Global Scientific JOURNALS

GSJ: Volume 7, Issue 1, January 2019, Online: ISSN 2320-9186
www.globalscientificjournal.com

TOXICOLOGICAL AND BIOCHEMICAL EVALUATION OF OIL FROM THE SEED OF DACROYDES EDULIS

***Efosa, J.O., Egielewa, S.J., Ojei, J.U. and Udoji, N.J.**

Department of Science Laboratory Technology, School of Applied Science and Technology, Auchi Polytechnic,P.M.B. 13,Auchi,Edo State, Nigeria.

*Corresponding author's email: salvationnumber1@yahoo.com

ABSTRACT

This study investigated toxicological, hematological and histopathological evaluations of albino rats treated with *D.edulis* seed oil using petroleum ether as extracting solvent. The objective of the present study was to investigate potential adverse effects, if any, of *D.edulis* seed oil using petroleum ether as extracting solvent.The study included histopathology of liver, kidney, and heart tissues, evaluation of biochemical parameters in plasma, liver, kidney, and heart tissues, *in vivo*enzymatic antioxidant studies (thiobarbituric acid reactive substances (TBARS), superoxide dismutase (SOD), and catalase (CAT)),electrolyte profile, liver enzyme assays, and lipid profile. Acute toxicity studies were carried out using twenty-five (25) male albino rats shared into five groups. Group A served as control while groups B, C, D, and E received doses of 100mg/kg, 250mg/kg, 5,000 mg/kg and 10,000mg/kg respectively.Sub-chronic studies were also performed on twenty-five (25) male albino rats divided into five groups and each received doses of 1,000mg/kg, 1,500mg/kg, 2,000mg/kg, 5,000mg/kg, and 0 mg/kg of the seed oil respectively. The acute and sub-chronic toxicity tests revealed that the extracts were tolerated with no observable adverse effects or mortality up to a dose

of 10,000 and 5000 (mg/kg) body weight respectively. The plasma obtained was used to determine the level of alanine transaminase (ALT), alkaline phosphatase (ALP) and aspartate aminotransferase (AST) and gamma glutamyl transferase (GGT). The seed oil did not compromise the activities of the liver, kidney, and heart tissues as the plasma levels of ALT,ALP, AST,and GGT, cholesterol, triglycerides, HDL, and LDL were not elevated ($p>0.05$) .The seed oil did not cause electrolyte imbalance in experimental animals as Na^+, K^+, HCO_3^-, Cl^- and creatinine were not significantly elevated in the plasma ($p>0.05$) but urea was significantly elevated when compared to the control ($p<0.05$). Antioxidant enzymes were not elevated in the liver, kidney, and heart tissues but TBARS activities were elevated. Haematological parameters were not adversely affected. Total bilirubin, conjugated bilirubin, total protein and albumin were not significantly elevated ($p>0.05$). Histopathological examination did not show significant biochemical lesions in the kidney and heart tissues but in the liver, there were mild biochemical lesions observed. *D. edulis* seed oil is relatively safe for domestic uses.

Keywords: Toxicological, biochemical, hematological, histopathology,*D.edulis*

INTRODUCTION

African pear (*D. edulis*) belongs to the family of Buseraceae. It is known as *Safou* in French,*ube* in Igbo, *elemi* in Yoruba, *eben* in Efik and *orumu* in Benin(Kengue and Nyangatou, 1990).There are two varieties of Dacryodes edulis in Nigeria viz; *Devar edulis* and *Devar parvicarpa* (Isaac and Ekpa,2009). The implication of high total cholesterol, Low-Density-Lipoprotein (LDL)-cholesterol, triacylglycerol and low High-Density Lipoprotein (HDL)-cholesterol in the development of cardiovascular disorders such as hypertension, arteriosclerosis, stroke and heart failure can never be over emphasized (Ghasi *et al*., 2000). There has been tremendous increase in the use of functional foods and, or nutraceuticals due to their beneficial effects on human health. Oil extracted from prickly pear seeds has been found to exhibit hypoglycemic and hypocholesterolemic effects (Ennouri *et al*., 2007). Changes in the lipoprotein composition of the plasma or serum could be attributed to the type of fat ingested in the diet. Bush pear oil is one of the most important rated versatile vegetable oil. It has been advocated that the *Safou* oil should take its place in the food industry, the pharmaceutical and the cosmetics industry as well as in other branches of industry where raw fat materials are needed.

