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Comparative Study on the Cardio protective Properties of Leaf and Seed Extracts of *Persea Americana* against Carbon Tetrachloride Induced Cardiotoxicity in Albino Rats

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ABSTRACT

The leaves and seeds of Persea americana are employed by traditional health providers for the treatment/management of various disorders including cardiovascular diseases in Nigeria. This study was carried out to investigate and compare the cardio protective potential of the aqueous and ethylacetate leaf and seed extracts of the plant using albino rats. The study was carried out using 19 groups of 5 rats. The rats were administered 200, 400, 600 and 800mg/kg body weight of extracts for 21 days by oral gavage. The normal and negative control received normal saline while the positive control received 10mg/kg body weight vitamin C. Thereafter, 2.5 ml/kg CCl_4 in olive oil (1:1) was administered to all groups except the normal control. The animals were sacrificed and blood and organs collected for analysis. Activities of the enzymes lactate dehydrogenase (LDH) creatine kinase (CK) and the concentration of the protein cardiac tropomin I (CTnI), total cholesterol (TC), triglyceride (TG) and low density lipoprotein (LDL) in the negative control were significantly ($P<0.05$) higher than that of groups given extracts, normal and positive controls. High density lipoprotein (HDL) was significantly ($P<0.05$) lower in negative control than the other groups. The cardio protective effects of the leaf extracts were higher than that of the seed extracts.

Keywords: Cardioprotection, Persea americana, carbon tetrachloride, albino rats.

INTRODUCTION

The use of plants in treating and managing ailments started with life [1]. These plants are able to produce various chemical compounds that have therapeutic actions in men. [2]. Diseases such as cardiovascular diseases (CVD) are treated/managed using the various plant parts. The World Health Organization (WHO)(1985) estimates that some Asian and African countries depend on herbal drugs for some aspects of primary health care. This is because herbal medicines are easily accessed and affordable. They are also seen as safe because they are of natural origin [3].

Persea americana (avocado) is used by traditional health providers for treating and managing cardiovascular diseases such as hypertension. It belongs to the family *lauraceae*. It is called ube beke/ube oyibo in Igbo, pia in Yoruba, orunwu in Edo, piya in Hausa and ebem mbakara in Efik.

Cardiovascular disease (CVD) is a disease that affects the heart and blood vessels. The disease has become an important health issue of public concern [4]. It is a leading cause of death worldwide [5]. Hypertension is the most common CVD and a major public health issue in developed and developing countries [6]. Generally drugs used in managing high blood pressure in Nigeria are expensive and imported. This has lead to an upsurge in the use of traditional medicine (e.g. *Persea americana*) in treating hypertension. The leaf and seed of *Persea americana* are used for this purpose. This work studied the cardioprotective

potential of the plant parts and compared their potentials.

MATERIALS AND METHODS

Chemicals, Reagents and Equipments

The chemicals and Reagents used for this analysis were of analytical grade and products of Randox USA, May & Baker, England and Merek, Germany. All equipments used were in good working condition.

Sample Preparation

Fresh leaves and fruits of *Persea americana* were collected from a farm at Umuaga, Udi Local Government Area of Enugu State. The *Persea americana* seeds were grated and air dried. The leaves were also air dried. The dried seeds and leaves were separately ground into fine powder and stored in air-tight receptacles.

Five hundred grams each of seed powder were soaked in 1000ml ethylacetate and 1250ml of deionized water respectively. Same amount (500mg) of leaf powder was also soaked in 1400ml of ethylacetate and 1750ml deionized water respectively. They were allowed to soak for 48hours with intermittent shaking. The suspensions were filtered using muslin cloth and concentrated using rotary evaporator. The extracts were stored in air-tight containers. The extracts generated were deionized water seed (DWS), deionised water leaf (DWL), ethylacetate seed (EAS) and ethylacetate leaf (EAL) extracts.

Experimental Design

Male albino rats, ninety five (95) in number were maintained on normal rat chew and water *ad libitum* and allowed to acclimatize for seven days. The rats were grouped into nineteen (19) with each group having five (5) rats.

Groups 1 and 2, were normal and negative controls and were given 0.5 ml/Kg body weight of normal saline while group 3 (positive control) received 10 mg/Kg body weight of vitamin C. Groups 4 to 19 were given 200, 400, 600 and 800 mg/Kg body weight deionised water and ethylacetate leaf and seed extracts. Administration was by oral gavage once daily for 21 consecutive days. On the 22nd day, 2.5 ml/Kg body weight carbon tetrachloride in olive oil (1,1) was administered intraperitoneally to all groups except group one. After 48 hours, their blood and hearts were collected under chloroform anesthesia for biochemical analysis.

Table 1: Summary

Group 1,	**0.5ml/Kg normal saline (normal control)**
Group 2,	0.5ml/Kg normal saline (negative control) +CCl_4
Group 3,	10 mg/Kg body weight vitamin C (positive control) + CCl_4
Groups 4 – 7,	200,400, 600, and 800 mg/Kg body weight ethylacetate leaf extracts + CCl_4.
Groups 8 – 11,	200, 400, 600 and 800 mg/Kg body weight ethylacetate seed extracts +CC
Groups 12 – 15,	200, 400, 600 and 800 mg/Kg body weight aqueous leaf extracts +CCl_4.
Groups 16 – 19,	200, 400, 600 and 800 mg/Kg body weight aqueus seed extracts +CCl_4

The administration of normal saline and extracts were done for 21 consecutive days and on the 22nd day, the blood, was collected under chloroform anesthesia for biochemical analysis [7].

