00^a$  mg/l), a phytochemical noted for the formation of kidney stone (Finkelstein and Goldfarb, 2006). *C. cajan* had the highest saponins ( $5.12 \pm 0.11^a$  %), with no significance difference in its phenol content ( $1.42 \pm 0.01^a$  %) and that of *V. u. subsp. Sesquipedalis* ( $1.47 \pm 0.00^a$  %). Among the four species of beans studied, there are no distinct beans with greatest or favored phytochemical contents. For instance those with high level of antioxidant phytochemicals has some anti-nutritional factors which could be deleterious when the beans specie is consumed in excess. To this vein, it will be advisable not to depend on only one beans. A mixed consumption pattern should be adopted in order to tap from the numerous phytochemicals present in the bean species. However, for preference, *C. Cajan* and *V. u. subsp. Sesquipedalis* has the least anti-nutrients which makes them a better choice despite low antioxidant phytochemical contents.

The results obtained for the antioxidant minerals analyzed for the four species of beans suggests that *V. u. subsp. Sesquipedalis* had the highest level of total mean concentration of the minerals ( $4.80 \pm 4.43^a$  mg/100g) followed by *C. cajan* ( $3.24 \pm 2.64^b$  mg/100g), with no significance difference for *P. vulgaris* L 'Red kidney' ( $2.42 \pm 2.38^b$  mg/100g) and *P. vulgaris* 'Black turtle' ( $2.39 \pm 2.22^b$  mg/100g) (Table 4). *V. u. subsp. Sesquipedalis* had the highest Fe content ( $2.73 \pm 0.11^a$  mg/100g), Zn content ( $12.06 \pm 0.06^a$  mg/100g), Mn content ( $1.48 \pm 0.02^a$  mg/100g) and Se

content ( $7.17 \pm 0.01^a$  mg/100g). This suggests that *V. u. subsp. Sesquipedalis* could be a better beans in terms of mineral composition as compared to the rest 3 species studied. Similarly, *C. cajan* had the highest Cu content ( $1.04 \pm 0.03^a$  mg/100g) which could be a good source of Cu for metalloproteins such as superoxide dismutase, quercetin 2,3dioxygenase, polyphenol oxidase etc.

The results obtained as a measure to assess the liver function response were presented in Figures 1-7. The effects on serum ALT level (Fig. 1) revealed a decrease in serum ALT levels (IU/L) when compared to the control at  $P < 0.05$ . The results suggests that the beans could be good to the liver as an elevated concentration of ALT in the serum suggests liver injury. Similar result was obtained for the serum AST level with no significance difference among the various groups. The decreases in the levels of ALT and AST in groups fed *P. vulgaris*, the red and black turtle beans is in line with the reports of Luka *et al.* (2013). In the ALP result, there was a significant increase ( $P < 0.05$ ) in the level of serum ALP in the group fed *V. u. subsp. Sesquipedalis* ( $174.30 \pm 10.48$  IU/L) compared to the control ( $125.43 \pm 5.34$  IU/L) (Fig. 3). ALP originates mainly from two sources: liver and bone (Pratt and Kaplan, 1999). High levels of ALP may indicate liver damage, blockage of the bile ducts, or a bone disease. Low ALP can caused by a variety of conditions, including zinc deficiency, malnutrition, and Wilson disease. The results obtained for the level of bilirubin, serum total protein, albumin, and globulin in all the groups fed with different species of beans as compared to the control followed the same pattern (Figs. 4-7). There was no significance differences when compared to the control. This suggest that the rats responded well with no observable effects.

The effects of the beans species on the kidney function through the determination of the urea and creatinine levels shows that there was a significant decrease in the levels compared to the control but with no significance differences across the groups (Fig. 13-14). An abnormal increase would have indicated negative effects but the reverse is the case which suggests that the beans improved renal functions and are good for consumption.

### **Conclusion**

The results obtained from the *in vitro* and *in vivo* studies suggests that the beans (*P. vulgaris* L 'Red kidney', *P. vulgaris* 'Black turtle', *C. Cajan*, and *V. u. subsp. Sesquipedalis*) are good sources of nutrients with no significant adverse effects. Although with some exhibition of differences in the physiological responses among the rats which somewhat is unfavorable based on biochemical interpretation, it is advisable to moderate and alternate the consumption of various beans species. Also, the results from the phytochemical studies gave insight why some species of beans are consumed more than the others. These species of beans contains high nutritional components that are of nutritional interest as evident in their effects in some biochemical parameters. The inclusion of these varieties of beans should be encouraged but should be moderated/alternated.

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