Years back, it was observed that plasma levels of potassium significantly decreased in the albino rats treated with oils extracted from *Xylopia aethiopica, Piper guineense* and *Tetrapleura tetrapter*a plants. Hypokalaemic effect was evident in albino rat(Nwaichi and Igbinogbaro,2012). Also two of the animals fed with *Tetrapleura tetraptera* diet died in the third week which could be attributable to possible hypernatremia. These problems mentioned above have influenced the toxicological evaluation of albino rat fed on the oil from the seed of*Dacryodesedulis*.

Till date not much has been done in relation to toxicity of oil from the seed and its possible pharmacological potentials and there is a growing interest in the possibility of using *D. edulis* oil as a source of edible oil. This has necessitated the need for a reasonable toxicological evaluation of this oil. So, this study is therefore designed to evaluate the toxicity and biochemical evaluation of the oil from the seed of *D. edulis* and to ascertain the toxicity and the nutritional status of the oil.

MATERIALS AND METHODS

Plant Materials

African pears were purchased from Uchi market,Auchi, Etsako West Local Government Area,Edo State,Nigeria. The plant was identified in Botany Department, University of Benin, Edo State, Nigeria. Sample was deposited at the departmental hebarium. Pre-extraction activities were carried out such as washing, and removal of foreign element. The fruits were split open with a sharp knife to remove the seed from the pulp. The seed sample was dried at a temperature of 70 ^{o}C in a Gallenkamp hot air oven model OV 160 for 48 hrs. The samples were milled with corona traditional grain mill REF 121 (100 μm mesh size).

Animal Materials

A total of fifty (50) healthy male albino rats of the Wister strain purchased at the animal house of Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Benin-City, Nigeria were used for the experiments. They were housed in metal cages with plastic base in the animal house of the Department of Pharmacology and Toxicology, University of Benin at room temperature. They were fed with commercially available standard pelleted feed and water *ad libitum*. The animals were acclimatized for fourteen days. The "Guide for the care and use of laboratory animals" issued by Institute of Laboratory Animal Research, Commission on Life Sciences, National Research Council (1996) was complied with and the study was approved by the Ethical Committee

of Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Benin-City, Nigeria.

Chemicals Used

Analytical grade chemicals were used and all were products of British Drug House (BDH), Poole, England. Triacylglycerol,total cholesterol, LDL-cholesterol and HDL-cholesterol diagnostic Kits were obtained from Randox Laboratories Ltd., Ardmore, United Kingdom.

Experimentation and Design

Acute studies were carried out ontwenty-five (25) male albino rats divided into five groups and were fed with graded doses of the seed oil of 100mg/kg,250mg/kg, 5,000mg/kg, 10,000mg/kg, and 0mg/kg respectively. The LD50 values were calculated using 'Probit Analysis' (Finney, 1971). Sub-chronic studies were performed on twenty five (25) male albino rats shared into five groups treated with 1,000mg/kg, 1,500mg/kg, 2,000mg/kg, 5,000mg/kg, and 0mg/kg of the seed oil respectively.

Blood Samples Collection and Analyses

Blood samples were collected by cardiac puncture into heparinized bottles and were centrifuged at 3,000rpm for 15min. The plasma obtained was stored into new plain sample bottles, frozen until required for biochemical assays.