Measurement of the Effect of the Extracts on Cardiac Markers

Creatine Kinase Assay by the Method of Stein (1985)

One vial of the enzyme/substrate/coenzyme reagent (ADP 2.0mmol/L, AMP 5.0mol/L Diadenosine pentaphosphate 10umol/l, NADP 2.0mmol/L; HK≥ 2.5U/mol, N-acetylcystein 20mmol/L, creatine phosphate 30mmol/L) was reconstituted by pipetting 2.5ml of the buffer/glucose reagent (Imidazole buffer 0.10 mol/L.pH6.7, glucose 20mol/L magnesium acetate 10mmol/L, EDTA 2.0mmol/L) into it and shaken to dissolve [8].

The spectrophotometer was turned on and the wavelength set at 340nm. 1.0ml of the substrate was introduced into a 1cm path length cuvette followed by

0.04ml of sample. The cuvette was introduced into the spectrophotometer and the initial reading taken at ambient temperature (28°C),. The reaction was timed and the absorbance was read again at 1, 2 and 3 minutes respectively. Creatine kinase activity was calculated using the following relationship.

Activity (U/L) = 8095 x ΔA (at 340nm/min).

Where ΔA = change in absorbance, 8095 = Factor.

Measurement of Lactate Dehydrogenese Activity by Rec (1972) method

Test tubes were labeled appropriately and set up in a rack. One vial of R1b (0.18mmol/L NADH) was reconstituted with 3.0ml of buffer/substrate Rla (50mmol/l phosphate buffer, pH 7.5, 0.6mmol/L pyruvate). Sample (0.04ml) was introduced into a tube followed by 1.0ml reagent and mixed. Initial absorbance was read at 30seconds and subsequently at 1, 2 and 3 min. Lactate dehydrogenate activity was calculated as follows,

LDH (U/L) = 4127 x ΔA

where ΔA – change in absorbance

4127 – factor.

Cardiac Troponin I (CTnI) Measurement by Etievent *et al.* (1995) Method

Coated wells were labeled appropriately and placed in the holder. Standards (0.2, 0.7, 5, 30 and 75 ng/ml troponin I), 100µl were dispensed into the appropriate wells followed by the samples in their own wells [9]. They were gently mixed for 120 seconds. Enzyme conjugate reagent (mouse monoclonal anti-TnI conjugated to horse radish peroxide in Tris-BSA solution), 100µl was dispensed into each well, mixed properly for 30sec and incubated for 90min at 25°C. Thereafter, the incubated mixture was removed by flicking plate contents into a waste bin. The wells were rinsed and flicked five times with distilled water. The wells were struck sharply into absorbent paper to remove water droplets [10].

Tetramethylbenzidine (TBN) reagent (100µl) was added to each well, mixed for 5 sec and incubated at room temperature for 20min. The reaction was stopped with the addition of 100µl of stopping solution (1N HCl) to each well, followed by mixing for 30sec. The absorbance was read at 450nm with a microtiter reader within 15 minutes. A standard curve was plotted from which the values of the samples were read.

Investigation of the Effect of the Extracts on the Lipid Profile

Triglycerides (TG), high density lipoprotein (HDL), total cholesterol (TC) and low density lipoprotein (LDL) were estimated as described by [11] [12]. Low density lipoprotein (LDL) was calculated using the relationship.

$$\text{LDL-C (mg/dl)} = \text{TC} - \frac{\text{TG}}{5} - \text{HDL-C}$$

Determination of Total Cholesterol by method of [8] [9].

Test tubes were set up on a rack in order. 0.01ml each of the standard, samples and distilled water were dispensed into the appropriate test tubes. 1.0ml each of reagent 1 (Pipes buffer 80mmol/1 pH6.8, 4-aminoantipyrine 0.25mmol/L, phenol 6.mmol/L, peroxidase $\geq$ 0.5U/ml; Cholesterol esterase $\geq$ 0.15U/ml, cholesterol oxidase $\geq$ 0.10U/ml) was then pipetted into each tube and mixed. The tubes were incubated at 37°C for 5 minutes. The absorbances of the content of the tubes were measured using a spectrophotometer at 546nm against the reagent blank [13].

The cholesterol content was calculated using the following relationship

$$\text{Cholesterol (mg/dl)} = \frac{\textit{absorbance of sample}}{\textit{absorbancre of standard}} \times \text{concentration of standard}$$

Determination of HDL – Cholesterol

Centrifuge tubes were labeled in order and placed on a rack. 200µl standard and sample were placed in appropriate tubes followed by 500µl of diluted precipitant R1, (Phosphotungstic acid 0.55mmol/L magnesium chloride 25mmol/L). They were mixed and allowed to stand for 10

minutes at room temperature. The tubes were thereafter centrifuged for 10 minutes at 4,000rpm and the supernatant collected.

