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Effect of Aqueous Seed Extracts of Two Varieties of *Phaseolus Vulgaris* on the Lipid Profile of Rats

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ABSTRACT

Comparative studies on the in vivo effect of aqueous seed extracts of Phaseolus vulgaris on serum lipid profile were carried out using the white and black varieties of the sample. Preliminary analysis reveals that the two varieties consist of varying amounts of phytochemicals. The extracts had significant favourable effect ($p<0.05$) on the serum total cholesterol, high density lipoprotein, low density lipoprotein and triacylglycerol concentrations in rats.
Keywords: Phaseolus vulgaris, lipid profile, high density lipoprotein, low density lipoprotein, total cholesterol, triacylglycerol

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INTRODUCTION

High consumption of legumes has been associated with 82% reduction in the risk of coronary heart diseases (Bazzano *et al.,* 2003). Beans are a good source of soluble dietary fiber (Anderson *et al*., 1994) which had been shown to reduce blood cholesterol in epidemiologic (Brown *et al*., 1999), clinical (Anderson, 1987 and Anderson *et al*., 1990), and animal (Rosa *et al*., 1998a and Rosa *et al*., 1998b) studies. In carefully controlled clinical studies where the macronutrient intake was matched and the fiber content in the bean fed group was at least twice that of the control diet, significant reductions in both total and LDL cholesterol occurred (Anderson *et al*, 1984; Anderson, 1987). Significant increases in HDL cholesterol (Anderson, 1987; Anderson *et al*., 1990; Shutler *et al*., 1989) and/or reductions in triglycerides (Anderson *et al*., 1990; Jenkins *et al*., 1988) were also seen in many but not all of the studies (Anderson *et al*., 1984; Anderson, 1987). In addition to cholesterol, recent attention has focused on high levels of plasma homocysteine as an independent risk factor for vascular disease (Boushey *et al*., 1995; Selhub *et al*., 1996). Using metaanalysis, Boushey *et al*. (1995) determined that individuals with elevated homocysteine had 1.7 to 2.5 times greater risk for developing cardiovascular disease. Plasma homocysteine had been shown to be inversely related to plasma folate levels and with intake of dietary folate and vitamin B6 (Selhub *et al*., 1996). Cleophas *et al*., (2000) posited that increasing the consumption of folate-containing foods may lower the prevalence of vascular disease in people with elevated homocysteine. Beans are rich sources of folate.

It is well known that diet plays an important role in the control of cholesterol homeostasis. In this context, it has been reported that legumes lower serum LDL-cholesterol (Duane, 1997). Although most of the studies have been carried out using soyabean, other legumes such as kidney beans, peas, chickpeas, etc have also shown hypocholesterolaemic properties (Sharma, 1987; Kingman *et al*. 1993; Zulet & Martinez, 1995; Zulet *et al*. 1999). Different components such as protein, amino acids and peptides, isoflavones, saponins, phytic acid, fibres, and protease inhibitors have been suggested as being responsible for the hypocholesterolaemic effect of legumes (Potter, 1995). With regards to the mechanisms underlying the effects of soyabean protein, Nagata *et al*. (1982) showed that soyabean protein increased faecal cholesterol excretion as a consequence of a reduction of intestinal absorption. In other studies, it had been suggested that soyabean protein increases cholesterol saturation of bile by increasing biliary secretion of cholesterol (Potter, 1995). Moreover, consumption of soyabean protein may be associated with an increased removal of LDL and VLDL by hepatocytes as compared with casein consumption (Khosla *et al*. 1991; Macarulla *et al*., 2001). Bourdon *et al* (2001) reported that fiber content of beans prolongs the postprandial presence of intestinally derived lipoproteins and augments the cholecystokinin response to the meal leading to hypocholesterolemic effect. Sources of viscous polysaccharides lower plasma cholesterol in humans, which contributes to the reduction in risk of cardiovascular disease associated with diets high in fiber-rich foods (Brown *et al*., 1999; Schneeman, 1999). Several studies have associated the cholesterol-lowering of beans with their content of viscous polysaccharides; however, the protein in beans may also contribute to the response (Shutler *et al*., 1989; Fruhbeck *et al*., 1997; Dabai *et al*., 1996; Duane, 1997)

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MATERIALS AND METHODS

Plant Material

White and black varieties of *Phaseolus vulgaris* bean were obtained from Nsukka in south eastern Nigeria and identified at the Bioresources and Development and Conservation Centre (BDCP) Nsukka, Nigeria where a voucher had been deposited.

Animals

Adult male wistar rats between 10 and 16 weeks old of average weight 160±13g were obtained from the animal house of the Faculty of Biological Sciences, University of Nigeria Nsukka. The animals were acclimatized for seven days under standard environmental conditions, with a 12 hour light/dark cycle maintained on a normal rat feed and water *ad libitum*.

