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**Research Article**

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# An Investigation of the Antihyperlipidemic and Antioxidant Activities of the Crude Extract and Fractions of *Phaseolus vulgaris* Linn on Experimental Rats

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**Abstract** This research aimed at investigating the antihyperlipidemic and antioxidants activities of the crude extract and fractions of *Phaseolus vulgaris Linn*. The crude extract (PVE) of dried pulverized plant material was obtained by maceration in methylene/methanol (1:1) while the solvent fractions were obtained by successive solvent-solvent partition in separating funnel between the crude extract suspended in aqueous medium and solvents of increasing polarity to obtained the n-hexane fraction (PVHF), ethylacetate fraction (PVEF), and butanol fraction (PVBF) in that order. Acute, sub-acute and chronic protocols were used in the investigation of antihyperlipidemic effects. DPPH scavenging and nitric oxide scavenging assays were used in the investigation of the extracts and fractions for the *in vitro* antioxidant activities study. The antioxidant activities of the extract and fractions were further determined *in vivo* in rats. Antioxidant enzymes and factors such as catalase, glutathione peroxidase, and lipid peroxidation activities were measured in carbon tetrachloride-treated rats treated with or without the extract and fractions studies. The findings of this study shows that the various extracts of *Phaseolus vulgaris L*. have antihyperlipidemic and antioxidant effects; the ethylacetate fractions showing substantially significant activity.

**Keywords** *Phaseolus vulgaris*, fractions, lipid peroxidation, antihyperlipidemia

## Introduction

Free oxygen radicals or reactive oxygen species (ROS) are generated naturally by biological systems as a result of various cellular activities or from exposure to xenobiotic. This radical plays an active role in the pathogenesis of many human diseases including cardiovascular diseases [1-2]. A large volume of scientific research suggests that in situations of oxidative stress, reactive oxygen species (ROS) are generated and a homeostatic environment between anti-oxidant and oxidation are known to be an important concept for maintaining a healthy biological system [3]. Reactive oxygen species (ROS) such as superoxide anions ($O_2^-$), nitric oxide (NO) and hydroxyl radical ($OH^-$) aids in the inactivation of enzymes and this result in damage in important cellular components such as coronary heart diseases [4].

Disease of coronary origins such as stroke, atherosclerosis, etc., continues to be the leading cause of death in most countries of the world [5]. One of the greatest risk factors in the severity and prevalence of coronary heart diseases is disorders of lipid metabolism known as Hyperlipidemia [6]. According to reports by the World Health Organization approximately 56% of coronary heart diseases are as a result of hyperlipidemia and this result in about 4.4million deaths each year worldwide [7]. Hyperlipidemia is a disorder of lipoprotein metabolism, including lipoprotein overproduction or deficiency and manifested by elevation of the serum total cholesterol, low-density lipoprotein

(LDL) cholesterol and triglyceride concentrations, and a decrease in the high-density lipoprotein (HDL) cholesterol concentration [8].

Modern drugs (Over 50%) are of natural product origin [9] and they play an important role in pharmaceutical industry drug development programme [10]. *Phaseolus vulgaris* L. (Leguminosae) is commonly known as kidney bean, various parts of the plant have been used extensively for the treatment of diabetes mellitus traditionally [11]. Previous studies have reported the hypolipidemic activities of the aqueous extract [12], as well as, the anti-inflammatory, antimutagenic, antioxidant, antimicrobial and antioxidant activities of the extract [13]. The present study aims to establish the antihyperlipidemic and antioxidants effects of various fractions of *Phaseolus vulgaris L*. fruit and determine the most active of the fractions.

## Materials and methods

### *Experimental animals*

Wistar albino male rats weighing about 120-200 g were procured from laboratory animal facility of the Department of Pharmacology and Toxicology, University of Nigeria, Nsukka. Animals were housed in steel cages under standard conditions and fed with standard pellets and water *ad libitum*. The animals were allowed to acclimatize for two weeks, prior to the commencement of the experiment. Guidelines followed in the handling of animals were in accordance with the ethical standards of the Institutional Animals Ethics (IAEC), as adopted by the ethical committee of the Faculty of Pharmacy, University of Nigeria, Nsukka, Nigeria.

### *Collection and Preparation of plant extract*

Fresh fruit of *Phaseolus vulgaris L.* were obtained from Obollo-Afor in Nsukka Local Government Area, Enugu State, Nigeria and authenticated by Mr. A. Ozioko, a *taxonomist* with the Bio-resources Development and Conservation Programme (BDCP) center, Nsukka, Enugu State, Nigeria. The plant materials were sun dried and then pulverized using the laboratory grinding machine at the Department of Crop Science, University of Nigeria, Nsukka. One (1) kg of the powdered extract was dissolved in 2.5 L of a mixture of methanol and methylene chloride in the ratio 1:1 to obtain the crude extract (PVE). A portion of the extract was suspended in distilled water and fractions were made by adding solvents with increasing polarity successively i.e. n-hexane (PVHF), ethylacetate (PVEF) and n-butanol (PVBF). The layers were separated accordingly and the fractions were dried.

