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Hepatoprotective and Haemopoeitic Activity of Ethanol Extract of *Persea americana* Seed in Paracetamol Induced Toxicity in Wistar Albino Rat

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ABSTRACT

The study was designed to evaluate the hepatoprotective and haemopoietic activity of *Persea americana.* Thrity-five wistar albino rats weighing 100-150g were divided into seven groups consisting of five animals in each. Group A served as normal control, group B served as experimental control while group C-G served as test group administered different doses of *Persea americana* seed extract. Hepatotoxicity was induced with paracetamol in groups. Experimental period lasted for 14 days after which the animals were sacrificed and blood collected for biochemical estimation. The hepatoprotective effect was evaluated by performing a determining the concentration in serum of alkaline phosphatase, asparate amino transferase, alanine aminotransferase, direct and total bilirubin spectrophotometrically and the haemopoietic effect was evaluated by carrying an assay on the haemoglobin, packed cell volume and total red blood cells by hematocrit method. The results obtained were presented as Mean+SD. ALP in negative control as (788.90±5.80), Normal control (647.76±18.61), the test groups ranged from 610.62±101.28-643.14±8.69. AST for negative control is (242.40±1.54), normal control (198.55±11.34), the test groups ranged from 112.35±4.35-109.65±2. ALT, Negative control is (86.23±0.37), Normal control (55.09±3.53), the test groups ranged from 46.61±3.30-34.23±1.34, DB, negative control is (8.70±0.17), normal control (7.31±0.14), the test groups ranged from 6.29±0.06-3.35±0.30 and TB for negative control (9.63±0.27), normal control (7.35±0.28), the test groups ranged from 9.11±0.18-4.11±0.22. RBC in negative control as (1050000.00±70710.67), normal control (4300000.00±141421.35), the test groups ranged from 49560000.00±212132.03-1050000.00±7071.67, HB in negative control as (3.7±0.14), normal control (12.85±0.912), the test groups ranged from 15.50±0.56-3.15±0.07, PCV for negative control was (10.50±0.70), normal control (37.50±2.12), the test groups ranged from 46.50±2.12-12.50±0.70, Reduction in liver enzymes and increase in haematological parameters in the test group indicated that *Persea americana* seeds have hepatoprotective and haematological properties.

INTRODUCTION

Humans are dependent upon plants, directly or indirectly. Plants provide food, clothing, fuel, shelter and many other necessities of life. Some of the plants that are more beneficial to man are the medicinal plants. Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform biological functions and to defend against attack from predators such as insects, fungi and herbivorous mammals (Tapsell *et al.*, 2006). Chemical compounds in plants mediate their effect on the human body through processes identical to those already well understood for the chemical compounds in conventional drugs; thus herbal medicines do not differ greatly from conventional drugs in terms of how they work. This enables herbal medicines to be as effective as conventional medicines, but also gives them the same potential to cause harmful side effects (Lai *et al.*, 2004).

This is also true for avocado tree of the Lauraceae family, is native to America and is cultivated from Equator to the Mediterranean climate. It has different names depending on the country where it grows; Alligator in Florida, Xiene in Mexico, Palta in Cocombia, Ecuador and Abacoteiro in Brazil and Avocado pear or (Ube Bekee) in Nigeria.

The avocado is a leafy tree up to 20 feet high with a trunk diameter of 60cm, with alternative and elliptical leaves and small flowers. The juice is a drupe (fleshy fruits with seed inside) greenish and thin skin whose taste reminiscent of walnut, has a very oily pulp, commonly used as food. Avocados are a good source of B vitamins, which help you fight off diseases and infection. They also give you vitamin C and E, plus natural plant chemicals that may prevent cancer (Kathleen *et al.*, 2004).

Avocado seeds have many antioxidants than most fruits and veggies in the market and polyphenols like green tea, plus they are full of more soluble fiber than just about any other food. In fact, Avocado seeds have 70% of the antioxidants found in the whole avocado, and avocado seed oil is also full of antioxidants, lower cholesterol and helps fight off disease. Avocado seeds help to prevent cardiovascular disease, lower cholesterol and prevent strokes (Paul *et al.*, 2012). Avocado seeds are great for Gastrointestinal tract problem and dysentery. Avocado seeds have a lot of phenolic compounds that help to prevent gastric ulcers and prevent bacterial and viral diseases. Avocado seeds contain a flavonoid that prevents tumor growth. It boosts immune

system high, because antioxidants keep free radicals at bay and slows aging process. Avocado seeds seem to have a good anti- inflammatory ability, can thus help with arthritics and other joint diseases (Paul *et al.,* 2012).

Hepatoprotective or antihepatotoxicity is the ability to prevent damage to the liver. This damage caused in the liver is known as hepatotoxicity which is the chemical-driven liver damage. The liver plays a central role in transferring and cleaning chemicals and is susceptible to the toxicity from these agents. Certain medicinal agents, when taken in overdoses and sometimes even when introduced within therapeutic ranges may injure the organ. Chemicals that cause liver injury are called hepatotoxins. Hepatotoxicity and drug-induced liver injury also account for a substantial number of compound failures, highlighting the need for drug screening assays, such as stem cell-derived hepatocyte-like cells, that are capable of detecting toxicity early in the drug development process (Greenhough *et al.,* 2012). Chemicals often cause subclinical injury to the liver, which manifests only as abnormal liver enzyme tests. Drug-induced liver injury is responsible for 5% of all hospital admissions and 50% of all acute liver failures (Mcnally *et al.,* 2006).

