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Comparative Study of the effect of Leaf and Seed Extracts of *Persea americana* on Liver Function Using Albino Rats

Ude, C.M[+1]., Agbafor, K.N[2]. and Eze-Steven, P.E[1].

[1]Department of Applied Biochemistry, Enugu State University of Science and Technology, Agbani, Enugu State, Nigeria.

[2]Department of Biochemistry, Ebonyi State University, Abakaliki, Ebonyi State, Nigeria.

ABSTRACT

Persea americana leaves and seeds are used in herbal medicine for the treatment/management of various ailments in Nigeria. The liver is a vital organ involved in numerous metabolic processes and uncontrolled administration of foreign compounds into the body can impair its functions. This study was conducted to compare the effect of administration of various doses of the aqueous and ethylacetate seed and leaf extracts of *Persea americana* on liver function using albino rats. The study was carried out using seventeen (17) groups of five (5) rats each. The rats wereadministered 200,400, 600 and 800 mg/kg body weight of extracts for twenty-one (21) days by oral gavage. The control group received 0.5ml/kg body weight of normalsaline. The animals were thereafter sacrificed and the liver and blood sample collected for analysis. The activities of alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) and the levels of bilirubin were significantly ($P<0.05$) lower in the deionised water leaf (DWL) extract (up to 600 mg/kg body weight) then in the control and ethylacetate seed (EAS) extract while albumin was significantly ($P<0.05$) higher. The seed extracts had significantly ($P<0.05$) higher values for the enzymes and significantly ($P<0.05$) lower value for albumin. A dose dependant increase in the effect of both leave and seed extracts, with the seed extract having more adverse effect was established.

Keywords: *Persea americana*, Liver function, Liver enzymes, Albino rats.

INTRODUCTION

Persea americana (avocado) is a tree classified as a member of the plant family *Lauraceae*. The three botanical races: West Indian, Mexican and Guatemalan are now cultivated worldwide [1,2,3,4,5]. Avocado pear is known locally as Pia ewe, Igba/apoka (Yoruba), ubebekee/ubeoyibo (Igbo), orumwu (Edo), piya (Hausa) and ebenmbakara (Efik) [6,7,8,9]. *Persea americana* is used by traditional health practitioners for the treatment and management of various ailments in men. These include their use as anti-diabetic, anti-trussive, anti-arthritic, convulsion, epilepsy and hypertension [10,11,12,13,14]. The leaf and seed of *Persea americana* are reported to have beneficial effect in the management of cardiovascular disease [15,16,17]. The medicinal and pharmacologic actions of medicinal plants are usually due to the presence of chemicals known as secondary metabolites and *Persea americana* leaf and seed are known to possess the chemicals in significant quantities [18,19,20]. The uncontrolled administration of chemical substances as is prevalent in the use of most herbal preparations may lead to adverse effect in the vital organs of the body including the liver. The liver which is the second largest organ in the body and situated under the rib cage on the right side carries out different kinds of biochemical, synthetic and excretory functions. Drugs are known to cause liver disease [21] and when this happens, the liver loses its ability to perform its natural functions. This work studied the effect of the aqueous and ethylacetate leaf and seed extract on the liver functions of albino rats.

Plate 1:*Persea americana* fruits, seeds and leaves. (Source: Researcher's photo)

MATERIALS AND METHODS

Chemicals, Reagents and Equipment

The chemicals and Reagents employed in this investigation were of analytical grade and products of Randox USA, May & Baker, England and Merck, Germany. All equipment used were in good working condition.

Sample Preparation

The seeds and leaves of *Persea americana* were collected from Umuaga in Udi Local Government Area of Enugu State, Nigeria. They were identified by Dr. S. C. Eze of the Department of Applied Biology and Biotechnology, Enugu State University of Science and Technology, Enugu. The male albino rats (120 – 150g) used were purchased from the Department of Animal Science, University of Nigeria, Nsukka. The *Persea americana* seeds were grated and air dried. The leaves were also air dried. The dried samples 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 deionized water respectively. The same quantity (500g) of leaf powder were 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. The extracts were then stored in air-tight receptacles.