Assay Methods

Malondialdehyde level was estimated by the method of Buege and Aust (1978).The method of Cohen *et al.,* (1970) was adopted for catalase.Superoxide dismutase (SOD) activity was determined according to the method of Misra and Fridovich (1989).Liver enzymes (ALT, AST, ALP, and GGT) were estimated using Selectra Pros machine.Total protein was determined by Biuret method as described by Okutucu *et al*. (2007).Plasma albumin was determined by Bromocresol Green method (Doumas *et al.*, 1987). Creatinine assay was determined by Jaffes method.Urea was determined by the method of Berthelot as modified bySims (2006).Plasma total cholesterol, LDL-cholesterol, HDL-cholesterol and triacylglycerol levels were determined using diagnostic kits from Randox Laboratories, U.K.Electrolytes concentrationswere determined using Ion Selective Electrode (ISE) method.Hematological evaluation was determined using an automated

Haematolizer.Histopathological analyses for liver, heart and kidney tissues were determined according to the method described byAwvioro (2002).

Statistical Analysis

Mean and standard deviation were calculated and all the data obtained were analyzed statistically using Graphpad Prism version 6.All results represented were Mean±Standard deviation of five (5) determinations.

RESULTS AND DISCUSSIONS

Acute and Sub-Chronic Toxicity

For acute and sub-chronic toxicity, the behavioral changes observed during the study include, restlessness, hyper-respiration, jerking, over-reactive (hyperactive) and itching. At acute toxicity study, no animal death was observed even at a dose of 10,000mg/kg of the seed oil. Similarly, at sub-chronic toxicity study, no death was observed even at a dose of 5,000mg/kg, which indicates that the respective seed oil is safe orally. *D. edulis* is known to be non-toxic (Ajibesin *et al*., 2002; Obasi and Okolie, 1993). Ajibesin (2011)supported this finding when hereported lack of toxic substances in the seed of the plant. Findings by Hewitt *et al.* (2000) reveal that within the periods of 24-48 h, rapid toxicity assessment can be ascertained, which gives credence to this work.

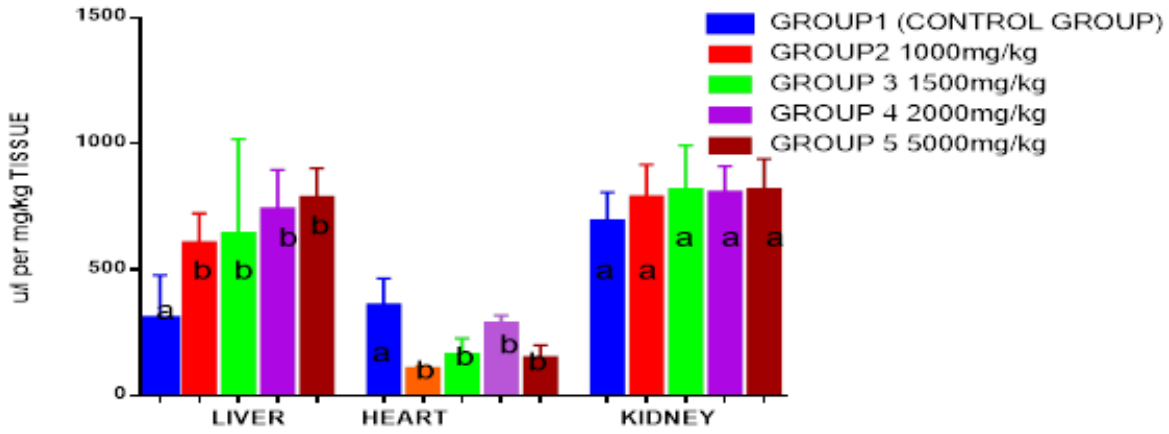


Fig. 1: *Invivo* Antioxidant assay results for seed oil Thiobraturic acid assay (liver, heart and kidney).