Haemopoiesis is the formation of blood cellular components. All cellular blood components are derived from haematopoietic stem cells. In a healthy adult person, approximately 1011-1012 new blood cells are produced daily in order to maintain steady state levels in the peripheral circulation. Haematopoietic stem cells (HSCs) reside in the medulla of the bone (bone marrow) and have the unique ability to give rise to all of the different mature blood cell types and tissues. HSCs are self-renewing cells, in the sense that when they proliferate, at least some of their daughter cells remain as HSCs, so the pool of stem cells does not become depleted. This phenomenon is called asymmetric division (Morrison *et al.,* 2006). The daughters of HSCs (myeloid and lymphoid progenitor cells), however, can commit to any of the alternative differentiation pathways that lead to the production of one or more specific types of blood cells, but cannot renew itself. The pool of progenitors is heterogeneous and can be divided into two groups, long- term self-renewing HSC and only transiently self-renewing HSC, are also called short terms (Weissman, 2015).

MATERIALS AND METHODS

Collection of plant material and identification

Seeds of *P. americana* was collected from the National Root Crops Research Institute farm, Umudike in Abia State in the month of May 2015. The plant was identified by a taxonomist, Dr. Osuagwu G.C of the Department of Plant Science and Biotechnology, Micheal Okpara University of Agriculture, Umudike (MOUAU).

Animals

A total of thirty-five Wistar albino rats of both sexes were used for the study. The rats were between the ages of 6-10 weeks old and the weight of the rats ranged from 100-160g. The animals were kept in a well-aerated laboratory cages in the Biochemistry Department animal house and were allowed to acclimatize to the environment for a period of two weeks before the commencement of the experiment. The animals were maintained on a standard animal feed and drinking water during the acclimatization.

Preparation of plant material

The seeds were chopped into small sizes to aid drying and oven dried at 40^{o}C and then milled into fine powder using a manual blender. 100g of the sample was soaked in 500ml of 80% ethanol for 72 hours and filtered through a filter paper Whatman no. 1. The residue was discarded and the filtrate was left to evaporate and the extract was kept in an air tight container until experimental period. The extract was reconstituted inappropriate volume of distilled water for administration orally.

Acute toxicity study

The median lethal dose (LD_{50}) of the plant extract was carried out in order to select a suitable dose for the evaluation of the hepatoprotective and hemopoietic activity. This was done using twenty mice. The mice were divided into five groups of four mice each and were treated with different concentration of 1000, 500, 250, 125, 62mg of the extract per body weight. The mice were observed for 48hours for signs of toxicity including deaths. The LD_{50} was then calculated from the results of the phase as the square root of the product of the lowest lethal dose and the highest non-lethal dose. i.e. geometric mean of the consecutive dose which 0 and 100% survival mice were recorded (Lorke,1983).

Experimental design

Wistar albino rats were divided into seven groups

Group I (NC): Normal control was administered a single daily dosage of normal saline (1.0 ml/kg body weight).

Group II (HC): Hepatotoxic control received Paracetamol (250mg/kg body weight).

Group III: Animals were given Paracetamol + ethanol fraction of *Persea americana* orally (500 mg/kg)

Group IV: Animals were given Paracetamol + ethanol fraction of *Persea americana* orally (250 mg/kg)

Group V: Animals were given Paracetamol + ethanol fraction of *Persea americana* orally (125 mg/kg)

Group VI: Animals were given Paracetamol + ethanol fraction of *Persea americana* orally (62 mg/kg)

Group VII: Animals were given Paracetamol + ethanol fraction of *Persea americana* orally (31mg/kg)

Biochemical Analysis

Determination of Alkaline phosphatase (ALP)

The serum alkaline phosphatase was determined by the method described by Englehardt *et al.*, 1970

Determination of Alanine Aminotransferase (ALT)

The serum alanine aminotransferase was determined by the method described by Schmidt *et al.*, 1963

Determination of conjugated bilirubin (CB) and total bilirubin (TB) in serum

The conjugated and total bilirubin were determined using the Max +Discovery ^{Tm} Total bilirubin Assay Kit and Diazyme's Direct Bilirubin Vanadate Oxidation assay by the colorimetric method described by Jendrassik and Grof,1938.

Determination of total leucocytes count by Haemocytometry

Total leucocytes count was determined by haemocytometry following the method described by Ochei and Kolhartkar (2008).

Packed cell volume (PCV) estimation

PCV was estimated as described by Ochei and Kolhatkar (2008).

Determination of Haemoglobin (Hb) concentration

This was done using cyanomethaglobin method as described by Ochei and Kolhartkar (2008).

PHYTOCHEMICAL ANALYSIS

Phytochemical screening was achieved using standard methods.

Statistical Analysis

Data obtained was statistically analyzed by one way ANOVA (LSD) using SPSS version 20 and reported as Mean+SD. Significant difference was accepted at 95% confidence level of probability.

RESULTS AND DISCUSSION

Phytochemical Screening

Table 1: Concentration of Phytochemicals present.