### *Phytochemical screening*

Chemical tests for the phytochemistry of the medicinal plants under study were carried out using the standard procedures as described by [14].

### *Acute toxicity ($LD_{50}$) study*

The acute toxicity ($LD_{50}$) study was carried on the crude extract of *Phaleosus vulgaris L.* using Lorke's method [15]. Animals (rats) of either sex received oral doses of 10, 100, 1000 mg/kg in phase I, while 1600, 2500, 3900, and 5000 mg/kg were given in phase II to the treated groups. The median lethal dose ($LD_{50}$) was estimated after observing the animals for 24 hours for obvious toxic symptoms or mortality.

### *HPLC-fingerprinting studies*

The various constituents present in PVE and their relative abundance was determined by comparing their peaks and peak areas with those of known standard compounds in an analytical HPLC 'finger- printing' procedure. A solution of 1 mg PVE/mL was prepared in HPLC grade methanol. The solution was centrifuged and HPLC analysis was carried out on the supernatant with a Dionex P580 HPLC system coupled to a photodiode array detector (UVD340S). Detection was at 235 nm. The separation column (125 X 4 mm; length _ internal diameter) was prefilled with Eurospher-10 C18 (Knauer, Germany), and a linear gradient of nanopure water (adjusted to pH 2 by addition of formic acid) and methanol was used as eluent.

### *Hypolipidemic studies*

Acute, subacute and chronic studies to determine the lipid lowering effects of various extract of *Phaseolus vulgaris* were carried out using the following experimental design: Fifty-five rats randomly divided into eleven groups of five rats each where used. Group 1 rats served as the positive control and were treated with 10 mg/kg of atorvastatin. Group 2 (untreated group), Group 3, 4, 5 where treated with the crude extract at 100 mg/kg, 200 mg/kg and 400

mg/kg respectively while group 6 and 7 where treated with PVHF at 100 mg/kg and 200 mg/kg with group 8 and 9 administered the PVEF at 100 mg/kg and 200 mg/kg and group 10 and 11 treated with PVBF at 100 mg/kg and 200 mg/kg respectively

***Acute study***

Each group where given various doses of the test drugs, an hour later intra-peritoneal injection of 200 mg/kg of Trition was administered to each rats [16, 17, 18, 19]. Twenty-four hours after treatment, blood was drawn from the retro-orbital plexus of the rats and biochemical analysis was done using randox lipid profile kit.

***Sub-acute study***

Hyperlipidemia was induced using Trition-X 100 at 100mg/kg to all rats in each groups, after 72hours of trition administration, the test extract was administered for 7days, on the $8^{th}$ day the animals where sacrificed and blood used for biochemical analysis [20].

***Chronic study***

Three rats where drawn at random from each group and analysis where done to determine the baseline value of lipid profile parameters. Each group where given a high fatty diet throughout the experimental duration (4weeks), after the first week the same three rats from each groups where tested to determine the level of induction of hyperlipidemia.

After achieving satisfactory hyperlipidemic state using high fat diet comprising of the chow enriched with high calorie and 1% cholesterol [21], all rats were given various doses of the test drugs for three weeks after which they were sacrificed and lipid profile analysis was done.

***Anti-oxidant activity***

Anti-oxidant activities where determine by in-vitro and in-vivo studies. In the in-vitro studies free radical scavenging assay was determine using DPPH radical scavenging activity and nitric oxide scavenging activity while in-vitro activities was determine by measuring tissue estimates of catalase, glutathione-peroxidase and lipid peroxidation activities.

***DPPH scavenging activity***

The free radical scavenging activity of the test substances were determined by measuring the change in absorbance of DPPH radicals at 517 nm [22]. The sample extract (0.2 mL) is diluted with methanol and 1.8 mL of DPPH solution (0.1 mM) is added. After 30 min, the absorbance is measured at 517 nm. The percentage of DPPH scavenging activity was determined as follows, DPPH Radical Scavenging Activity (%) = [(A0−A1)/A0] where A0 is the absorbance of control and A1 is the absorbance of sample.

***Nitric oxide scavenging activity***

Nitric oxide scavenging activity was measured spectrophotometrically by using Greiss reaction [23]. Extract, prepared in ethanol, was added to different test-tubes in varying concentrations (25mg- 400mg). Sodium nitroprusside (5mM) in phosphate buffer was added to each test tube to make volume up to 1.5ml. Solutions were incubated at 25ºC for 30 minutes. Thereafter, 1.5ml of Griess reagent (1% sulphanilamide, 0.1%naphthylethylenediamine dichloride and 2% phosphoric acid) was added to each test tube. The absorbance was measured, immediately, at 546 nm and percentage of scavenging activity was measured with reference to ascorbic acid as standard.

***In-vivo study***

Fifty five rats were randomly divided into eleven groups of five rats each; Group 1 rats served as the positive control and were treated with ascorbic acid. Group 2 (untreated group), Group 3, 4, 5 where treated with the crude extract at 100mg/kg, 200mg/kg and 400mg/kg respectively while group 6 and 7 where treated with n-hexane fraction at 100mg/kg and 200mg/kg with group 8 and 9 administered the ethylacetate fraction at 100mg/kg and 200mg/kg and group 10 and 11 treated with butanol fraction at 100mg/kg and 200mg/kg respectively.