Experimental Design

Male albino rats, eighty five (85) in number were maintained on normal rat chew and water *ad libitum* and allowed to acclimatize for seven days. The rats were grouped into seventeen (17) with each group having five (5) rats and administered normal saline and extracts as follows

Group 1, 0.5ml/kg normal saline (normal control)
Group 2-5, 200, 400, 600 and 800 mg/Kg body weight ethylacetate leaf extracts.
Groups 6-9, 200,400, 600, and 800 mg/Kg body weight ethylacetate seed extracts.
Groups 10 – 13, 200, 400, 600 and 800 mg/Kg body weight aqueous leaf extracts.
Groups 14 – 17, 200, 400, 600 and 800 mg/Kg body weight aqueous seed extracts.

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

Determination of Alkaline Phosphatase Activity by Rec (1972) method

Tests tubes were labelled appropriately and set up on a rack. One vial of substrate (Rlb, 10mmol/L p-nitrophenylphosphate) was reconstituted by introducing 3.0ml of buffer (Rla, 1mol/l diethanolamine buffer pH 9.8, 0.5mmol/L MgO_2) into it. Each sample (002ml) was introduced into the appropriate tube followed by 1.0ml of reagent and mixed. The initial absorbance was read at 405nm against air. The reaction was timed and absorbance reading taken again after 1, 2 and 3 minutes. Alkaline phosphatase activity was calculated as follows,

ALP (U/L) = 2760 x ΔA

Where ΔA = change in absorbance

2760 – factor

Determination of AlanineAminotransferace (ALT) Activity by Reitman and Frankel (1957) Method

Test tubes were labelled blank and samples and placed on a rack. 0.1ml each of distilled water and sample were pipetted into the appropriate test tubes followed by 0.5ml of reagent 1 (R1, phosphate buffer 100mmol/L pH 7.4, L-alanine 200mmol/L, α-ketoglutarate 2.0mmol/L). The tubes were shaken to mix and incubated for exactly 30 minutes at 37° C. 0.5ml of reagent 2 (2,4-dinitrophenylhydrazine 2.0mmol/L) was then added to each tube, mixed and further incubated for 20 minutes at 24°C. This was followed by the addition of 5.0ml of sodium hydroxide solution (0.4 mol/L) to each tube and mixing. The absorbance of the samples were read at 546nm against the reagent blank after 5 minutes. ALT activity was obtained by reference to the ALT activity table.

Determination of Aspartate Aminotransferase (AST) Activity by Reitman and Frankel (1957) Method

Test tubes were labelled blank and sample and placed in a rack. 0.1ml of distilled water was pipetted into the blank tube followed by 0.1ml of sample to the sample tubes. 0.5ml each of reagent 1 (Phosphate buffer 100mmol/L pH7.4, L-aspartate 100mmol/L, α-ketoglutarate 2mmol/L) was added to the tubes followed by 200ml of sample, mixed and incubated for 30 minutes at 37°C. 0.5ml each of reagent 2 (2,4-dinitrophenylhydrazine 2mmol/L) was then added to each tube, mixed and allowed to stand at 25°C for 20 minutes. 5.0ml of sodium hydroxide (0.4mol/L) was then added to each tube, mixed and the absorbance read at 546nm against the reagent blank using a spectrophotometer. AST activity was obtained by reference to AST activity tableprovided.

Determination of Albumin Concentration by the Bromocresol Green Method (Tietz, 1987)

The contents of one BCG concentrate (succinate buffer 75mmol pH 4.2, bromocresol green 1.9mmol, Brij 35 preservative) was diluted with 87.0ml of distilled water. Test tubes were labeled blank, standard and sample and stood on a rack. Volumes (0.01ml) each of distilled water, standard and sample were respectively pipetted into appropriate test tubes followed by 3.0ml each of BCG reagent. They were mixed and incubated at 25°C for 5 minutes.

The absorbance was read at 650nm against the reagent blank. The albumin concentration was calculated using the following formula.

$$\text{Albumin (g/dl)} = \frac{absorbance\,of\,sample}{absorbance\,of\,standard} \times \text{concentration of standard}$$

Determination of Bilirubin Concentration by Jandrassik and Grof (1938) Method

Test tubes were labeled sample blank and sample and placed on a rack. 200µl of reagent 1 (Sulphanilic acid 29mmol/L, Hydrochloric acid 0.17N) was added to each tube followed by 50µl of reagent 2 (38.5mmol/L sodium nitrite) to only the sample tubes. 2000µl 0.9% NaCl solution was then added to all tubes, followed by 200µl of sample. They were mixed and incubated at 25°C for 10 minutes. The absorbance of the samples were read at

546nm in a spectrophotometer against the sample blank. The bilirubin concentration was calculated using the following relationship.