Compound	Concentration (%)
Alkaloids	2.92±0.028
Flavanoids	4.76±0.053
Saponins	3.22±0.055
Steroids	1.58±0.05
Tanins	0.18±0.00
Phenol	2.47±0.039

4.1.2 Acute toxicity

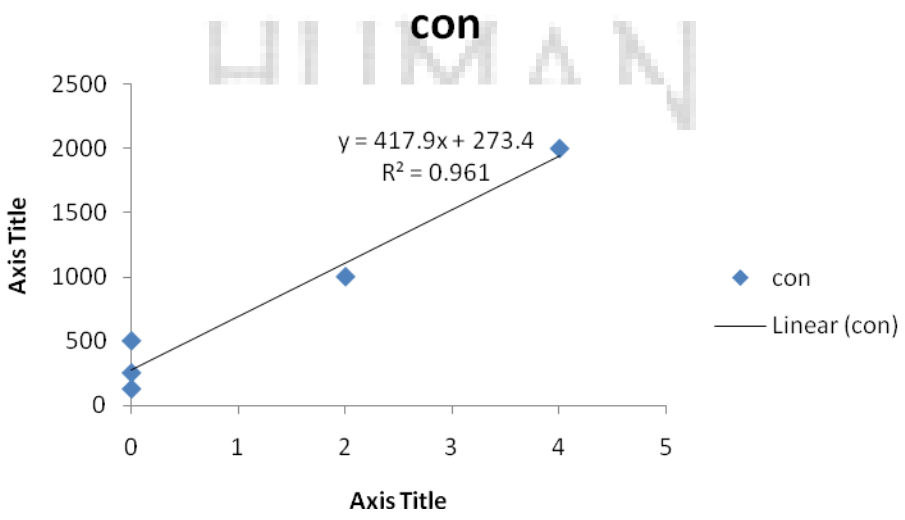
Table 2: LD50 RESULT

Group	CONC.(Mg/Kg.B.W)	Number of animals	Number of mortality
A	2000	4	4
B	1000	4	2
C	500	4	0
D	250	4	0
E	125	4	0

$$LD_{50} = \sqrt{\text{least conc. with highest mortality} \times \text{highest conc. without mortality}}$$

$$LD_{50} = \sqrt{2000 \times 500}$$

$$= 707 \text{ mg/kg B.w.}$$



LIVER FUNCTION TEST (LFT)

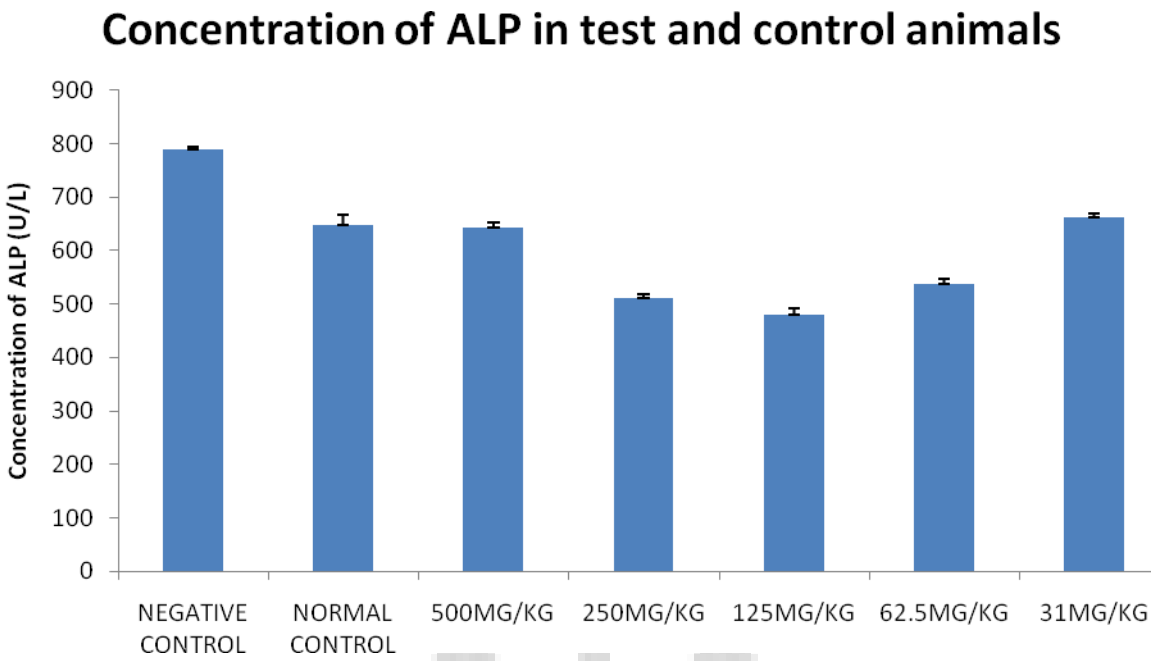


Fig. 1. Concentration of ALP in test and control animals

Result indicated that ALP concentration was significantly ($p<0.05$) higher in the negative control when compared to every other group. Also, ALP concentration was significantly ($p<0.05$) higher in the normal control when compared to 250, 125, 62.5, 31mg/kg body weight. ALP concentration was also significantly ($p<0.05$) higher in the 250mg/kg group when compared with the 125mg/kg and significantly ($p<0.05$) lower in 62.5mg/kg and 31mg/kg. ALP concentration in all the test groups was significantly different. Thus, giving the value for ALP in negative control as (788.90±5.80), Normal control (647.76±18.61), 500mg/kg (643.14±8.69), 250mg/kg (511.76±5.36), 125mg/kg (481.10±10.77), 62.5mg/kg (538.52±7.22) and 31mg/kg (610.62±101.28).