Preparation and Extraction of Sample

Known weights of the white and black bean varieties were extracted by continuous boiling for five hours using aqueous solvent. The extracts were first filtered with four layers of muslin cloth before filtering with a Whatman no. 1 filter paper. The aqueous extracts were subsequently concentrated to obtain a semi-solid extract and weighed. A quantity each of the semi-solid extracts dissolved in normal saline was used for animal treatment.

Phytochemical Analysis

The phytochemical analysis of aqueous extracts of the white and black varieties of *Phaseolus vulgaris* seeds were carried out according to the methods of Harbourne (1973), and Trease and Evans (1989).

Acute Toxicity Study (LD_{50}) of the Extract

The acute toxicity study (LD_{50}) of the extract was determined using the method of Lorke (1983).

Experimental Design

A total of twenty four (24) adult male wistar rats were divided into four (4) experimental groups of six (6) rats each. Each group was further divided into two subgroups of three (3) rats each. They were administered (by oral intubation) varying doses of the white and black bean extracts for seven (7) days. Group one fed with normal rat diet and water ad libitum is the normal control. Group two were fed with normal diet and 100mg/kg b.w. of the white and black aqueous seed extracts. Group three were fed with normal rat diet and 300mg/kg b.w. of the two extracts. Group 4 were fed with normal rat diet and 500 mg/kg b.w. of the two extracts. After treatment, blood was drawn via ocular puncture and the serum was used to determine the lipid profile. Total cholesterol, high density lipoprotein, low density lipoprotein and triacylglycerol were determined using standard methods.

Statistical Analysis

The results were analyzed with one way ANOVA expressed as Mean ± SD. The Fischer LSD post hoc test was used to test the difference between mean of treated and control groups were regarded significant at $P<0.05$.

RESULTS

Percentage Yield of Extracts

Table 1: Percentage Yield of Aqueous Extracts of White and Black Varieties of *Phaseolus vulgaris*

Extract	Percentage yield (%)
White Variety	5.45
Brown Variety	7.65

The percentage yields of the white and brown varieties were found to be 5.45% and 7.65% respectively.

Phyochemical Analysis

Table 2: Qualitative Result of Phytochemical analysis of the white and black varieties of *Phaseolus vulgaris*

Phytochemical	Composition	
	White Variety	Black Variety
Alkaloids	+	+
Flavonoids	+	+
Tannins	+++	++
Reducing sugar	+++	++
Cyanogenic Glycosides	+	++
Saponins	+++	++
Soluble carbohydrate	+++	++
Glycosides	++	++
Steroids	++	+

+++ = Relatively high abundance of compound; ++ = Moderate presence; + = low presence

From the result of phytochemical analysis, tannins, alkaloids, reducing sugar, saponins, glycosides, flavonoids, carbohydrate and steroids were all present.

Table 3: Quantitative Result of Phytochemical analysis of the white and black varieties of *Phaseolus vulgaris*

Phytochemical	Composition	
	White Variety	Black Variety
Alkaloids	2.46 ± 0.002	1.45±0.002
Flavonoids	0.89 ± 0.005	2.53±0.003
Tannins	0.05 ± 0.003	0.05±0.003
Reducing sugar	1.43±0.004	4.43±0.004
Cyanogenic Glycosides	0.07±0.008	0.06±0.004
Saponin	446.09±0.003	327.0±0.003
Soluble carbohydrate	11.87±0.002	13.08±0.004
Glycosides	3.81±0.005	3.76±0.003

The result shows the relative values of the identified phytochemical present in the aqueous extracts.

Acute Toxicity Studies

The aqueous extracts showed no toxicity at the dosage of 5000mg/kg b.w.

Lipid Profile

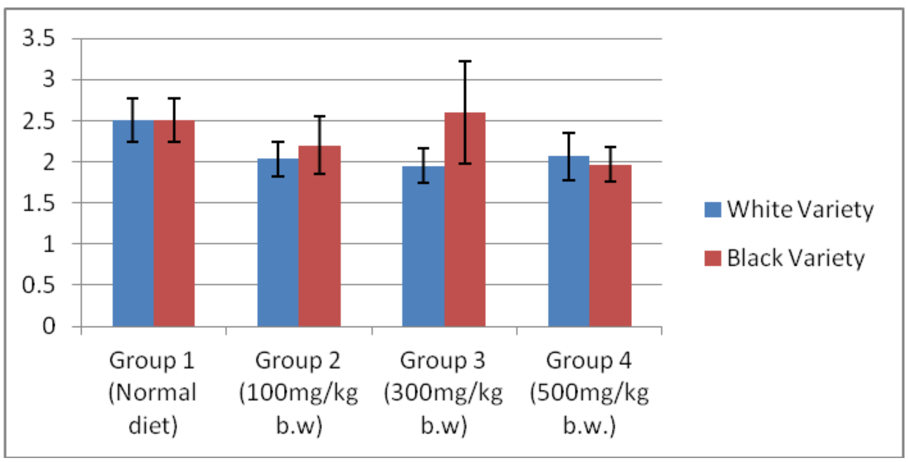

Figure 1: Effect of acute feeding of aqueous extracts of white and black varieties of *Phaseolus vulgaris* on the total cholesterol concentration in rats