The TBARS activities (liver, heart, and kidney tissues) are observed to increase significantly in all groups(1,000mg/kg, 1,500mg/kg, 2,000mg/kg, and 5,000mg/kg) when compared to control($p<0.05$).This result could be attributed to the fact that the extract from the seed undergoes lipid peroxidation, which could trigger oxidative stress in the tissues. Lipid peroxidation of polyunsaturated fatty acids form products such as malondialdehyde (MDA), which is regarded as a carcinogen (Sehrawat and Sultana, 2006). The results from this study suggest that treatment of albino rats with *D. edulis* seed oil for 28 days significantly increase MDA levels in the respective tissues.

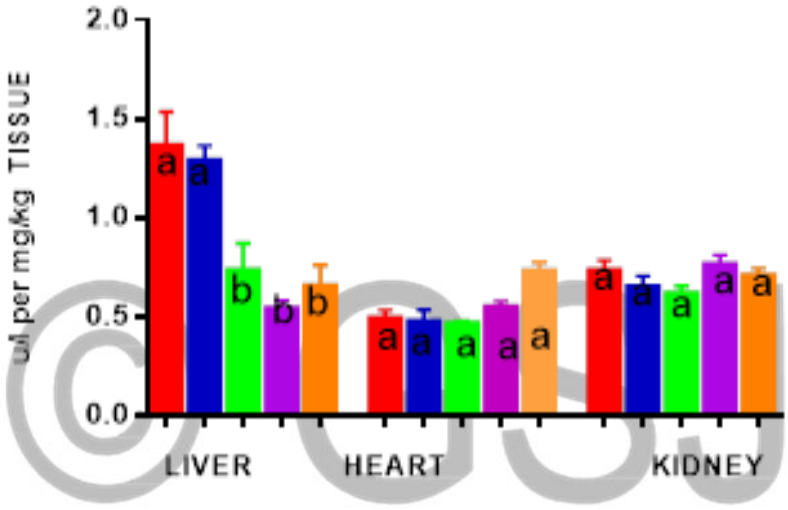


Fig. 2:*In vivo* antioxidant assay results for seed oil catalase (liver, heart and kidney)

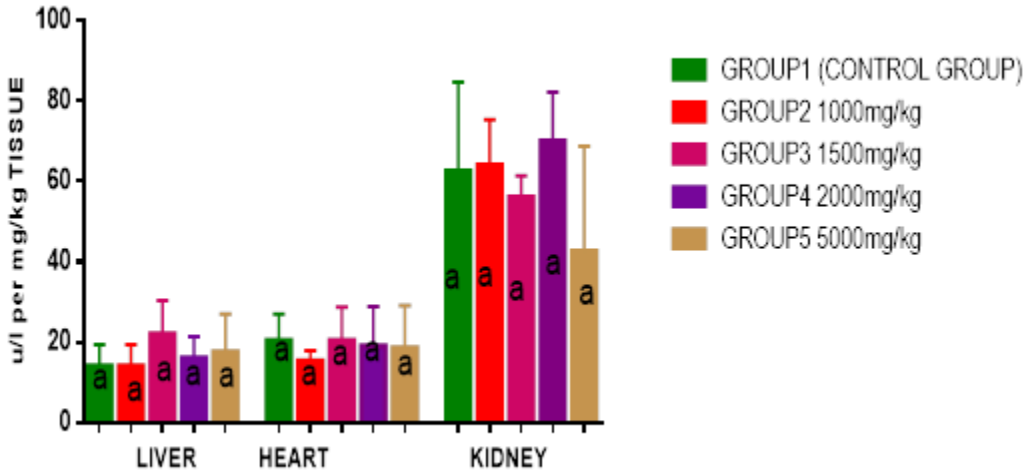


Fig. 3:*In vivo* antioxidant assay results for seed oil sod (liver, heart and kidney)

The superoxide dismutase (SOD) and Catalase (CAT) activities (liver, heart, and kidney tissues) increase significantly in all groups when compared to control($p>0.05$).The most important enzymes for removal of ROS in the cell are SOD and CAT. SOD catalyzes the dismutation of superoxide anion to hydrogen peroxide and molecular oxygen (Frdovich, 1986). Increased SOD and/or CAT synthesis is correlated with increased tolerance to oxidative stress (Arisi*et al.,1998*; Mittler,2002). This clearly indicates that SOD and/or CAT protect eucaryotic metabolic enzymes against damage by ROS. These buttress the finding of this work that *D.edulis* seed oil does not cause oxidative stress in the liver of male albino rats of Wistar strain.