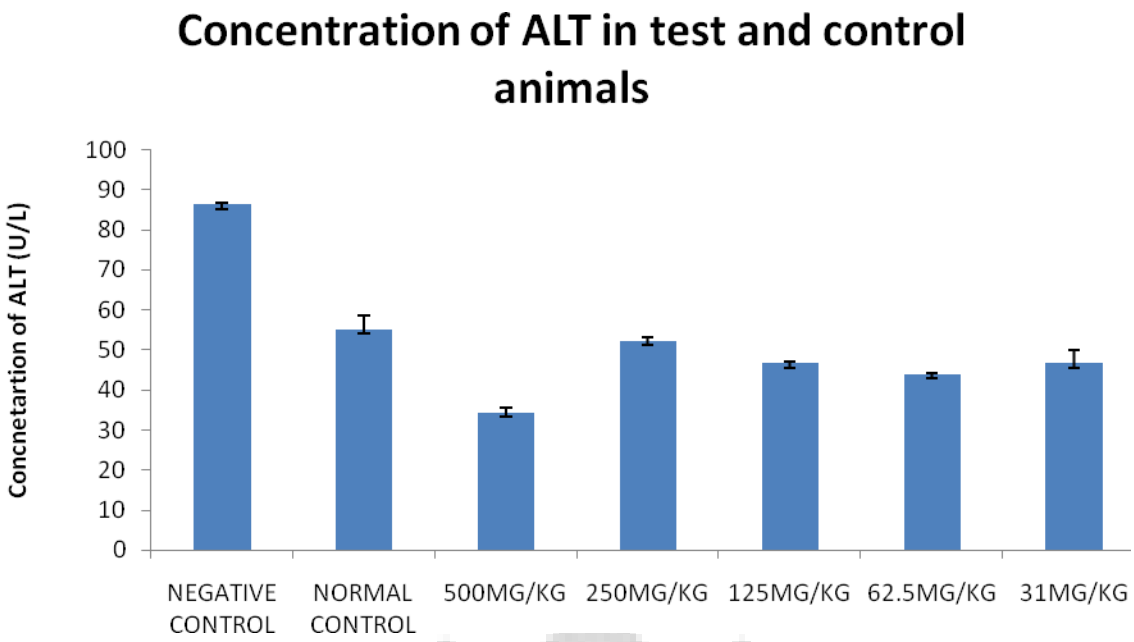


Fig. 2. Concentration of ALT in test and control groups

Result indicated ALT concentration was significantly ($p<0.05$) higher in the Negative control when compared to other group. ALT concentration was significantly ($p<0.05$) higher in the normal control when compared to 500, 250, 125, 62.5, 31mg/kg body weight. ALT concentration was also significantly ($p<0.05$) lower in the 500mg/kg group when compared to other test groups, while ALT concentration was significantly ($p<0.05$) higher in 250mg/kg group when compared to other test groups. Thus, giving the value of ALT concentration in negative control as (86.23±0.37), Normal control (55.09±3.53), 500mg/kg (34.23±1.34), 250mg/kg (52.07±1.18), 125mg/kg(46.60±0.57), 62.5mg/kg (43.83±0.46) and 31mg/kg (46.61±3.30).

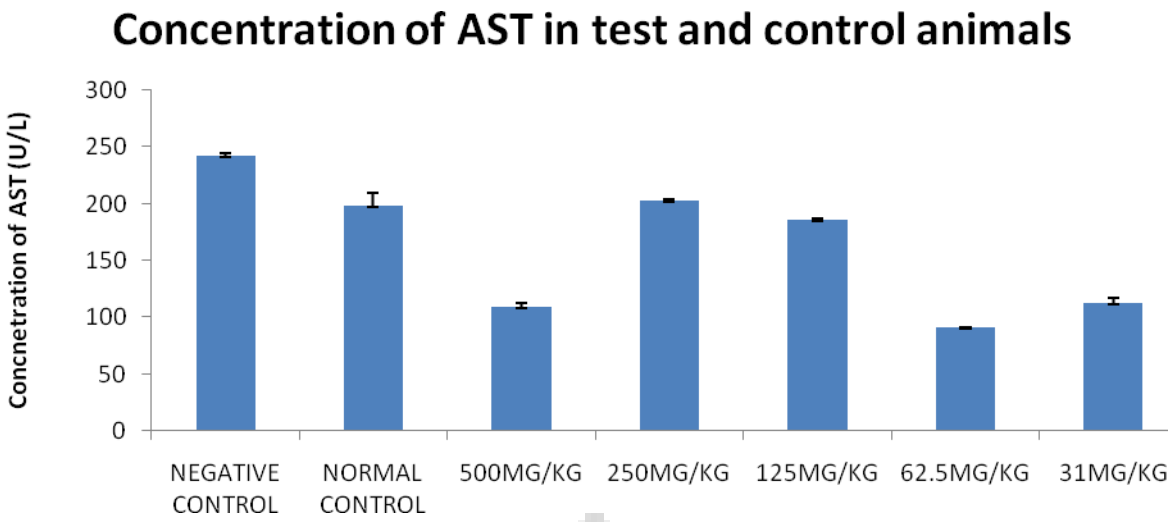


Fig. 3. Concentration of AST in test and control groups

Result indicated AST concentration was significantly ($p<0.05$) higher in the negative control when compared to other groups. AST concentration was also significantly ($p<0.05$) higher in normal control when compared to 500, 125 62.5, 31mg/kg and significantly ($p<0.05$) lower in 250mg/kg. 250mg/kg was significantly ($p<0.05$) higher when compared to other test groups. Thus, giving the value for AST concentration in the negative control as (242.40±1.54), normal control (198.55±11.34), 500mg/kg (109.65±2.99), 250mg/kg (202.67±0.72), 125mg/kg (185.75±0.88), 62.5mg/kg (90.89±0.75) and 31mg/kg (112.35±4.35).