Cholesterol CHOD – PAP assay was performed by first labeling sets of test tubes, blank, standard and sample respectively. 100µl each of distilled water, standard and sample were pipetted into the appropriate test tubes followed by 1000µl of the colour reaction reagent. They were mixed and incubated for 5 minutes at 37°C. The absorbance of the sample and standard were measured at 546nm against the reagent blank within 60 min. The HDL-cholesterol was calculated using the following formular.

$$\text{HDL – Cholesterol (mg/dl)} = \frac{absorbance\ of\ sample}{absorbancre\ of\ standard} \times \text{concentration of standard}$$

Determination of Triglycerides

One vial of enzyme reagent Rlb (4-amino phenazone 0.5mmol/L; ATP 1.0mmol/L, lipase ≥ 150U/mol, glycerol-kinase ≥ 0.4U/ml; Glycerol-3-phosphate oxidase ≥ 1.5U/ml, peroxidase ≥ 0.5U/ml) was reconstituted by pipetting 15.0ml of buffer Rla (buffer reagent pipes buffer 40mmol/L pH 7.6, 4-chlorophenol 5.5mol/L, magnesium ions 17.5mmol/L) into the vial. It was shaken to dissolve and kept protected from light.

Test tubes were labeled blank, standard and sample and stood on a rack. 10µl each of standard and samples were introduced into appropriate test-tubes followed by 1000µl of the reconstituted reagent [14]. The tubes were shaken to mix and incubated at 37°C for 5 minutes. The absorbance of the samples and standard were read at 546nm against a reagent blank. The triglyceride concentration was calculated using the relationship.

$$\text{TG} = \frac{absorbance\ of\ sample}{absorbancre\ of\ standard} \times \text{concentration of standard (mg/dl).}$$

STATISCAL ANALYSIS

Data generated were expressed as mean ± standard deviation. The difference among groups was determined using SPSS for windows software programme (version 20). Group comparison were done employing analysis of variance (ANOVA) test. P-values less than 0.05 ($P<0.05$) were regarded as significant.

RESULTS

Effect of the Extract of *Persea americana* on lactate dehydrogenate activity

LDH activity in the negative control was significantly ($P< 0.05$) higher than that of normal and positive controls. There was no significant ($P> 0.05$) difference in the activity of the enzyme in the normal and positive control groups. LDH activity in the DWL and EAL extract treated groups showed significant ($P<0.05$) dose dependent decrease with the 600 mg/Kg dose producing the highest effect. LDH activity in the DWS and EAS treated groups also showed a dose dependent significant ($P<0.05$) decrease compared with the negative control. LDH activity was significantly ($P<0.05$) lower in the DWL and EAL extract treated groups than in the DWS and EAS treated groups. (Figure 1).

Effect of the Extracts of *P americana* on Creatine Kinase (CK) Activity

The activity of creatine kinase (CK) was significantly ($P<0.05$) higher in the negative control than in the normal and positive control groups. There was no significant ($P>0.05$) difference in the activity of the enzyme in the normal and positive control groups. There was significant ($P<0.05$) difference in the activity of the enzyme in the negative control and the groups treated with the extracts. The activity of the enzyme in the DWL and EAL treated groups showed a dose dependent significant ($P<0.05$) decrease up to 600 mg/Kg dose compared with the negative control group. Creatine kinase activity in the DWL and EAL extract

groups was significantly (P<0.05) lower than that of the DWS and EAS extract treated groups at 600 mg/Kg dose. (Figure 2)

Effect of *Persea americana* Extract on Cardiac Troponin I (CTnI) level

CTnI level in the negative control was significantly (P<0.05) higher than those of the normal and positive control groups. The concentration of CTnI in the positive control group was significantly (P<0.05) higher than that of normal control group. A dose dependent significant (P<0.05) decrease in the concentration of CTnI was recorded for all the extracts up to 600 mg/Kg dose group with the lowest decrease recorded for the DWL extracts at that concentration compared with the negative control. The level of CTnI in the DWL and EAL treated groups were significantly (P<0.05) lower than those of DWS and EAS treated groups. (Figure 3).

Effect of *Persea americana* Extracts on Total Cholesterol (TC)

TC concentration in the negative control was significantly (P<0.05) higher than those of normal and positive control groups. TC concentration in the normal and positive control groups was not significanly (P>0.05) different. A dose dependent significant (P<0.05) decrease in TC concentration was recorded for all the extracts treated groups compared with the negative control. The concentration of TC in the DWL and EAL treated groups was significantly (P<0.05) lower than those of the DWS and EAS groups up to 600 mg/Kg dose. (Figure 4).

Effect of *Persea americana* Extracts on Triglyceride (TG) level

TG concentration in the negative control was significantly (P<0.05) higher than those of the normal and positive control groups. TG concentration in the normal and positive control groups were not significantly (P>0.05) different. A dose dependent significant (P<0.05) decrease in TG concentration for all the extract treated groups compared with the negative control group was recorded. TG concentration in the DWL and EAL treated groups at 600mg/Kg body weight dose was significantly (P<0.05) lower than those of the other extract treated groups. TG concentration in all the extract treated groups were significantly (P<0.05) higher than those of the normal and positive control groups except for DWL extract treated group at 600 and 800 mg/Kg body weight dose. (Figure 5).