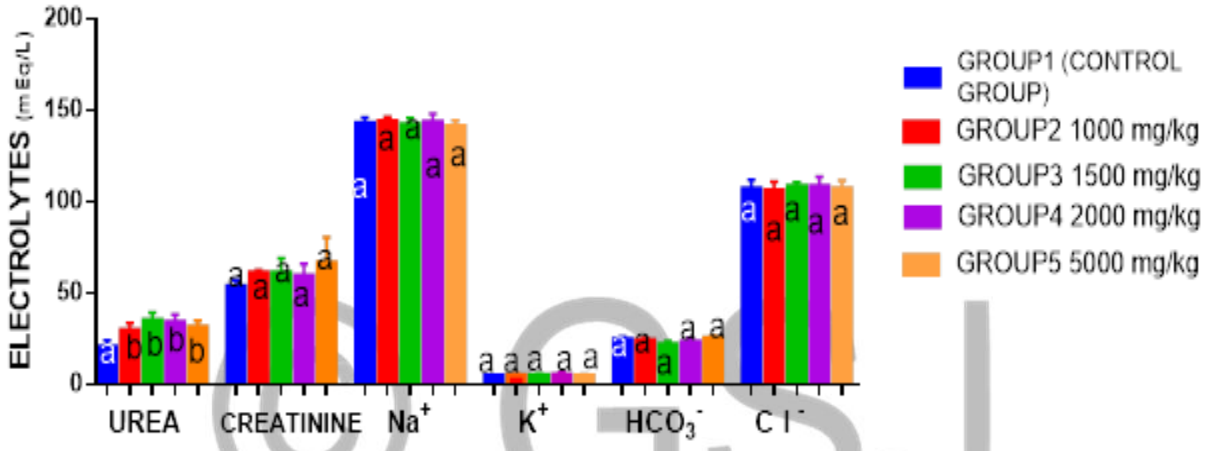


Fig. 4:Plasma urea, creatinine and electrolyte for seed oil

Electrolytes of Na^+, K^+, HCO_3^- and Cl^-show no significant difference in all treatment groups when compared to control ($p>0.05$). Urea concentration reveals significant difference in all treatment groups when compared to the control ($p<0.05$). Creatinine level shows no significant difference in all treatment groups when compared to the control ($p>0.05$). Plasma concentrations of creatinine and urea could be used as indicators of nephrotoxicity (Saka *et al*., 2012). Low clearance of creatinine or/and urea indicates a diminished impaired ability of the kidneys to filter these waste products from the blood and excrete them in urine. As their clearance values decrease, their blood levels increase. Hence, an abnormally elevated blood creatinine is diagnostic of impaired renal function. This study revealed that seed oil causes a significant rise in serum urea and non-significant increase in creatinine levels. This study suggests that seed oil could not be ascertained to promote nephrotoxicity or cause impaired renal function as there was no increase in plasma creatinine and electrolyte which would have given credence to this claim.