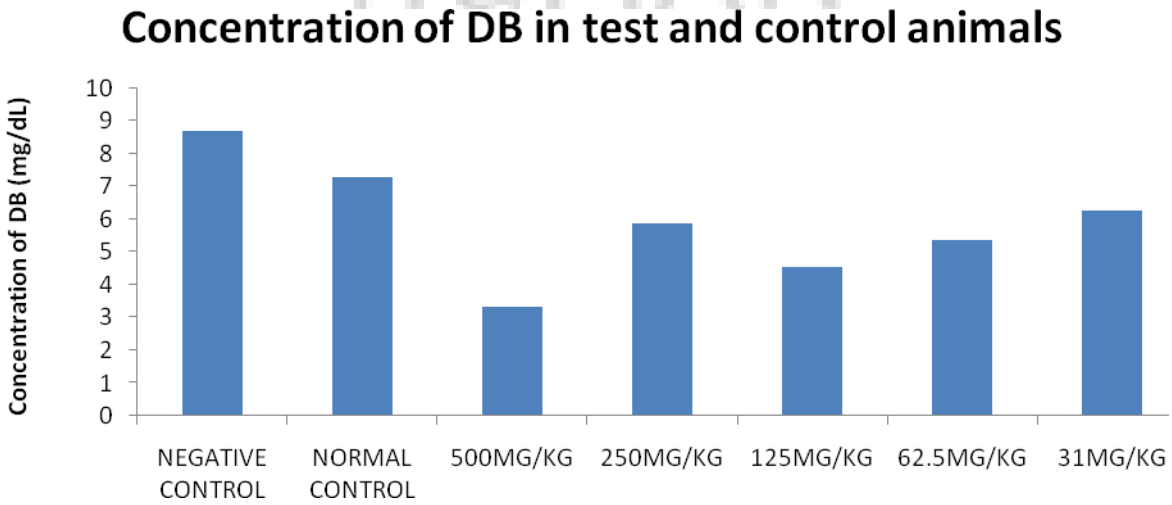


Fig. 4. Concentration of direct bilirubin in test and control groups

Result indicated that Direct bilirubin concentration was significantly ($p<0.05$) higher in the negative control when compared to other groups. Direct bilirubin concentration was also significantly ($p<0.05$) higher in normal control when compared to other groups. Also, direct bilirubin concentration was significantly ($p<0.05$) higher in the 250mg/kg group when compared with the 500mg/kg, 125mg/kg, 62.5mg/kg and significantly ($p<0.05$) lower when compared to the 31mg/kg. Thus, giving the value for DB in negative control as (8.70±0.17), normal control (7.31±0.14), 500mg/kg (3.35±0.30), 250mg/kg (5.90±0.35), 125mg/kg (4.54±0.08), 62.5mg/kg (5.36±0.08) and 31mg/kg (6.29±0.06).