Bilirubin (mg/dl) = 14.4 x absorbance

Statistical Analysis

All the data generated were expressed as mean ± standard deviation. The differences among experimental and control groups were determined using SPSS for windows software programme (version 20). Group comparism were done employing analysis of variance (ANOVA) test. P-values less than 0.05 (P<0.05) were regarded as significant.

RESULTS

Effect of Administration of *Persea americana* Extracts on Rat Serum Alkaline Phosphatase (ALP) Activity.

Alkaline phosphatase activity in the normal control was significantly (P<0.05) lower than those of DWS and EAS treated groups while the activity in the normal control was significantly (P<0.05) higher than those of DWL and EAL extract treated groups up to 400 mg/Kg body weight dose and DWL up to 600 mg/Kg body weight dose.ALP activity in the DWL and EAL extract treated groups were significantly (P<0.05) lower than those of the DWS and EAS extract treated groups. The activity of all extract treated groups were significantly (P<0.05) higher than the normal control at 800 mg/Kg body weight dose (Figure 1).

Effect of Administration of *Persea americana* Extracts on Rat Serum Alanine Aminotransferase (ALT) Activity.

ALT activity in DWS and EAS extract treated groups at all doses were significantly (P<0.05) higher than that of the normal control groups. The activity of the enzyme in the DWL extract treated group was significantly (P<0.05) lower than the normal control at 600 mg/Kg body weight dose. ALT activity in the DWL and EAL extract treated groups were significantly (P<0.05) higher than that of the normal control group at 800 mg/Kg body weight (Figure 2).

Effect of Administration of *Persea americana* extracts on Rat Serum Aspartate Aminotransferase (AST) Activity

AST activity in the EAS and DWS extract treated groups at all doses were significantly (P<0.05) higher than that of the normal control group. The activity of the enzyme showed a dose dependent (P<0.05) decrease for the DWL and EAL extract treated groups up to 600 mg/Kg body weight dose compared with the normal control group. AST activity of DWL and EAL extract treated groups were significantly (P<0.05) higher than that of the normal control at 800 mg/Kg body weight dose (Figure 3).

Effect of Administration of *Persea americana* Extracts on Rat Serum Bilirubin Concentration.

Bilirubin concentration in the DWS and EAS extract treated groups were significantly (P<0.05) higher than that of the normal control group from 400 to 800 mg/Kg body weight dose. Bilirubin concentration of the DWL extract treated group was significantly (P<0.05) lower than that of normal control up to 400 mg/Kg body weight dose while the value for normal control was not significantly (P>0.05) different from those of the EAL and DWL extract treated groups at 600 mg/Kg body weight dose (Figure 4).

Effect of Administration of *Persea americana* Extracts on Rat Serum Albumin Concentration.

Albumin concentration in DWL extract treated group was significantly (P<0.05) higher than that of normal control up to 400 mg/Kg body weight dose while the concentration in EAL extract treated group was significantly (P<0.05) higher than that of the normal control group at 400 and 600 mg/Kg body weight doses. Albumin concentration in DWS and EAS extract treated groups showed dose dependent significant (P<0.05) decrease compared with normal control (Figure 5).