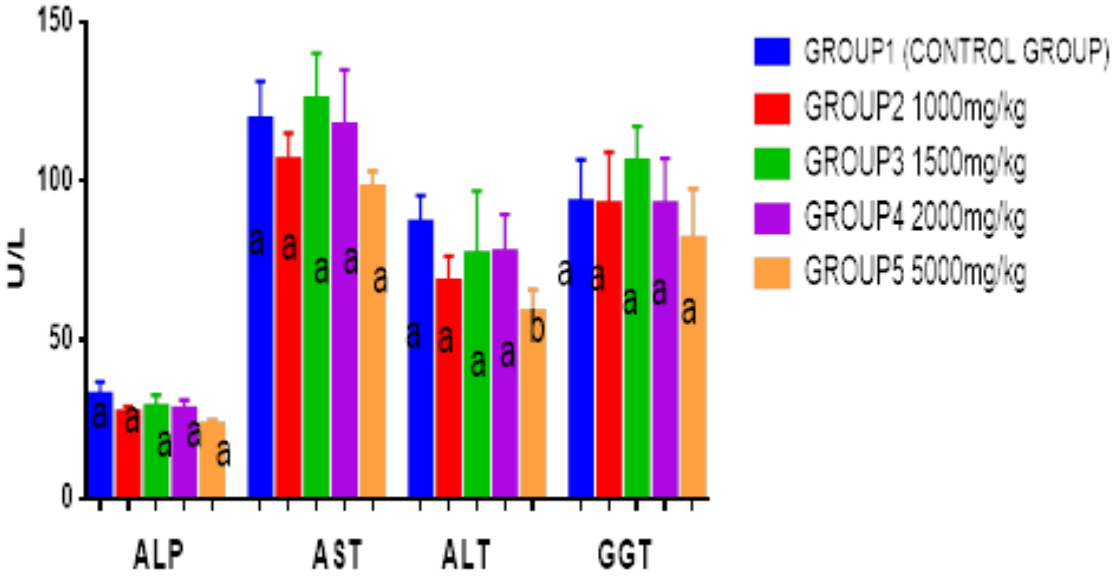


Fig. 5**:** Seed oil liver function test (ALP, ALT, AST and GGT)

Gamma glutamyl transferase (**GGT)** shows no significant difference in all treatment groups with respect to control ($p>0.05$). Other liver enzymes, that is, alanine aminotransferase **(ALT),** aspartate aminotransferase **(AST),** alkaline phosphatase **(ALP)** reveal the same trend or pattern. The non-elevated plasma levels of these enzymes observed in this study suggest non-impaired liver function as increased activities of plasma ALP is due to increased synthesis in the presence of increasing biliary pressure. Plasma alkaline phosphatase is known to increase when there is biliary obstruction as seen in cholestatic disease of the liver. GGT is a membrane-bound enzyme and an elevated level in plasma is an indicator of cell or tissue damage (Vasudha *et al.*,2006). These statementsjustify the claims that the seed oil of *D. edulis*is not toxic to the liver, as marker enzymes were not elevated.

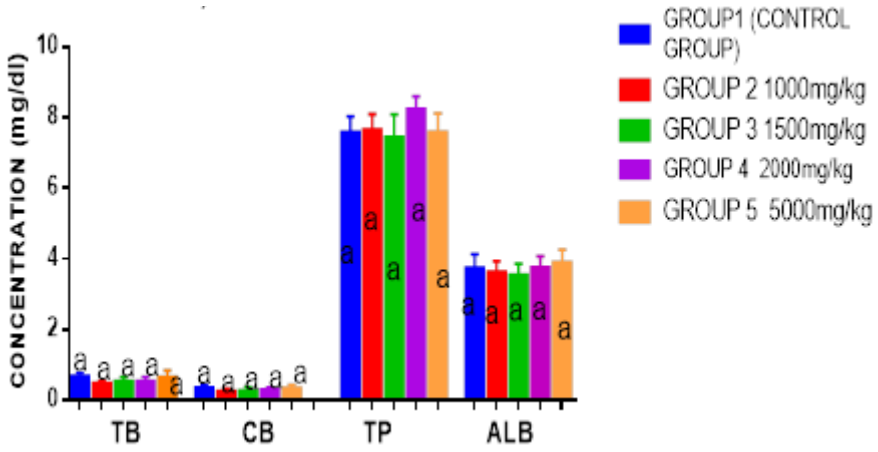


Fig. 6**:** Plasma total bilirubin, conjugated bilirubin, total protein, and albumin for seed oil

The plasma total protein value is not significant in all the treatment groups when compared with the control ($p>0.05$) as shown above. Generally, this study infers that *D. edulis* seed oil did not impair protein metabolism.