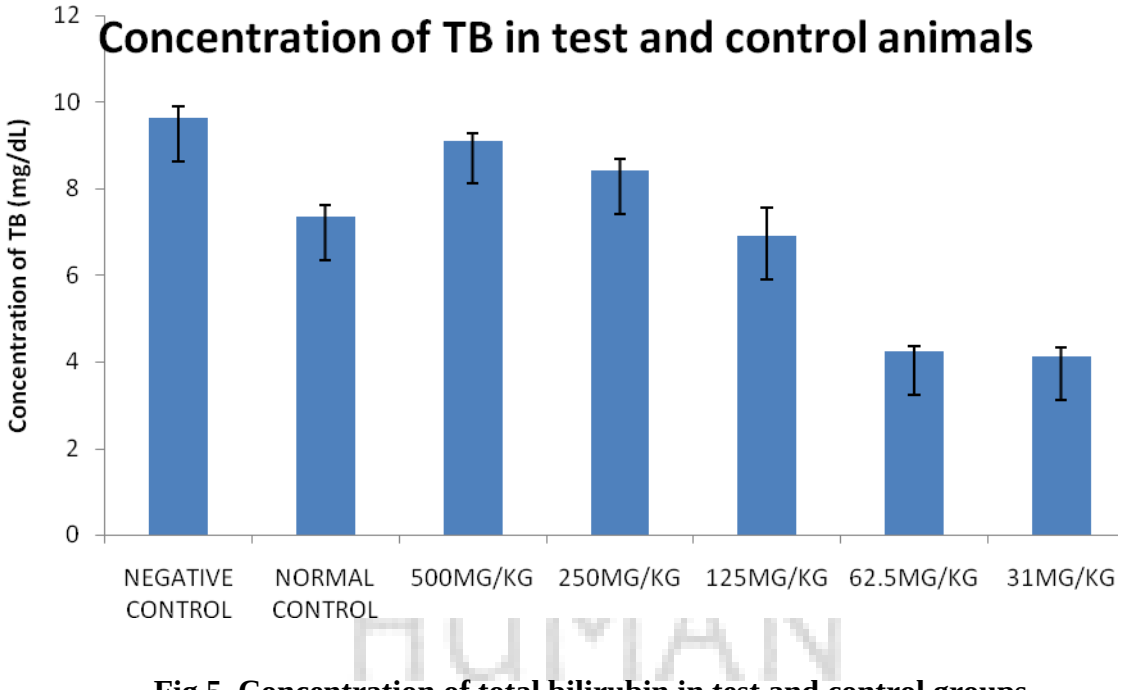


Fig 5. Concentration of total bilirubin in test and control groups

Total bilirubin concentration was significantly ($p<0.05$) higher in the negative control when compared to other groups. Total bilirubin concentration was also significantly ($p<0.05$) higher in normal control when compared to 125, 62.5, 31mg/kg body weight and significantly ($p<0.05$) lower when compared to 500 and 250mg/kg body weight. Total bilirubin concentration was significantly ($p<0.05$) higher in 500mg/kg when compared to other test groups. Thus, giving the value of total bilirubin in negative control as (9.63±0.27), normal control (7.35±0.28), 500mg/kg (9.11±0.18), 250mg/kg (8.40±0.29), 125mg/kg (6.89±0.68), 62.5mg/kg (4.23±0.12), 31mg/kg (4.11±0.22).

HAEMATOLOGICAL PARAMETER

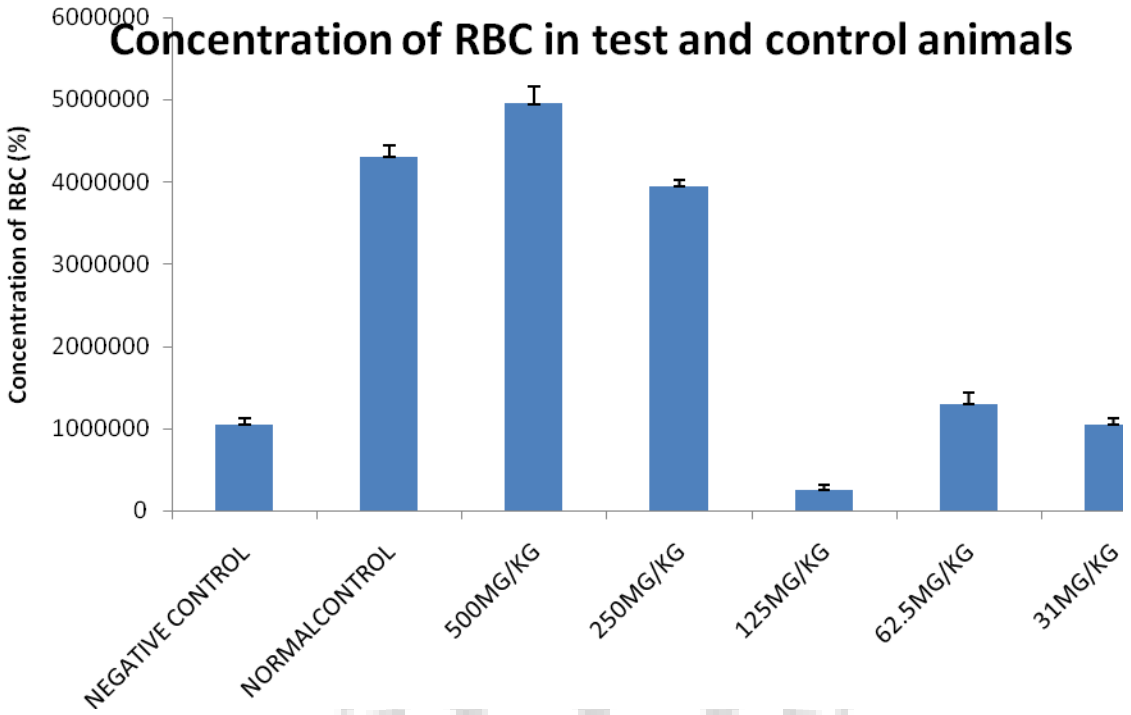


Fig. 6. Concentration of RBC in control and test groups

Result for RBC indicated that the concentration of RBC was significantly ($p<0.05$) lower in negative control when compared to normal control, 500mg/kg, 250mg/kg, 62.5mg/kg and significantly ($p<0.05$) higher when compared to125mg/kg and 31mg/kg. The concentration of RBC was significantly ($p<0.05$) higher in normal control when compared to other groups and significantly lower when compared to 500mg/kg. RBC was also significantly ($p<0.05$) higher in 250mg/kg group when compared to 125, 62.5, 31mg/kg group. The concentration of RBC in 125mg/kg was significantly lower when compared to other groups while 62.5mg/kg was significantly ($p<0.05$) was significantly higher when compared to 31, 125mg/kg and negative control. Thus, giving the value of RBC in negative control as (1050000.00±70710.67), normal control (4300000.00±141421.35), 500mg/kg (49560000.00±212132.03), 250mg/kg (3950000.00±70781.67), 125mg/kg (2650000.00±7071.67), 62.5mg/kg (1300000.00±141421.35) and 31mg/kg (1050000.00±7071.67).