Test and control group animals were intoxicated with $CCl_4$ for 2 days followed by administration of test and control substances for 7days. After the treatments, the animals were starved overnight and sacrificed under mild chloroform anesthesia. Blood and liver tissues were harvested and use for further analysis [24].

***Estimation of catalase activity***

Volume of 2.5 ml of phosphate buffer is pipette into a test tube along with 2 ml of hydrogen peroxide and 0.5 ml of sample. To 1 ml portion of the reaction, add 2 ml of dichromate acetic acid reagent. The absorbance at 240 nm is determined into 4 places at a minute interval [25].

***Estimation of lipid peroxidation***

Lipid peroxidation was determined spectrophotometrically by measuring the level of the lipid peroxidation product, malondialdehyde (MDA). A volume of 0.1 ml of liver tissue homogenate samples were treated separately with 2 ml of TBA-TCA-HCl reagent (1:1:1 ratio, 0.37% TBA, 0.25 N HCl, 10% TCA) and incubated at 95$^{o}$C for 40mins and cool in water. 0.1ml of 20% SDS (sodium dodecyl sulphate) was added and the absorbance at 532 nm was determined against a blank [26].

***Estimation of gluthathione peroxidase***

Glutathione peroxidase (GPx) activity was determined spectrophotometrically [27]. A volume of 0.1 ml of liver tissue homogenate samples was added to 3 mls of phosphate buffer solution, 0.05 ml of gluaiacol and 0.03 ml of hydrogen peroxide. The absorbance was taken at 436nm for 2 mins at 30secs interval.

***Statistical Analysis***

Significant difference between control and experimental groups were obtained by one way ANOVA using Graph pad Prism followed by Dunnet's test. All data obtained were expressed as Mean ± SEM (standard error of mean). Graphical representation was done using Graph pad Prism. P-values <0.05 were considered significant.

## Results

***Fractionation and phytochemical analysis***

After extraction the crude extract yielded a residue of 58.6g (11.72%) while the various fraction yields are as follows n-hexane 4.85g (8.27%), ethylacetate 6.72g (11.47%) and butanol 4.87g (8.31%). Phytochemical analysis showed the presence of various chemical constituents as shown in Table 1.

**Table 1:** Phytochemical screening of all fractions of *Phaseolus vulgaris L*

| Phytochemicals | Crude Extract | n-hexane Fraction | Ethylacetate Fraction | Butanol Fraction |
|---|---|---|---|---|
| **Tannins** | +++ | ++ | +++ | ++ |
| **Saponins** | +++ | +++ | +++ | +++ |
| **Flavonoids** | ++ | +++ | +++ | ++ |
| **Steroids** | + | + | + | + |
| **Alkaloids** | + | + | + | - |
| **Terpernoids** | + | - | - | + |
| **Carbohydrates** | + | ++ | ++ | + |
| **Resins** | - | - | - | - |
| **Proteins** | - | - | - | - |
| **Reducing Sugar** | ++ | ++ | + | + |

*+ = activity present*

*- = no activity present*

***Acute toxicity study***

No death was recorded in the acute toxicity testing for all animals and there were also no obvious signs of toxicity in all treatment groups during both phase of the study when observed within 24 hours of post-administration of the crude extracts of *Phaselous vulgaris L.* The $LD_{50}$ of the extracts was greater than 5000 mg extract/kg body weight.

***HPLC analysis***

Qualitative and quantitative analysis of the crude extract of *Phaselous vulgaris L.* showed the presence of various compounds as seen in Figure 1 and Table 2.