Aqueous extracts of *Phaseolus vulgaris* had significant cholesterol lowering effect at the different doses administered. The decrease were however not significant across the treatment groups and between the two extracts.

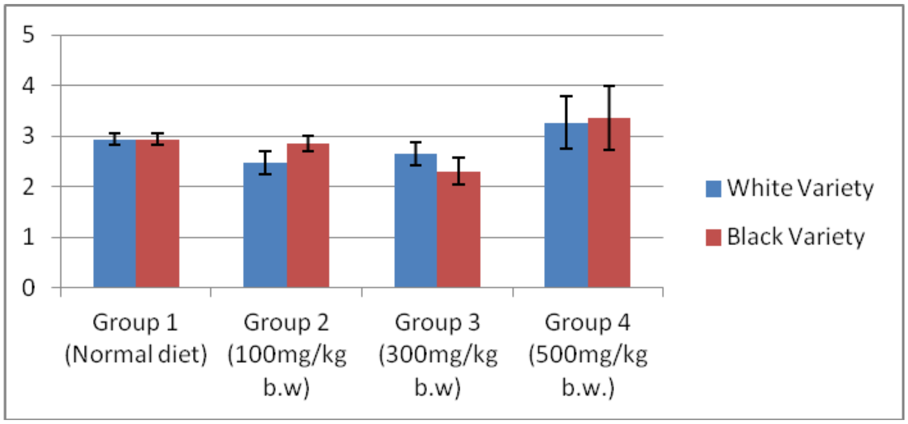

Figure 2: Effect of acute feeding of aqueous extracts of white and black varieties of *Phaseolus vulgaris* on the high density lipoprotein concentration in rats

At high doses of the extracts, the two varieties had significant increase in high density lipoprotein when compared to the control. However, the increase was not significant between the aqueous extracts of the two varieties.

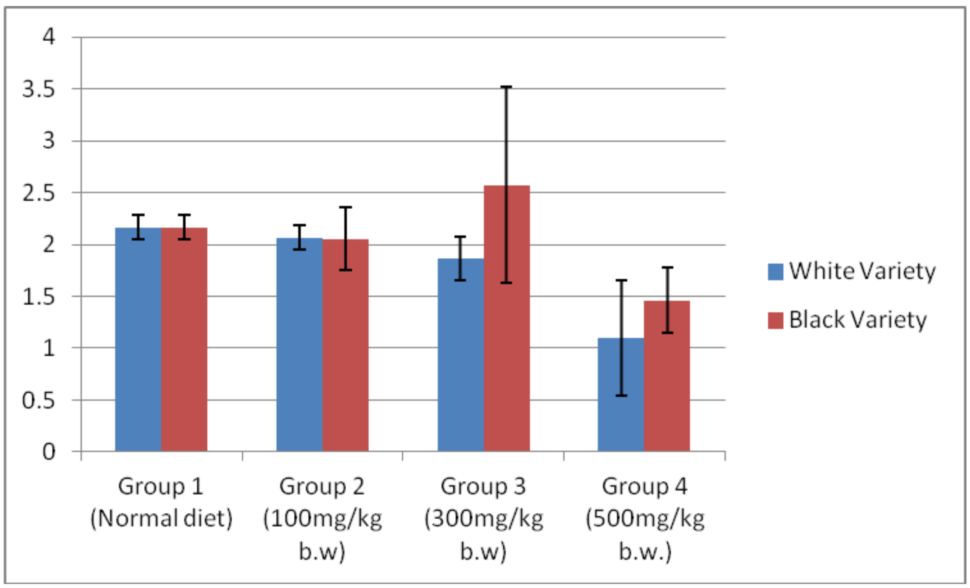

Figure 3: Effect of acute feeding of aqueous extracts of white and black varieties of *Phaseolus vulgaris* on the low density lipoprotein concentration in rats

At high doses of the extracts, there was significant decrease in low density lipoprotein. The white variety showed significant decrease in LDL when compared to the black variety at 500mg/kg b.w