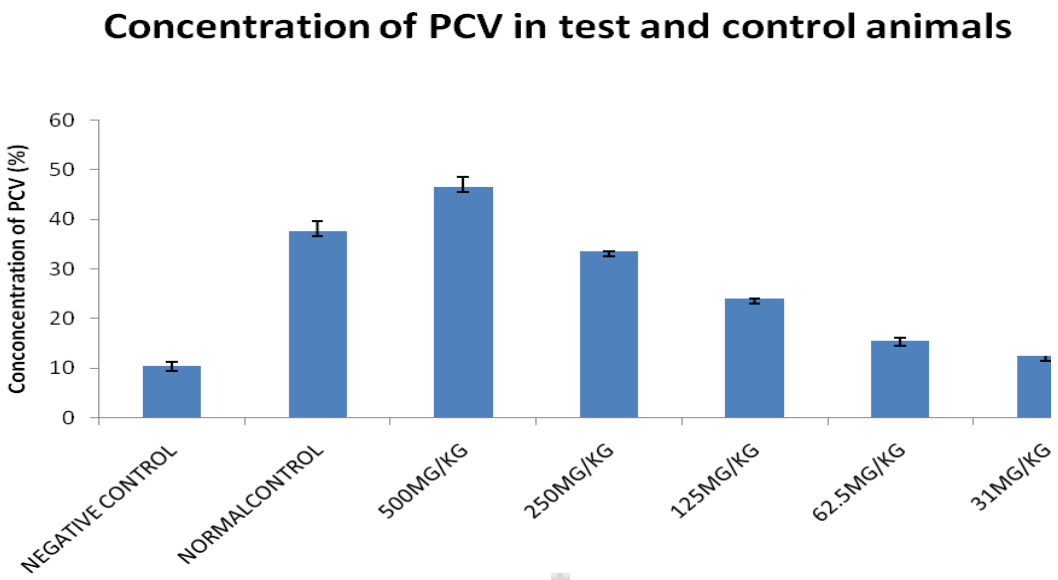


Fig. 7. Concentration of PCV test and control groups

Result for PCV concentration indicated that PCV was significantly ($p<0.05$) lower in the 500mg/kg group when compared to other groups. PCV concentration was also significantly ($p<0.05$) higher in normal control when compared to other groups and significantly ($p<0.05$). The PCV concentration was also significantly ($p<0.05$) higher in 250mg/kg group when compared to 62.5mg/kg and 32mg/kg. Thus, giving the value of PCV in negative control as (10.50±0.70), normal control (37.50±2.12), 500mg/kg (46.50±2.12), 250mg/kg (33.50±0.70), 125mg/kg (24.00±0.00), 62.5mg/kg (15.50±0.70), 31mg/kg (12.50±0.70).

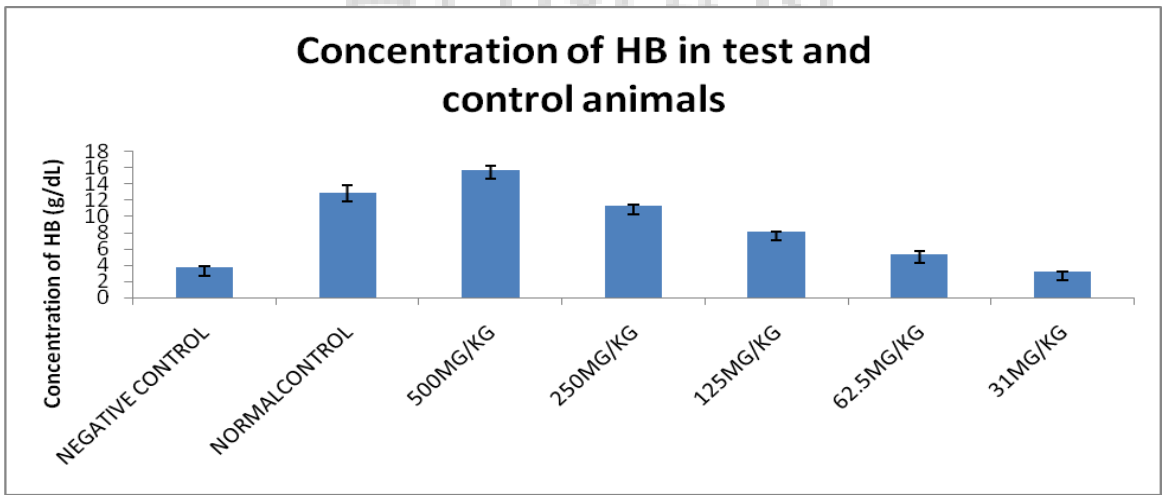


Fig. 8. Concentration of HB test and control groups

Result for HB concentration indicated that HB was significantly ($p<0.05$) higher in the 500mg/kg group when compared to other groups. Also, HB concentration was significantly ($p<0.05$) higher in normal control when compared to 250mg/kg, 125mg/kg, 62.5mg/kg and 31mg/kg group. HB concentration was also significantly higher in 250mg/kg when compared to the 125mg/kg, 62.5mg/kg and 31mg/kg group. Thus, giving the value of HB in negative control as (3.7±0.14), normal control (12.85±0.912), 500mg/kg (15.50±0.56), 250mg/kg (11.2±0.28), 125mg/kg (8.0±0.70), 62.5mg/kg (5.30±0.42), 31mg/kg (3.15±0.07).

DISCUSSION

Phytochemicals are important chemicals found virtually in all the plants and their different parts and at different concentration. The result for the phytochemicals present in *Persea americana* seed showed the presence of flavonoid (4.764%), some of the general characteristics of flavonoid include potent water soluble super antioxidants and free radical scavenger; they prevent oxidative cell damage, have strong anticancer activity and protect against all stages of carcinogens (Sala *et al.*, 1995). Flavonoids in intestinal tract lower the risk of heart disease and inflammation. Flavonoids were highest in the *Persea americana*.