Effect of *Persea americana* Extracts on High Density Lipoprotein Cholesterol (HDL) Concentration

HDL concentration in the negative control group was significantly (P<0.05) lower than those of the normal and positive control group. HDL concentration in the positive control group was significantly (P<0.05) higher than that of the normal control group. HDL concentration in all the extract treated groups were significantly (P<0.05) higher than that of the negative control group. The concentration of HDL in DWL and EAL extract treated groups were significantly (P<0.05) higher than those of DWS and EAS extract treated groups up to 600 mg/kg body weight dose. HDL concentration in all the extracts treated groups were significantly (P<0.05) lower than those of the normal and positive controls (Figure 6).

Effect of *Persea americana* Extracts on Low Density Lipoprotein Cholesterol (LDL) Concentration.

LDL concentration in the negative control group was significantly (P<0.05) higher than those of the normal and positive control groups. LDL concentration in the normal and positive control groups was not significantly (P>0.05) different. LDL concentration in all the extract treated groups were significantly (P<0.05) lower than that of negative control group and significantly (P<0.05) higher than those of the normal and positive control groups. LDL concentration in the DWL and EAL extract treated groups were significantly (P<0.05) lower than those of DWS and EAS extract treated groups at 600 mg/Kg body weight dose. (Figure 7).

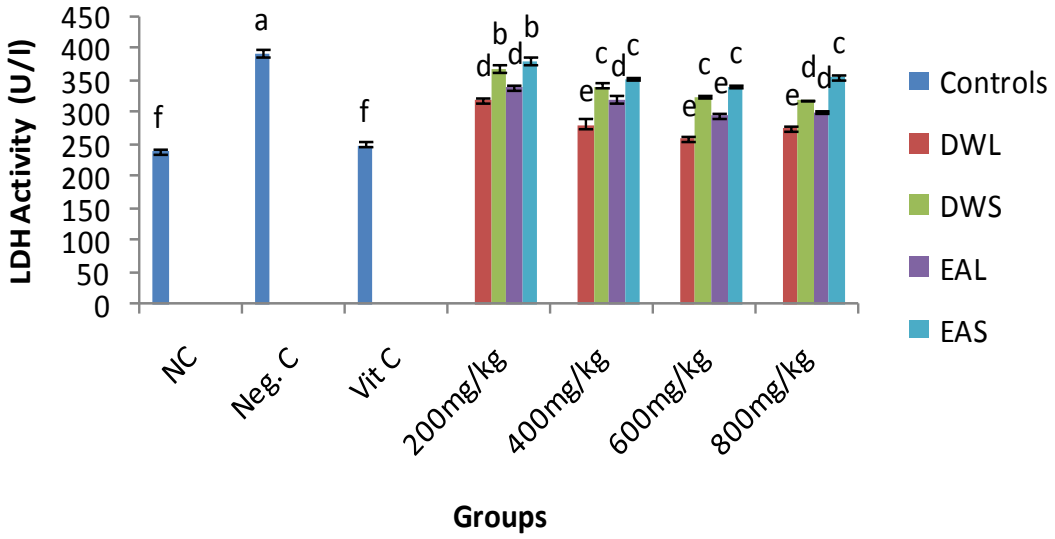

Figure 1, Serum lactate dehydrogenase (LDH) activity in albino rats administered aqueous and ethylacetate extract of the leaves and seeds of *Persea americana* before induction of organ damage with carbon tetrachloride.
The data are presented as mean ± SD. vit C = vitamin C, NC = normal control. Neg C = negative control. Bars with the same letter are not significantly different (P<0.05).