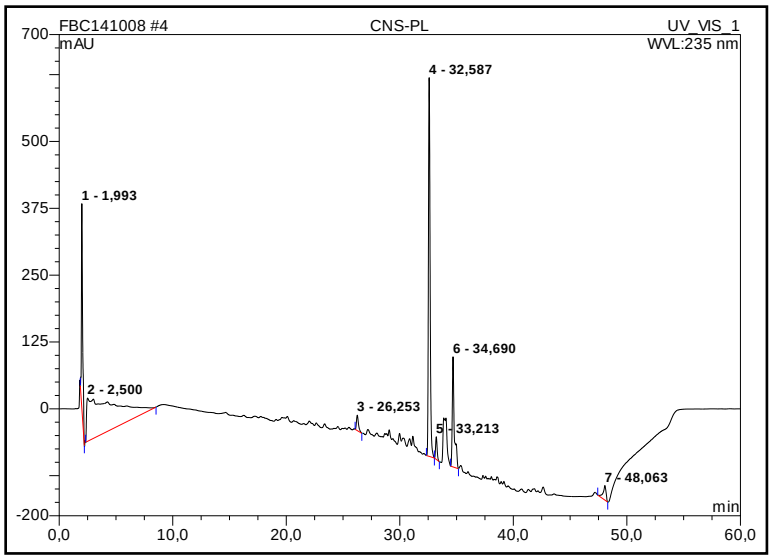


*Figure 1: HPLC-profile studies*

**Table 2:** HPLC-Analysis Table Showing Compounds Name

| No. | Ret. Time | Peak Name | Height | Area | Rel. Area | Amount | Type |
|---|---|---|---|---|---|---|---|
| | **min** | | **mAU** | **mAU*min** | **%** | | |
| **1** | 1.99 | GLUCOBRASIN | 383.059 | 47.782 | 10.59 | n.a. | BMB |
| **2** | 2.50 | WAOLSAURE | 80.823 | 220.220 | 48.79 | n.a. | BMB |
| **3** | 26.25 | DAIDZIN | 28.757 | 5.884 | 1.30 | n.a. | BMB |
| **4** | 32.59 | CATECHIN | 708.202 | 120.661 | 26.73 | n.a. | BMb |
| **5** | 33.21 | CATECHIN | 42.345 | 6.579 | 1.46 | n.a. | bMB |
| **6** | 34.69 | n.a. | 206.117 | 42.214 | 9.35 | n.a. | BMB |
| **7** | 48.06 | n.a. | 27.313 | 8.022 | 1.78 | n.a. | BMB |
| **Total:** | | | 1476.616 | 451.361 | 100.00 | 0.000 | |

***Acute study results***

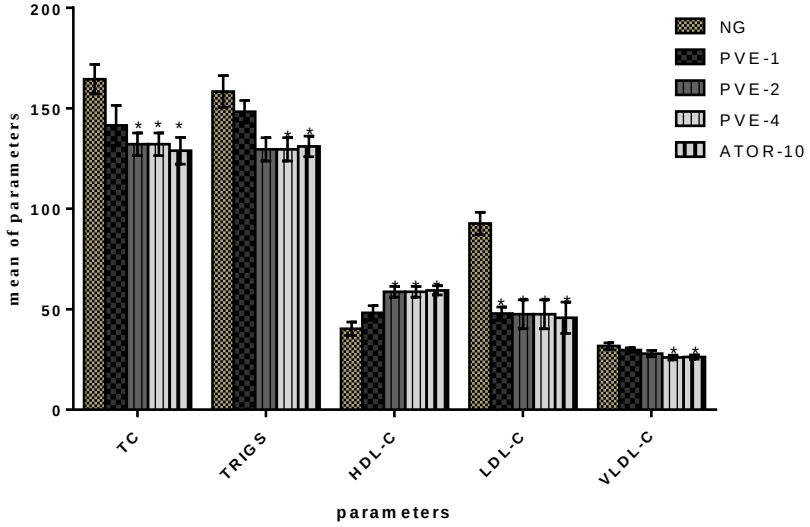


*Figure 2: The effects of crude extract of Phaseolus vulgaris L. on lipid profile parameters*

Serum triglycerides, total cholesterol, LDL-C, VLDL-C were found to have a dose dependent significant (*P≤0.05*) decrease with the crude extract, hexane fraction and ethylacetate fractions when compared with the untreated groups, with the ethylacetate fractions showing a comparable level of decrease as the positive control. There was also a significant (*P≤0.05*) increase in HDL-C level for crude extract, PVEF and PVHF while the PVBF showed significance (*P≤0.05*) only with serum triglycerides and VLDL-C levels. As seen in Figures 2 and 3.

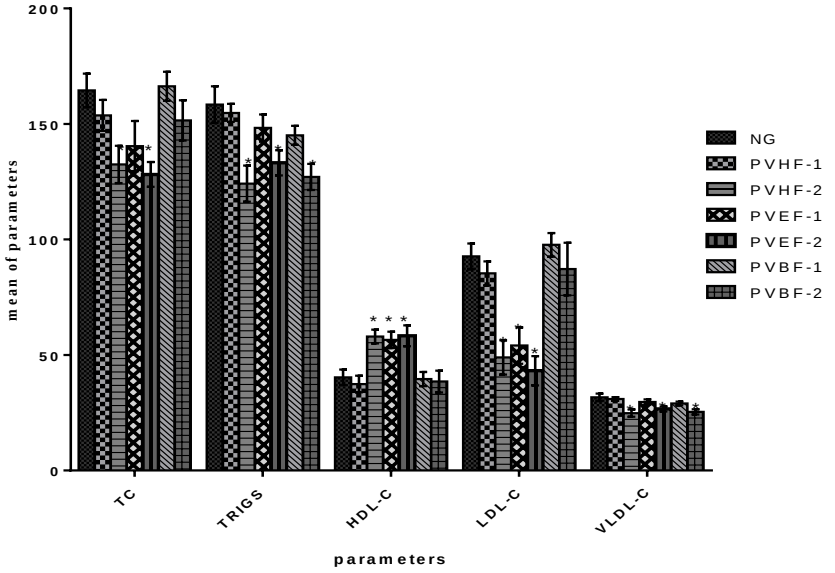


*Figure 3: The effects of various fractions of Phaseolus vulgaris L. on lipid profile parameters on Wistar rats*