The result also showed the presence of saponins and some of the general characteristics of saponins include formation of foams in aqueous solution, haemolytic activity, cholesterol binding properties (Sodipo *et al.*, 2000). The presence of alkaloid was also noticed in the phytochemical analysis of *Persea americana* seed. Alkaloids are important therapeutically significantly plant secondary metabolites, isolated pure form of alkaloids and their synthetic derivatives are used as basic medicinal agents for their analgesic and bacterial effects (Stay *et al.*, 1988). The result of the phytochemical analysis of *Persea americana* also showed the presence of phenols, phenols have been extensively researched as disease preventives (Duke, 1992).

The presence of tannins and steroids were also noticed in the phytochemical analysis, tannin noted for astringency and bitter taste, hastens the healing of wounds and inflamed mucous membrane (Okwu *et al.*, 2004). Steroids are antioxidants *in vitro*, and have a link with reproduction in humans (Rice-evans *et al.*, 1995).

The *Persea americana* seed is dosage dependent. The signs of toxicity were noticed 15 hours after the administration, there was decreased locomotive activity, decreased feed intake, postration and weakness. There was 100% mortality in the group administered with 2000mg/kg, whereas the group administered with 1000mg/kg showed 50% mortality. The result of the LD_{50} was 707.1mg/kg.

The administration of paracetamol to the animals resulted in significant increase in the serum ALP, the value is follows; Negative control is (788.90±5.80), Normal control (647.76±18.61), 500mg/kg (643.`14±8.69), 250mg/kg (511.76±5.36), 125mg/kg (481.10±10.77), 62.5mg/kg (538.52±7.22), 31mg/kg (610.62±101.28). AST for negative control is (242.40±1.54), normal control (198.55±11.34), 500mg/kg (109.65±2.99), 250mg/kg (202.67±0.72), 125mg/kg (185.75±0.88), 62.5mg/kg (90.89±0.75), 31mg/kg (112.35±4.35). ALT, Negative control is (86.23±0.37), Normal control (55.09±3.53), 500mg/kg (34.23±1.34), 250mg/kg (52.07±1.18), 125mg/kg(46.60±0.57). 62.5mg/kg (43.83±0.46), 31mg/kg (46.61±3.30)

DB, negative control is (8.70±0.17), normal control (7.31±0.14), 500mg/kg (3.35±0.30), 250mg/kg (5.90±0.35), 125mg/kg (4.54±0.08), 62.5mg/kg (5.36±0.08), 31mg/kg (6.29±0.06), and TB for negative control (9.63±0.27), normal control (7.35±0.28), 500mg/kg (9.11±0.18), 250mg/kg (8.40±0.29), 125mg/kg (6.89±0.68), 62.5mg/kg (4.23±0.12), 31mg/kg (4.11±0.22).

However there was no significant rise in the serum ALP, ALT, AST, DB, and TB in the group that was given an ethanol fraction of *Persea americana*. Paracetamol which was used to induce hepatotoxicity in experimental animals leads to covalent binding of its toxic metabolite N-acetyl P benzoquinoneimine to sulfydryl group of proteins. This causes an exhaustion of reduced glutathione in the liver resulting in cell necrosis and lipid peroxidation (Sinyth *et al.,* 2006). An increase in the level of transaminases and ALP is an indication of cellular leakage and loss of functional integrity of the hepatic cell membrane. (Poole et al., 1989) *Persea americana* seeds are very rich in anti-oxidants (Paul *et al.,* 2012). This could be possible reason why it has a hepatoprotective and haemopoietic activity.

Investigation of haematological parameters represents a useful process in the diagnosis of many diseases as well as investigation of the extent of damage to the blood (Onyeyili *et al.,* 1991). This is relevant since blood constituent's change in relation to the physiological conditions of the

animals. Haematological studies are important because blood is the major transport system of the body, and evaluation of the haematological profile usually furnishes vital information on the body's response to injury of all forms, including toxic injury (Schalm *et al.*, 1975, and Ihedioha *et al.*, 2004). Haematological constituents reflect the physiological responsiveness of the animal to its internal and external environments which include feed and feeding (Esonu *et al.*, 2001) as well as drugs (Iheukwumere *et al.*, 2007).

The purpose of this research work was to determine the effects of oral administration of the extract of *Persea americana* seed at different concentrations on some haematological parameters in Wistar rats. The varied level of significance noticed in the haematological parameters evaluated in this study (post-administration) between the control and the test groups, and even within the test groups shows that there is a significant connection between the extracts (at the various concentrations tested) and the degree of haematological effects observed. The outcome of this present study shows that extract of *Persea americana*, particularly at 500mg/Kg concentration, has significant impacts on the various haematological parameters investigated as evident in the active proliferations of blood components to varied extent as measured in the test groups, compared to the control. The appreciable increase in the values of the haematological parameters investigated may be associated with the inherent-haematopoietic-stimulating properties possessed by the extract of *Persea americana*.

CONCLUSION

This study has demonstrated hepatoprotective and a haemopoietic activity of ethanol extract of *Persea americana* in protecting the liver from damage and being able to produce more red blood cells, this effect may be due to the reduction of oxidative stress and its ability to reduce elevated level of serum marker enzymes because of some vital phytochemical and antioxidant it possess.

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