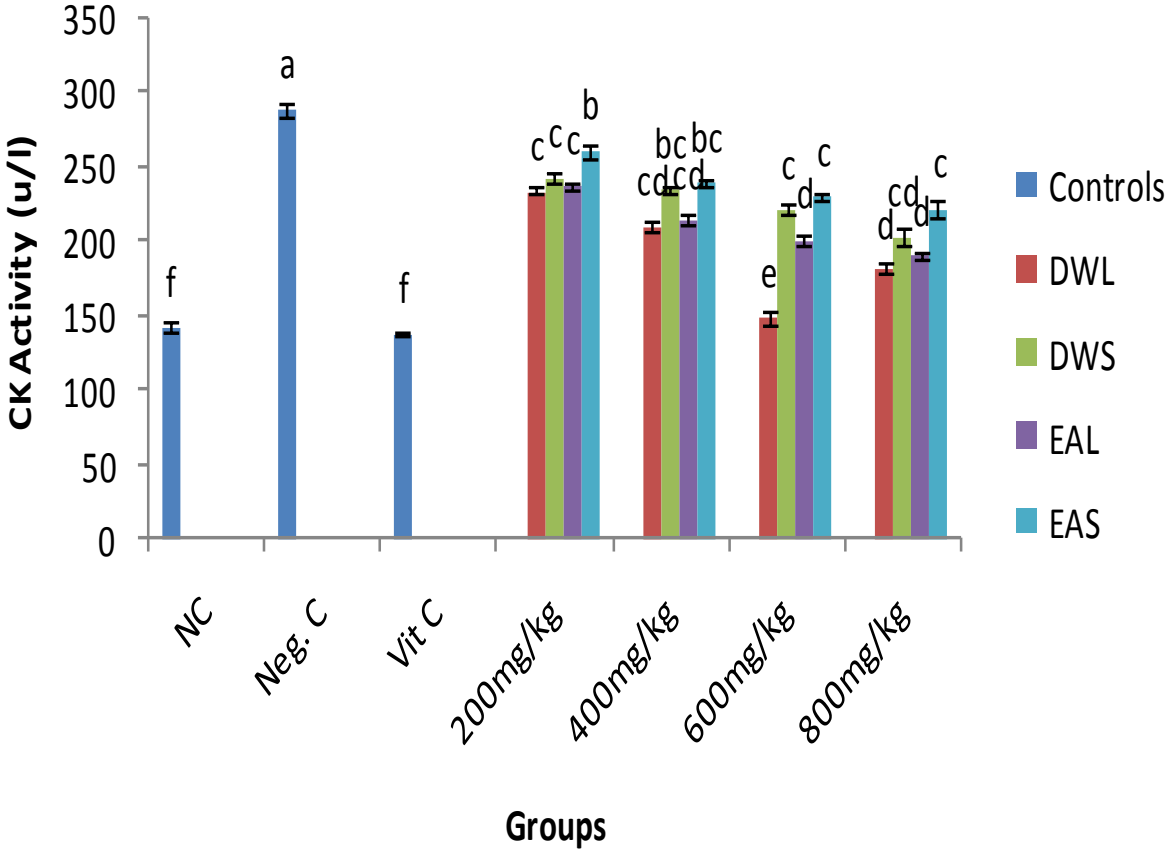

Figure 2, Serum creatine Kinase (CK) activity in albino rats adminstered different doses of aqueous and ethylacetate extracts of the leaves and seeds of *Persea americana* before induction of organ damage with carbon tetrachloride.
The data are presented as mean ± SD. vit C = vitamin C, NC = normal control and Neg C. = negative control. Bars with the same letter are not significantly different (P<0.05).

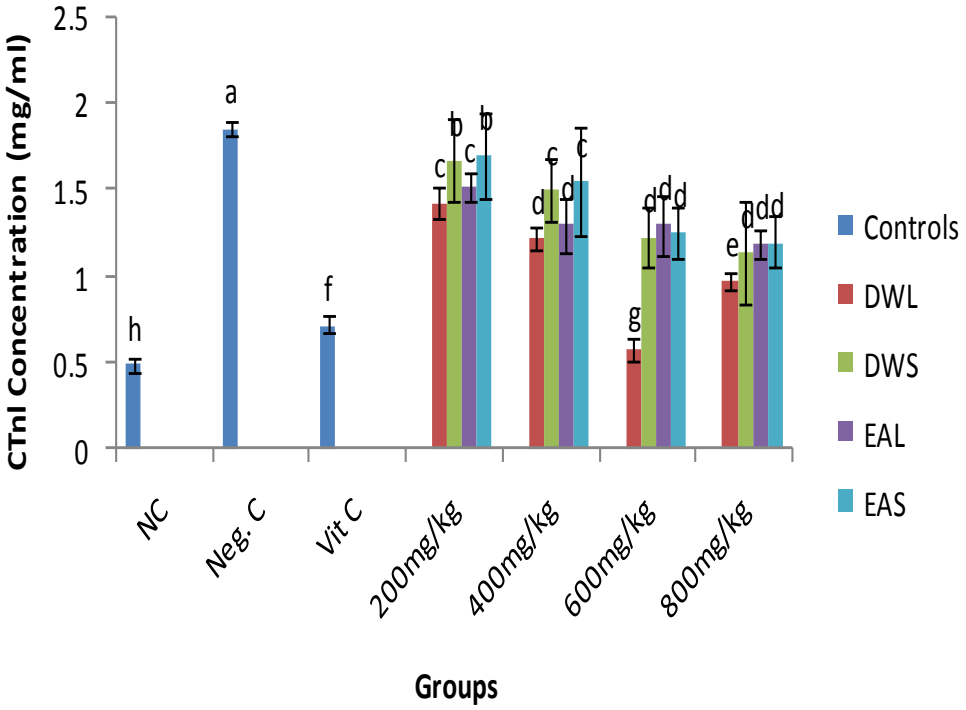

Figure 3, Serum cardiac troponin I (CTnl) level in albino rats administered different doses of aqueous and ethylacetate extracts of the leaves and seeds of *Persea americana* before induction of organ damage with carbon tetrachloride.
The data are presented as mean ± SD. vit C = vitamin C, NC = normal control and Neg C. = negative control. Bars with the same letter are not significantly different (P<0.05).