***Subacute study***

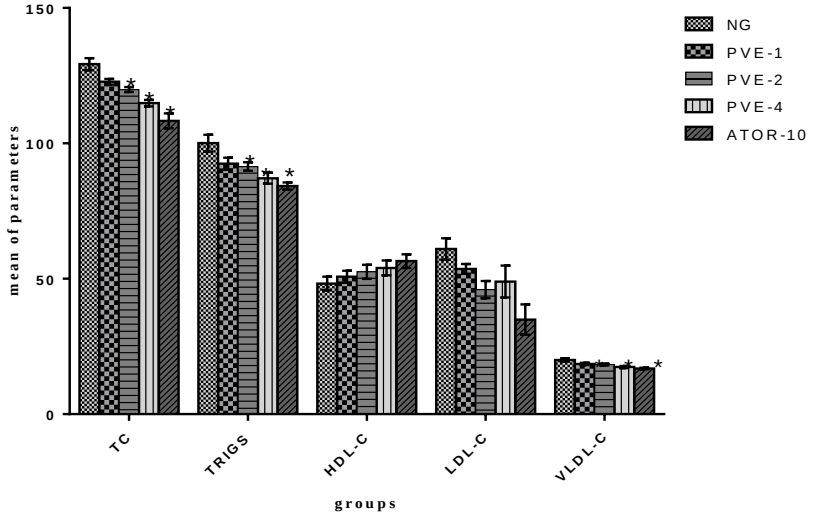


*Figure 4: The effects of crude extract of Phaseolus vulgaris L. on lipid profile parameters*

Serum triglycerides, total cholesterol and VLDL-C levels were found to decrease significantly (*P≤0.05*) with crude extract, PVEF and PVBF fractions when compared with the untreated group, this decrease was dose dependent, the PVHF showed a non-significant (*P≥0.05*) decrease. There was also a non-significant (*P≥0.05*) increase for serum HDL-C level with all extracts when compared with the negative and a non-significant (*P≥0.05*) decrease of LDL-C for all treated groups when compared with the negative. As seen in Figures 4 and 5.

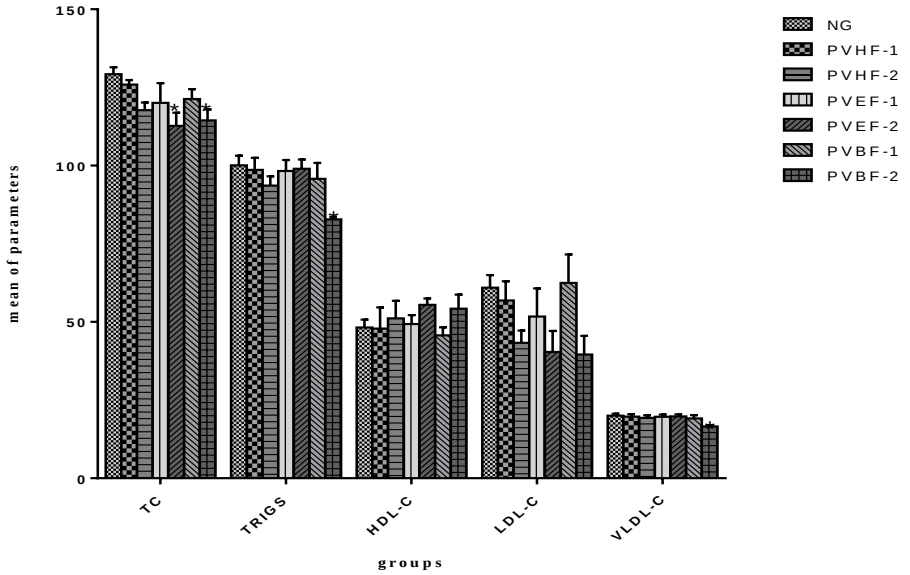


*Figure 5: The effects of various fractions of Phaseolus vulgaris L. on lipid profile parameters of Wistar rats*

***Chronic study***

Serum triglycerides, total cholesterol ,LDL-C and VLDL-C levels were found to decrease significantly (*P≤0.05*) with PVE, PVEF and PVBF fractions when compared with the untreated group, this decrease was dose dependent, the PVHF showed a non-significant (*P≥0.05*) decrease. There was also a significant (*P≤0.05*) increase for serum HDL-C level with PVE, PVHF and PVBF when compared with the untreated groups and a non-significant (*P≥0.05*) decrease with the PVEF. As seen in Figures 6 and 7.