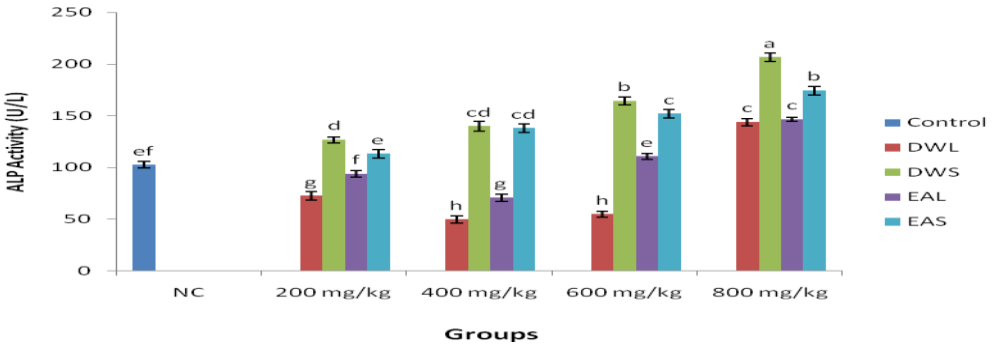

Figure 1 Serum alkaline phosphatase (ALP) activity in albino rats administered aqueous and ethylacetate seed and leaf extracts of *Perseaamericana*
The data are presented as mean ± SD. NC = normal control. DWL = aqueous leaf, DWS = aqueous seed extracts. EAL = ethylacetate leaf, EAS = ethylacetate seed extracts. Bars with the same letters are not significantly different (P<0.05).

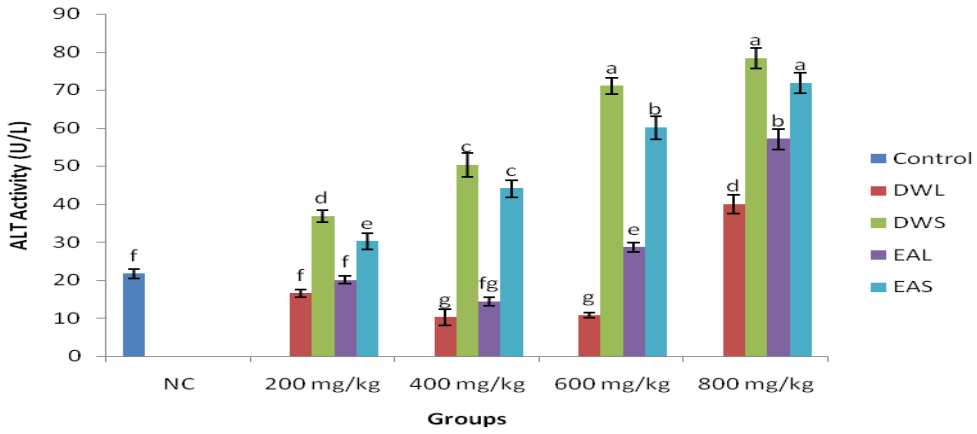

Figure 2, Serum alanine aminotransferase (ALT) activity in albino rats administered aqueous and ethylacetate seed and leaf extracts of *Perseaamericana*.
The data are presented as mean ± SD. NC = normal control. DWL = aqueous leaf, DWS = aqueous seed extracts. EAL = ethylacetate leaf, EAS = ethylacetate seed extracts. Bars with the same letters are not significantly different (P<0.05).

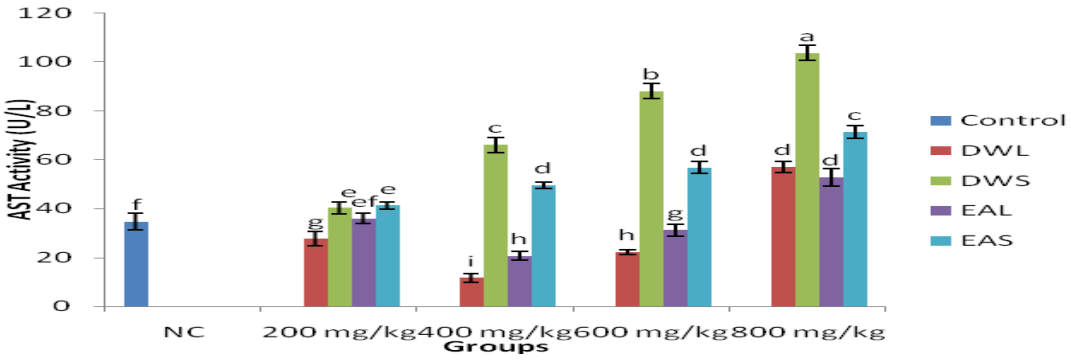

Figure 3, Serum aspartate aminotransferase (AST) activity in albino rats administered aqueous and ethylacetate seed and leaf extracts of *Perseaamericana*.
The data are presented as mean ± SD. NC = normal control. DWL = aqueous leaf DWS = aqueous seed extracts. EAL = ethylacetate leaf, EAS = ethylacetate seed extracts. Bars with the same letters are not significantly different (P<0.05).