The serum level of albumin fractions decrease non-significantly in treatment groups 1 and 2 (1000mg/kg and 1500mg/kg) respectively while it increases insignificantly in groups 3 and 4 treatments (2000mg/kg and 5000mg/kg) respectively ($p>0.05$). This implies that albumin metabolism by the liver is not affected by the administration of seed oil of *D. edulis* at lower concentrations.

Total bilirubin values decreased non-significantly ($p>0.05$) for rats in all the treatment groups when compared with the control.

Thetotal (unconjugated) bilirubin is not water soluble, as such; albumin binding aids its transportation across the watery plasma. Direct (conjugated) bilirubin is water soluble and when present in the blood can be filtered through glomerulus appearing in the urine. Secretion of conjugated bilirubin in bile across the biliary canalicular membrane is a rate limiting process and is sensitive to liver damage. Evident from this study reveal that there was no significant difference in the bilirubin (total and conjugated) concentrations ($p>0.05$) between the control and the treated rat groups. This implies that seed oil of *D. edulis* had little or no effects on the excretion of bilirubin. According to Ahmed *et al.* (1992) any change in the concentrations of plasma protein and albumin indicate a change in the normal liver functions. Serum protein and albumin for both oils are not altered when compared with the plasma of those of the experimental control animals. This study infers that *D. edulis* seed oil does not impair protein metabolism.

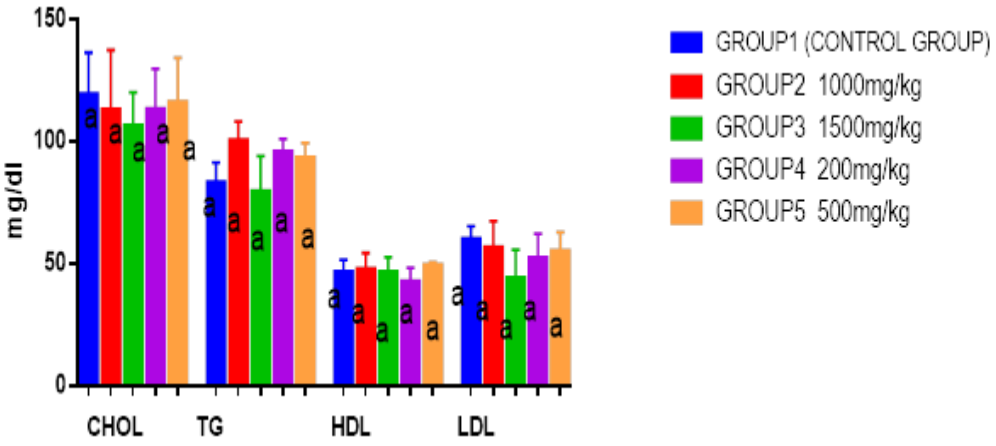


Fig. 7**:** Seed oil lipid profile

The lipid profiles (TG, CHOL, HDL and LDL) reveal the same pattern as they are not significantly different from the control group (p>0.05).Also supporting the above inferences is the fact that supplementation of the diet with *Dacryodes edulis* fruit seed oil (DFSO) does not produce any remarkable alterations in the serum total-cholesterol, LDL-cholesterol, HDL-cholesterol, VLDL-cholesterol and triacylglycerol levels of the albino rat (Alonso *et al*., 2001; Gaiva *et al*., 2003). The implicationof high total cholesterol, Low-Density-Lipoprotein (LDL)-cholesterol, triacylglycerol and low High-Density Lipoprotein (HDL)-cholesterol in the development of cardiovascular disorders such as hypertension, arteriosclerosis, stroke and heart failure can never be over emphasized (Ghasi *et al*., 2000).