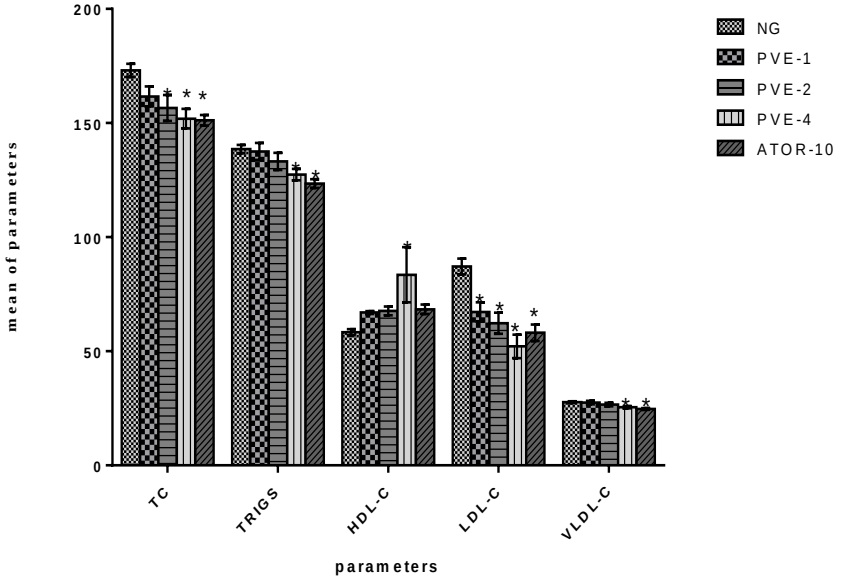


*Figure 6: The effects of crude extract of Phaseolus vulgaris L. on lipid profile parameters of Wistar rats*

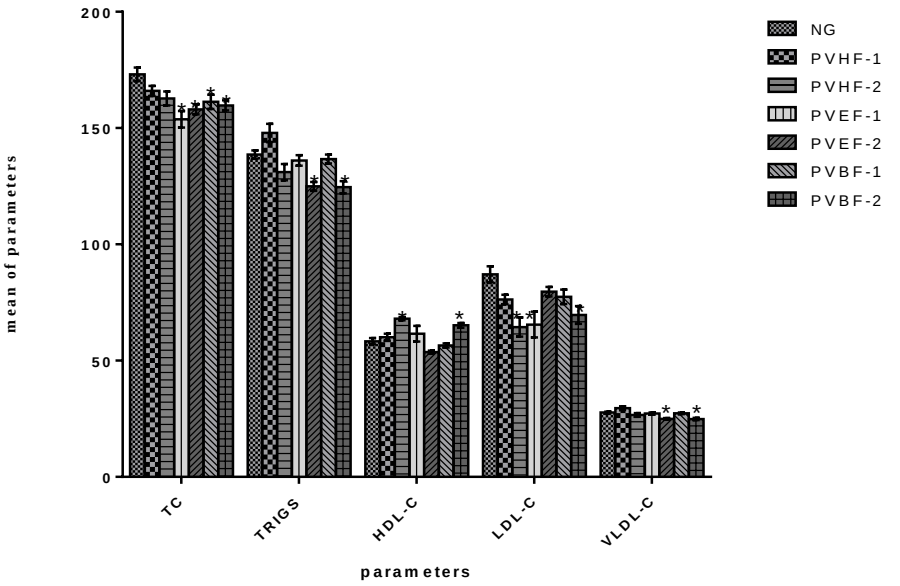


*Figure 7: The effects of various fractions of Phaseolus vulgaris L. on lipid profile parameters of Wistar rats*

***DPPH scavenging assay***

The highest scavenging assay of DPPH are 80.61% seen with PVEF and PVBF at 400 mg/kg while the highest percentage for the crude extract was 78.57% at 400 mg/kg and 75.51% also at 400 mg/kg for PVHF. The PVEF and PVBF showed a similar scavenging as ascorbic acid (85.71%). As seen in Figure 8.

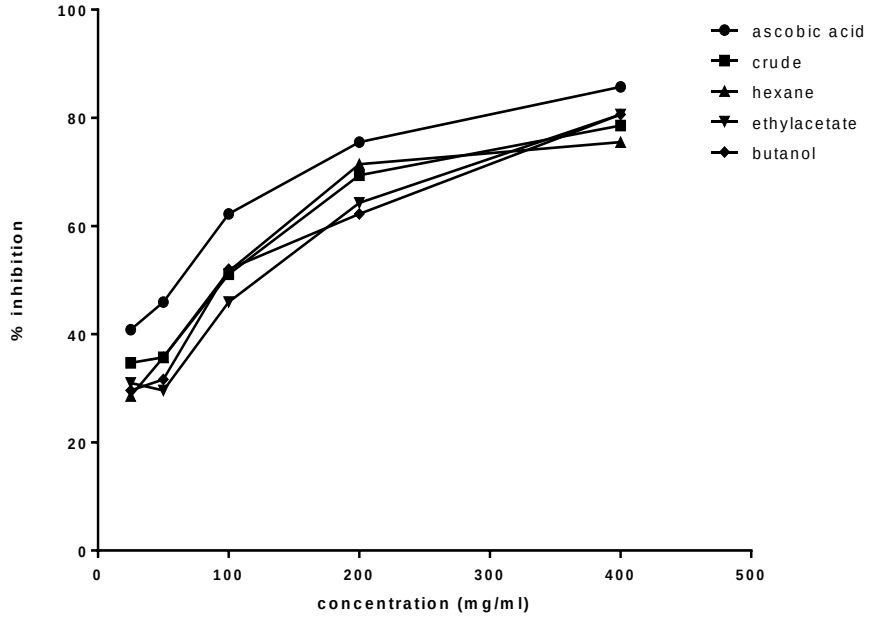


*Figure 8: DPPH scavenging activity of various extract of Phaseolus vulgaris L*

***Nitric oxide scavenging assay***

The highest scavenging assay of nitric oxide are 75.86% seen with PVHF at 200 mg/kg while the PVEF showed highest scavenging activity at 400 mg/kg (70.69%) with the PVBF fraction at 400 mg/kg have the highest percentage (67.24%) for the PVE the highest percentage (74.13%) was at 50 mg/kg with ascorbic acid highest scavenging activity occurring at 200 mg/kg (77.59%) (Figure 9).