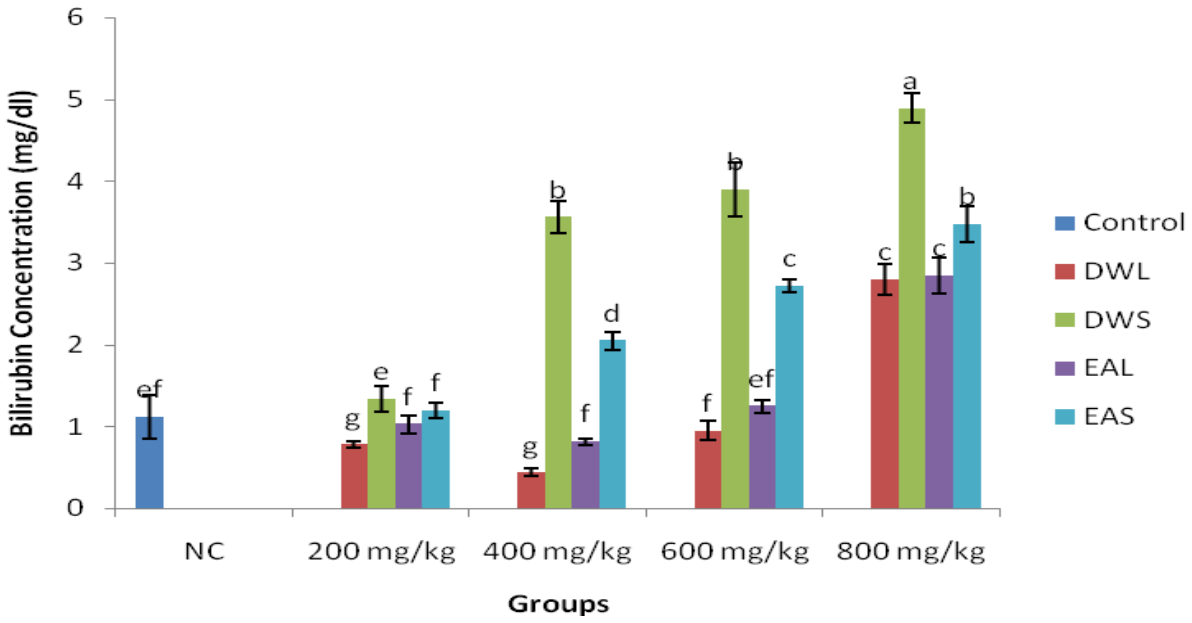

Figure 4;Serum bilirubin level in albino rats administered aqueous and ethylacetate seed and leaf extracts of *Perseaamericana.*
The data are presented as mean ± SD. NC = normal control, DWL = aqueous leaf, DWS = aqueous seed extracts. EAL = ethylacetate leaf, EAS = ethylacetate seed extract. Bars with the same letter are not significantly different (P<0.05).