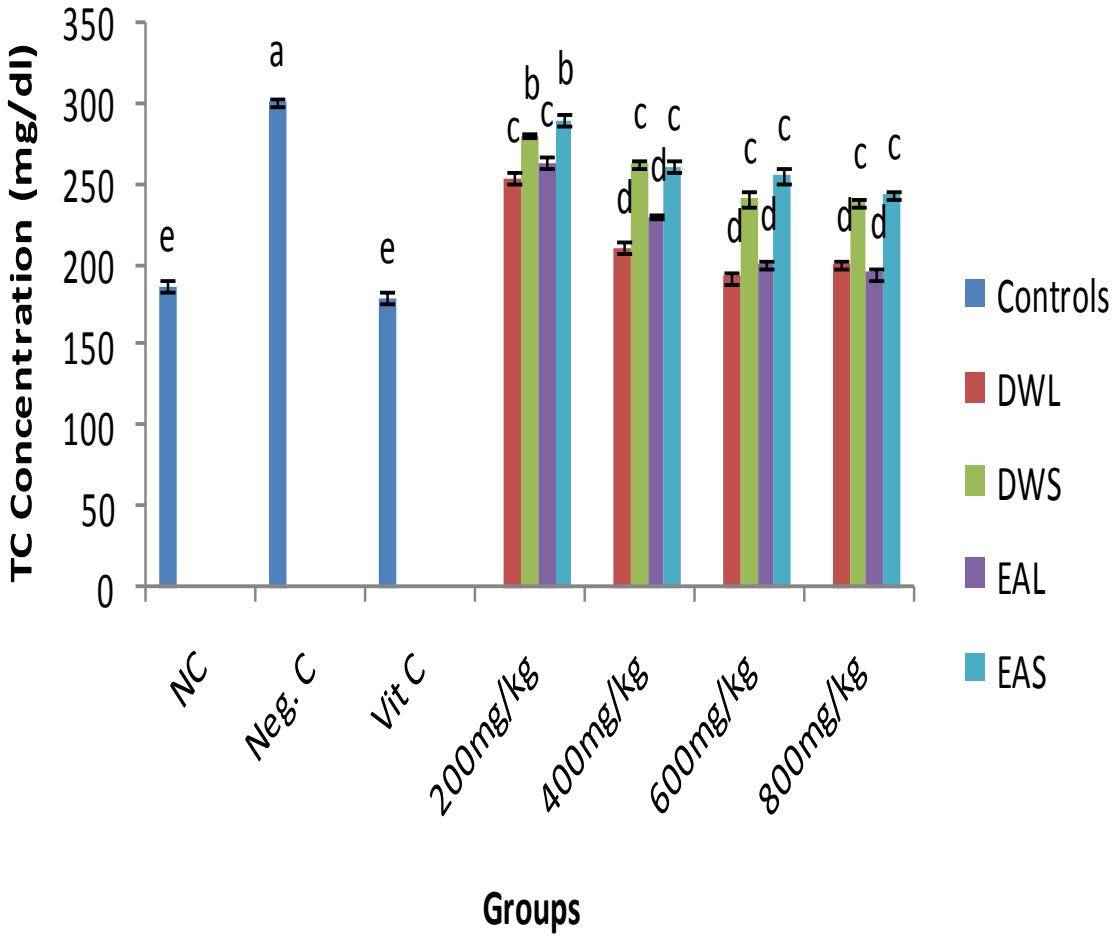

Figure 4, Serum total cholesterol level in albino rats administered different doses of aqueous and ethylacetate extracts of the leaves and seeds of *Persea americana* before induction of organ damage with carbon tetrachloride.
The data are presented as mean ± SD. vit C = vitamin C, NC = normal control and Neg C. = negative control. Bars with the same letter are not significantly different (P<0.05).