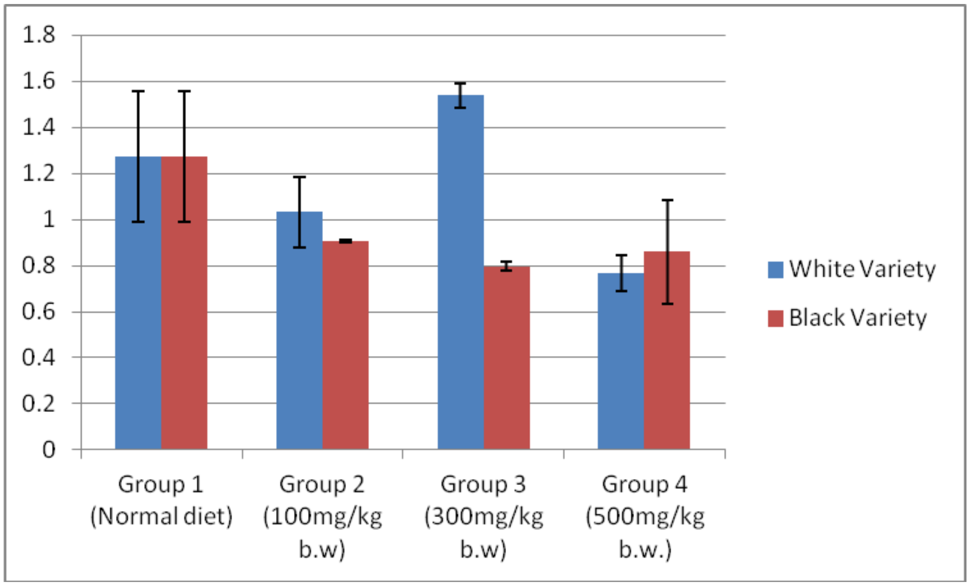

Figure 4: Effect of acute feeding of aqueous extracts of white and black varieties of *Phaseolus vulgaris* on the serum triacylglycerol concentration in rats.

The aqueous extracts showed marked decrease in triacylglycerol concentration when compared with the control. There was no significant difference in decrements between the two extracts.

DISCUSSION

Phytochemical analysis shows that the two varieties of Phaseolus vulgaris contain alkaloids, saponins, glycosides, reducing sugar, tannins, flavonoids, and soluble carbohydrate. They do not differ significantly in their contents of glycosides, soluble carbohydrate and tannins but not alkaloids, flavonoids, reducing sugar, and saponin. The high amount of phytochemicals probably accounts for their cholesterol lowering effect and may contribute to the vast pharmacological properties of white and black beans. Saponins have been shown to reduce cholesterol by forming insoluble complexes with cholesterol and bile, making them unavailable for absorption (Oboh and Osagie, 2003). The percentage yield for the black variety is higher (7.65%) when compared to the white variety (5.45%). Result of lethal toxicity test (LD_{50}) indicated at a dose of 5000mg/kg, both aqueous extracts did not exhibit toxic effect and hence, are considered safe for consumption. Result on figure one reveals that the two extracts had significant lowering effect on the serum total cholesterol; however, the white variety had more effect than the black variety at the doses studied. High concentration of both extracts significantly ($p<0.05$) increased the high density lipoprotein concentration. The lowering effect of the two extracts on low density lipoprotein concentration is dose dependent. The white variety had significant decrease on LDL concentration when compared with the black variety. Similarly, the two extracts were shown to lower the serum triacylglycerol significantly and in dose dependent manner when compared to the control. The deviations obtained in group three could be due to experimental error. The effects of the aqueous extracts of *Phaseolus vulgaris* on serum lipid profile could be attributed to its rich composition of antinutrients and phytochemicals which help to modulate physiological lipid concentration. Lipid profile appears to be a significant factor in the development of atherosclerosis and includes an increase in triacylglycerol, LDL and total cholesterol (Orchard, 1990). From the results obtained, there was gradual decrease in these lipids in groups treated with the aqueous extracts of the white and black varieties when compared with the control. These results agree with that reported by Carew *et al*., 2003 who observed a reduction in cholesterol and triacylglycerol after treatment with velvet beans and attributed the effect to the availability of saponins. Sugano *et al*. (1990) observed serum cholesterol and triacylglycerol levels in rats fed with soy beans and they attributed the lowering effect to saponins. Similar results could be expected in animals fed with the two varieties of *Phaseolus vulgaris* due to the presence of saponins as shown by the phytochemical analysis. The results of this study also reveals that the reduction in lipid profile in both extracts were dose-dependent. This is in agreement with the report of Jayaweera *et al* (2007) that 20% and 25% extracts of velvet beans reduced the serum total cholesterol by 15% and 16% respectively.

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