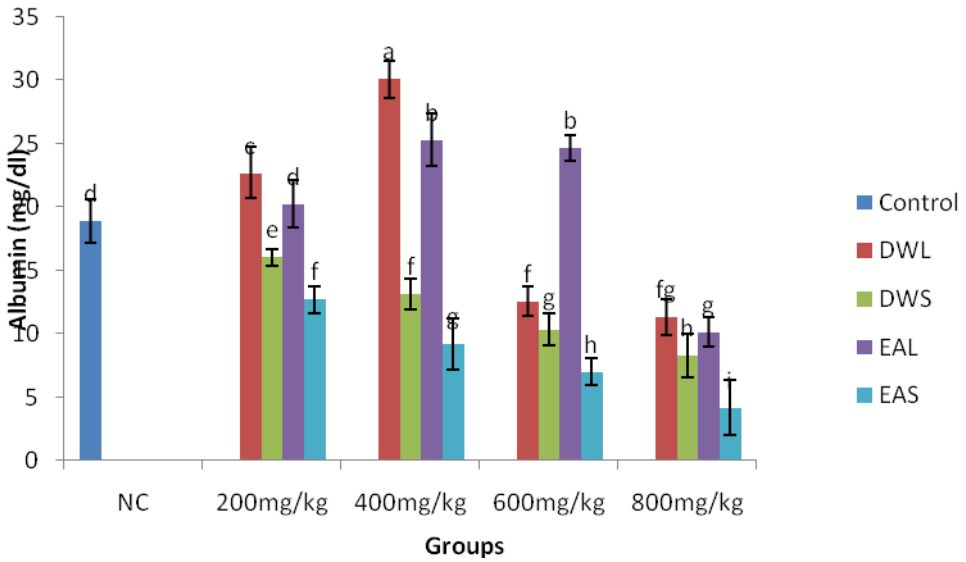

Figure 5, Serum albumin level in albino rats administered aqueous and ethylacetate seed and leaf extracts of *Perseaamericana*.
The data are presented as mean ± SD. NC = normal control, DWL = aqueous leaf, DWS = aqueous seed extracts. EAL = ethylacetate leaf, EAS = ethylacetate seed extract. Bars with the same letter are not significantly different (P<0.05).

DISCUSSION

The study of the effect of the extract of liver showed a dose dependent significant (P<0.05) increase in the activities of the liver enzymes ALP, ALT and AST in the DWS and EAS extract treated groups compared with the normal control (Figures 1, 2, 3). This suggests that the seed extracts of *P. americana* may be toxic to the liver. These enzymes are found in the liver and during liver damage, they are released into the bloodstream leading to elevation in their levels. The enzymes are used as specific markers (especially ALT and AST) for hepatocellular necrosis [8]. [22],[23] applying 500 mg/Kg body weight aqueous and phenol seed extracts on albino rats recorded increased levels of the liver enzyme ALT, AST and ALP, a result akin to the findings in this report. The activity of the enzymes in the EAL and DWL extracts treated groups were significantly (P<0.05) lower than the normal control group up to 600 mg/Kg body weight dose but significantly (P<0.05) higher above 600 mg/Kg body weight dose. This suggests that at lower doses, the leaf extracts may not be toxic but at doses from 800 mg/Kg body weight, they may be toxic to the liver. Olaniyan (2014) reported that administration of liquid extracts of *P. americana* leaves on hypertensive patients led to reduced plasma levels of total cholesterol, and LDL-cholesterol and increased values for ALT and AST. This report supports the findings in this work suggesting that while the extracts may be beneficial in lowering TC and LDL, it may be toxic to the liver especially at higher doses. Bilirubin concentration in the DWS and EAS extract treated groups showed a dose dependent significant (P<0.05) increase compared with the normal control group (Figure 4). Bilirubin is derived from haemoglobin degradation from red blood cells. The liver processes it for excretion through stool. Inability of the liver to process bilirubin for excretion leads to its accumulation and spilling into circulation leading to elevation in its value. Increase in the level of bilirubin in the DWS and EAS extract groups therefore suggest that the seed extracts of *P americana* may have damaged the liver cells preventing them from performing their excretory function. On the other hand, the bilirubin level in the DWL and EAL extract treated groups were substantially not significantly (P>0.05) different from the normal control group up to 600 mg/Kg body weight dose but significantly (P<0.05) higher at the 800 mg/Kg dose. By implication, the leaf extract may not be toxic to the liver up to 600 mg/Kg dose after which it may be toxic. Albumin concentration in the DWS and EAS extract treated groups showed a dose dependent significant (P<0.05) decrease compared with the normal control (Figure 5). The liver performs a lot of synthetic functions and albumin is quantitatively the most important plasma protein synthesized by the liver. It is a useful marker of hepatic function [8,9]. Traumatization of the liver results in its inability to perform its synthetic functions. The low level of albumin displayed by the administration of the seed extracts suggests that the seed extracts were injurious to the liver. The concentration of albumin in the DWL and EAL extract treated groups were significantly (P<0.05) higher than the normal control group up to 600 mg/Kg body weight dose after which it was significantly (P<0.05) lower at 800 mg/Kg body weight dose. This suggest that the leaf extracts may be relatively safe up to 600 mg/Kg body weight dose but toxic from 800 mg/Kg body weight dose.

CONCLUSION

This study has shown that the leaf extracts of *Perseaamericana* may not be toxic to the liver especially at doses up to 600mg/kg body weight while the seed extracts appears to be toxic at lower doses. The use of the leaf extracts is therefore more advisable than the seed extract.

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