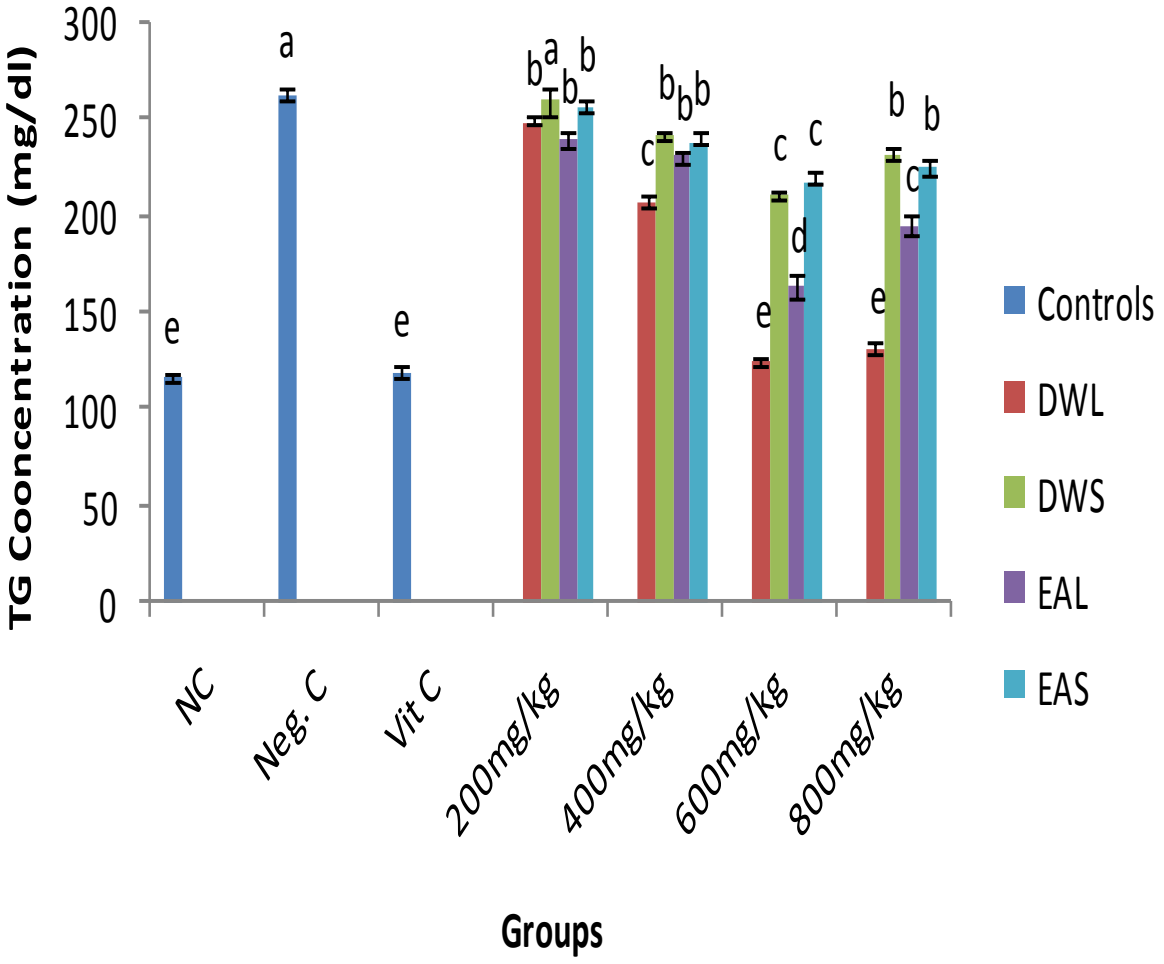

Figure 5, Serum triglyceride level in albino rats administered different doses of aqueous and ethylacetate extracts of the leaves and seeds of *Persea americana* before induction of organ damage with carbon tetrachloride.
The data are presented as mean ± SD. vit C = vitamin C, NC = normal control and Neg C. = negative control. Bars with the same letter are not significantly different (P<0.05).

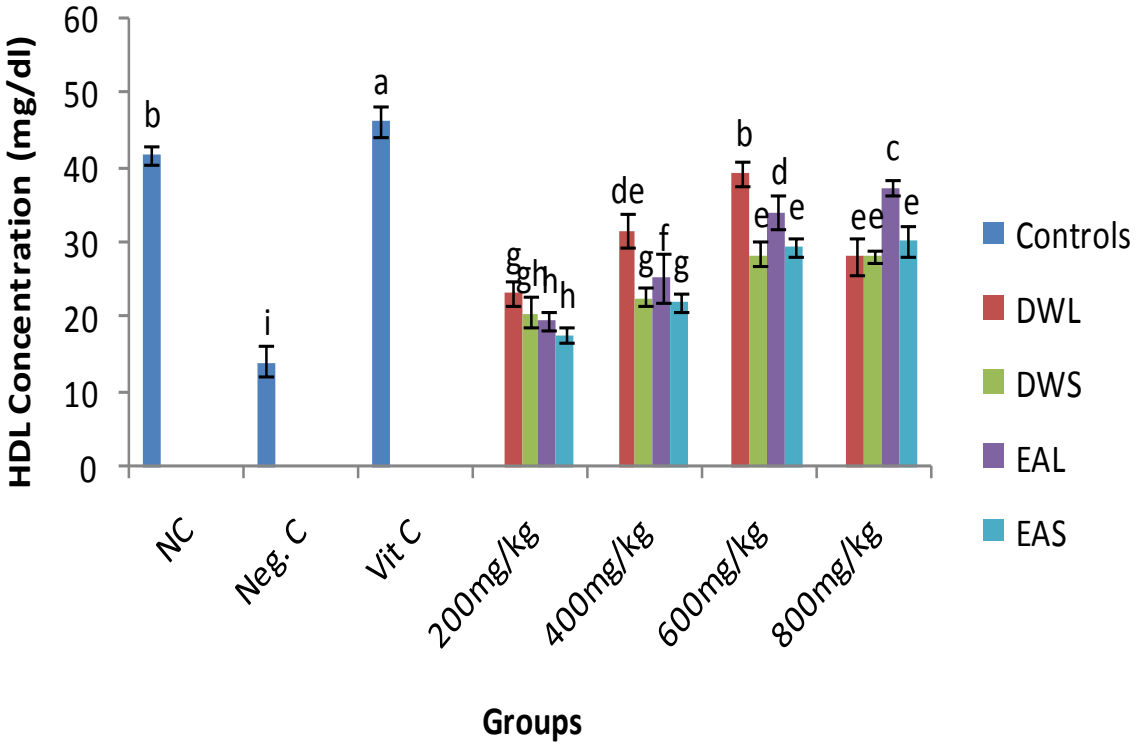

Figure 6, Serum high density lipoprotein (HDL) level in albino rats administered different doses of aqueous and ethylacetate extracts of the leaves and seeds of *Persea americana* before induction of organ damage with carbon tetrachloride.
The data are presented as mean ± SD. vit C = vitamin C, NC = normal control and Neg C. = negative control. Bars with the same letter are not significantly different ($P<0.05$).