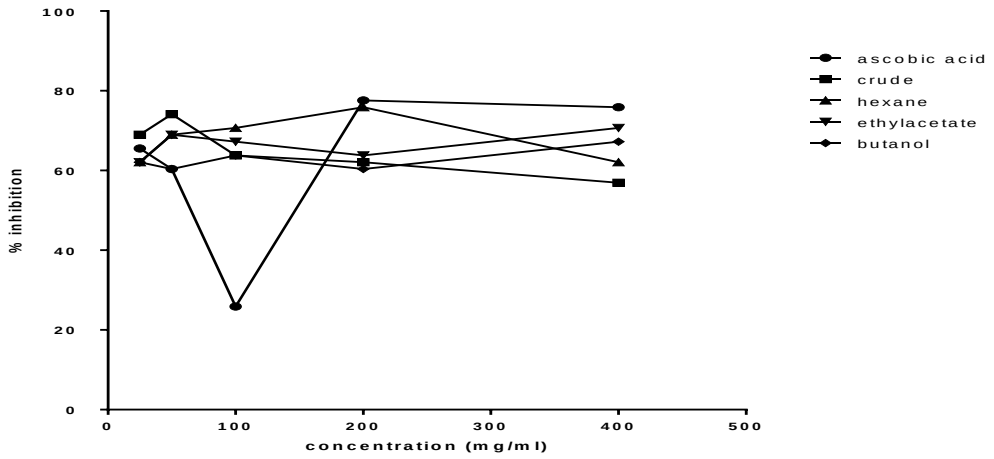


*Figure 9: Nitric oxide scavenging assay of various extract of Phaseolus vulgaris L.*

***Lipid peroxidation assay***

Lipid peroxidation levels estimated by thiobarbituric acid reaction showed no significant increase or decrease in the serum MDA of both the treated and untreated group (Table 3 and 4).

***Catalase activity***

A significant increase in catalase activity was observed with extracts treated with lower dose (100 mg/kg) of the PVE when compared with the untreated groups, while there was a non-significant (*P≥0.05*) increase for other groups with only the PVBF at 200 mg/kg showing a non-significant (*P≥0.05*) decrease when compared to the untreated group (Table 3 and 4).

***Glutathione Peroxidase assay activity***

A non-significant (*P≥0.05*) increase in glutathione peroxidase activity was observed with extracts treated with the PVE when compared with the untreated groups, while there was significant (*P≤0.05*) increase for other groups (Table 3 and 4).

**Table 3:** Effects of crude extract of *Phaseolus vulgaris L.* on antioxidant enzymes (IU/L)

| | Catalase | GPX | Lipid Peroxidation |
|---|---|---|---|
| **NG** | 0.65± 0.41 | 0.40±0.04 | 1.40±0.10 |
| **Ascorbic acid** | 2.32±0.73* | 0.99±0.15* | 2.18±0.45 |
| **PVE 100 mg/kg** | 2.09±1.68* | 0.43±0.04 | 2.44±0.57 |
| **PVE 200 mg/kg** | 1.16±0.14 | 0.34±0.02 | 1.55±0.12 |
| **PVE 400 mg/kg** | 0.85±0.12 | 0.51±0.28 | 0.53±0.16 |

*All values expressed as Mean ± SEM, where n=5, all data were analyzed by using one way ANOVA followed by Dunnet's test. p<0.05 was taken to be significant*

**Table 4:** Effects of various extract of *Phaseolus vulgaris L.* on antioxidant enzymes (IU/L)

| | Catalase | GPX | Lipid Peroxidation |
|---|---|---|---|
| **NG** | 0.65± 0.41 | 0.40±0.04 | 1.40±0.10 |
| **PVHF 100 mg/kg** | 0.68±0.16 | 1.25±0.12* | 1.57±0.11 |
| **PVHF 200 mg/kg** | 0.82±0.27 | 1.40±0.21* | 1.54±0.42 |
| **PVEF 100 mg/kg** | 0.62±0.09 | 1.13±0.21* | 1.03±0.36 |
| **PVEF 200 mg/kg** | 0.40±0.01 | 1.20±0.09* | 1.48±0.06 |
| **PVBF 100 mg/kg** | 0.75±0.17 | 1.68±0.06* | 1.25±0.22 |
| **PVBF 200 mg/kg** | 0.89±0.22 | 1.21±0.17* | 0.52±0.10 |

*All values expressed as Mean ± SEM, where n=5, all data were analyzed by using one way ANOVA followed by Dunnet's test. p<0.05 was taken to be significant*

**Discussion**

This study was carried out in order to establish the lipid lowering activity of various extract of the fruits of *Phaseolus vulgaris L.* The HPLC- fingering printing studies revealed the presence of various compounds such as daidzin and catechin. Dazidin is a natural organic compound in the class of phytochemicals known as isoflavones. It can be found in various plant materials such as soya bean leaves [28] and it is known to be a potent antioxidant compound [29]. Catechin is a plant secondary metabolite. It belongs to the group of flavan-3-ols (or simply flavanols), part of the chemical family of flavonoids [30]. High concentrations of catechin can be found red wine, black grapes broad beans etc. and its consumption has been associated with a variety of beneficial effects including increased plasma antioxidant activity (ability of plasma to scavenge free radicals, fat oxidation and resistance of LDL to oxidation [31].