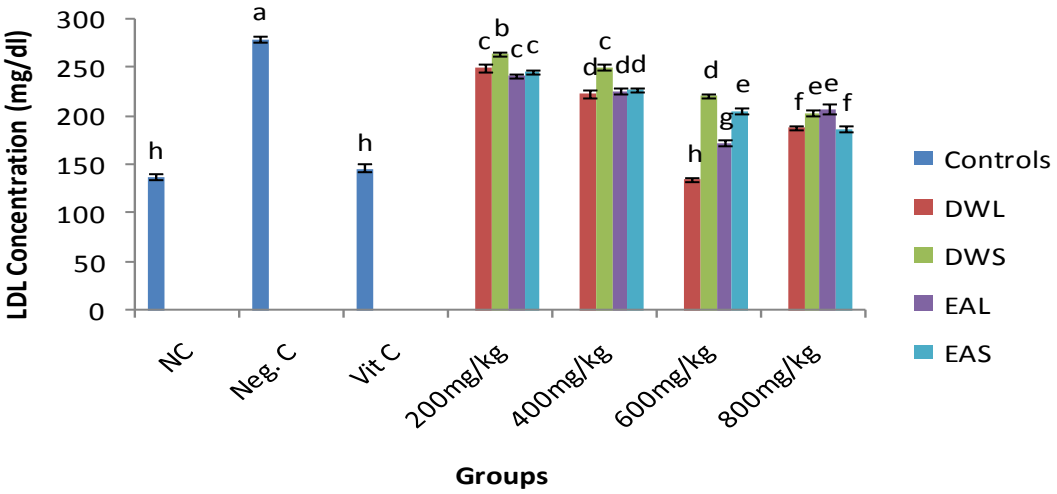

Figure 7, Serum low density lipoprotein (LDL) level in albino rats administered different doses of aqueous and ethylacetate extracts of the leaves and seeds of *Persea americana* before induction of organ damage with carbon tetrachloride.
The data are presented as mean ± SD. vit C = vitamin C, NC = normal control and Neg C. = negative control. Bars with the same letter are not significantly different (P<0.05).

DISCUSSION

The observed elevation in the activities of LDH and CK and level of CTnI in the serum of the negative control group compared with the normal control group, showed that the CCl_4 induced heart tissue damage which led to the elevation in the makers (figure 1,2,3). Carbon tetrachloride is known to cause tissue damage by generating free radicals (Adaramoye, 2009; Agbafor *et al.,* 2015). The observed decrease in the activities of LDH and CK and the level of CTnI in groups pretreated with *Persea americana* extracts compared with the negative control group suggests that the seed and lead extracts of *Persea americana* protected the cardiac tissue from the damaging effect of CCl_4. The extracts can thus be said to be cardioprotective. The reduction in the activities of LDH and CK and the level of CTnI in the extract treated groups was dose dependent. The DWL and EAL extract treated groups gave the lowest reduction at 600mg/kg body weight dose than the DWS and EAS extract groups. This suggests that the leaf extract were more effective in protecting the heart tissues. However, the protective effects of the extracts was not as effective as that of vitamin C (positive control) since the activities of LDH and CK and level of CTnI was significantly (P<0.05) higher than that of the positive control group. The cardio protective action of the leaf and seed extracts of *Persea americana* may be ascribed to the presence of antioxidant in the extracts.

Lipid profile showed that the concentration of TC, TG and LDL were significantly (P<0.05) higher in the negative control group while HDL concentration was significantly (P<0.05) lower compared with the normal control group (figure 4, 5, 6,7). This signified the

generation of adverse effect by CCl_4. Elevation of LDL, TC and TG and decrease of HDL-C(dyslipidemia) is a major risk factor for cardiovascular disorder (Raju *et al.,* 2014). The concentration of TC, TG and LDL in the extracts treated groups were significantly ($P<0.05$) lower than the negative control group while HDL was significantly ($P<0.05$) higher. This reversal in the trend exhibited by the extract treated groups again showed the protective action of the leaf and seed extracts. The leaf extracts were more effective in the reversal up to 600mg/kg body weight dose than the seed extracts. This suggests that although the seed and leaf extracts protects the heat tissue, the leaf extracts maybe more beneficial.

CONCLUSION

Although the seed and leaf extracts of *Persea americana* showed cardioprotecion capacity, the leaf extract appears to be more beneficial. The use of the leaf extracts for the management/treating of hypertension is therefore preferable than seed extract.

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