In experimental studies hyperlipidemia is usually induced by genetic and dietary manipulations or through the use of certain synthetic detergents such as trition. Trition is a non-ionic detergent, Literature reports that triton induces acute hyperlipidemia by raising the serum lipoproteins levels. It actually acts on serum lipoprotein lipase by some unknown mechanism and induces hyperlipidemia. For this study anti-hyperlipidemic activity was investigated through an acute study, sub-acute study and a chronic study using triton and dietary manipulations.

The results of this present hyperlipidemic study are consistent with the findings of [12] that reported that administration of *Phaseolus vulgaris L.* resulted in hypolipidemic effects. In all models of hyperlipidemic study, there was a significant decrease in serum levels of lipid profile parameters when compared with the untreated groups albeit to varied degree. In the acute study models, the decrease in serum triglycerides, total cholesterol, LDL-C and VLDL-C is in these order PVEF > PVHF > PVE > PVBF with the PVBF showing a non-significant decrease (Figure 2 and 3), in sub-acute study serum TG, TC and VLDL-C showed a decrease in this order PVEF >PVBF > PVE > PVHF with the PVHF showing a non-significant decrease, while all the fractions showed a non-significant decrease in LDL-C and an increase in HDL-C level (Figure 4 and 5). Finally in the chronic study there was a decrease in serum TG. TC, LDL-C and VLDL-C in these order PVEF > PVBF > PVE > PVHF levels (Figure 6 and 7).

The lipid lowering effects of the various extract of *Phaseolus vulgaris* may be due to a number of factors that can act indivividually or collectively leading to the lipid lowering effects of the test substances. This factor includes interferences with production of lipoprotein, an increased in expression of hepatic LDL receptors which leads to an increased removal of LDL-C from the blood and inhibition of intestinal absorption of cholesterol [32, 33, 34].

The scavenging activity of DPPH, a stable free radical is a widely used index and a quick method to evaluate antioxidant activity [35]. DPPH is an electron acceptor, it accepts electron donated by an antioxidant compound. The DPPH radical contains an odd electron which is responsible for absorbance at 517nm which leads to a discoloration of DPPH in the presence of an antioxidant [36]. The degree of discolouration indicates the scavenging potential of the extract or antioxidant in terms of hydrogen donating ability [37]. The highest scavenging assay of DPPH was seen with both ethylacetate and butanol fraction (80.61%) at 400 mg/kg whiles the highest percentage for the crude extract was 78.57% at 400 mg/kg and 75.51% also at 400 mg/kg for hexane fraction. The ethylacetate and butanol showed a similar scavenging as ascorbic acid (85.71%). As seen in Figure 8.

Nitric oxide plays an important role in various inflammatory processes and production of a sustained level of nitric oxide can lead to tissues toxicity and vascular collapse [38]. Nitric oxide toxicity increases greatly when it reacts with superoxide radical forming peroxy nitrate anion ($ONOO^-$) [39]. The highest scavenging assay of nitric oxide are 75.86% seen with hexane fractions at 200 mg/kg while the ethylacetate fractions showed highest scavenging activity at 400 mg/kg (70.69%) with the butanol fraction at 400 mg/kg have the highest percentage (67.24%) for the crude extract the highest percentage (74.13%) was at 50 mg/kg with ascorbic acid highest scavenging activity occurring at 200 mg/kg (77.59%) (Figure 9).

Catalase, malonedialdehyde (MDA), glutathione, and peroxidase are amongst several other compounds/enzymes that are there primary expression is an indication of the body defense mechanism [40, 41]. In the presence of diseased state, there is a reduction in the activity of these enzymes [42]. There was no significant difference in lipid peroxidation activity (Table 3 and 4), although a non-significant increase was seen with the crude extract at lower

dose level while there was a significant increase in glutathione peroxidase activities with the various extract except the crude extract with the butanol fraction showing the greatest activity. With catalase activity there was a significant difference with the crude extract alone at lower dose as seen in (Table 3 and 4).

**Conclusion**

The results of these studies show that the various extracts of *Phaseolus vulgaris L*. have anti-hyperlipidemic and antioxidant effects with the ethylacetate fractions showing substantially significant activity in acute, sub-chronic and chronic models of hyperlipidemia. It also showed consistent free radical scavenging activity in both *in-vivo* and *in-vitro* antioxidant models. Further studies are encouraged in elucidating the exact mechanism of action of the ethylacetate fractions of *Phaseolus vulgaris L.*, Also, studies on the effects of these extracts on other body tissues and organs can be done.

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