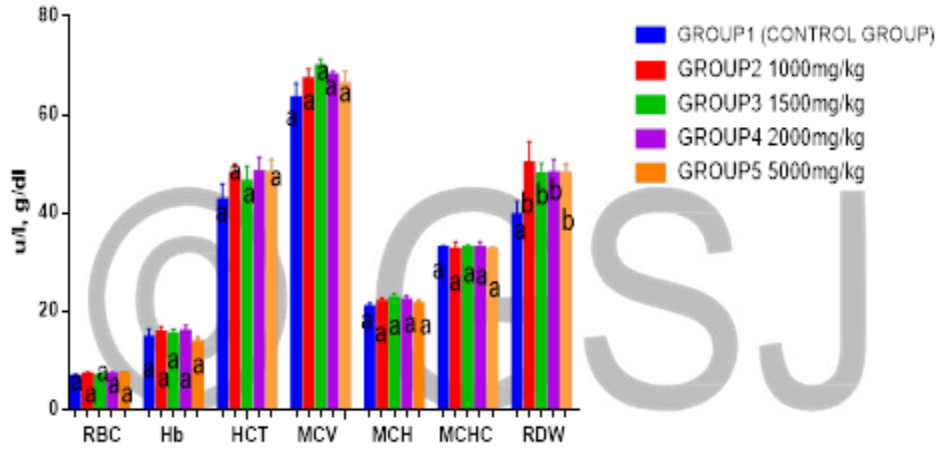


Fig. 8**:** Haematology (red cell parameters) for seed oil

Assessment of haematological parameters can be used to explain haematological functions of a chemical compound or plant extracts in an organism (Yakubu *et al.*, 2007). During the 28 days of the experimental study, the level of red blood cell parameters RBC**,** Hgb**,** HCT**,** MCV**,** MCH**,** MCHC for seed oil did not change significantly among the normal rat model in the control group (p>0.05), except for RDWwhich is significantly different at all groups of treatment as shown above.This is an indication that *Dacryodes edulis* seed oil does not contain factors that are deleterious to normal blood formation. This finding reflects that the seed oil possess erythropoietin stimulating activity that can improve hematopoietic activity of the cell and hence can play a vital role in management and or prevention of anaemia (Fig. 8). This appears to be the first study to investigate and document changes in red cell parameters of male albino rat fed on *D. edulis* seed oil.

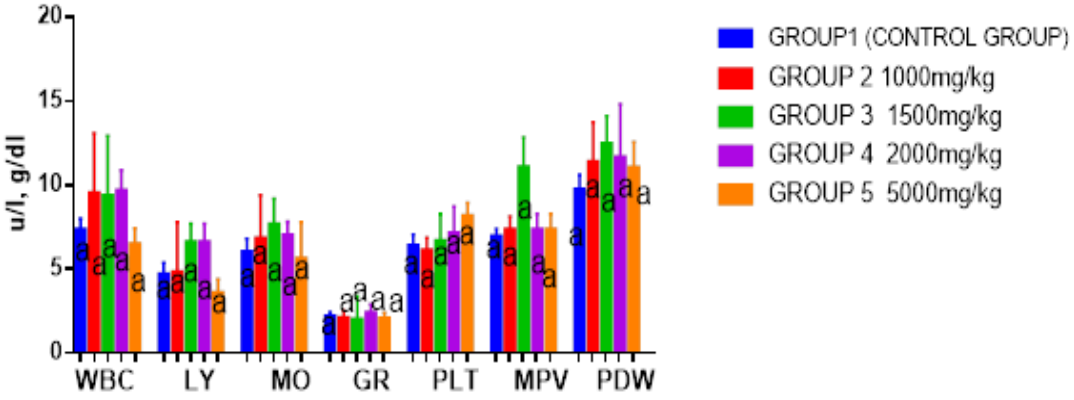
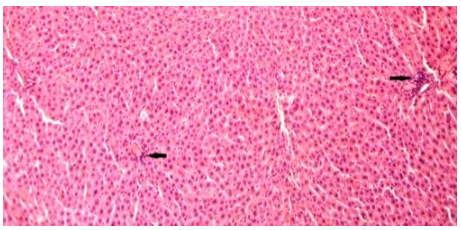


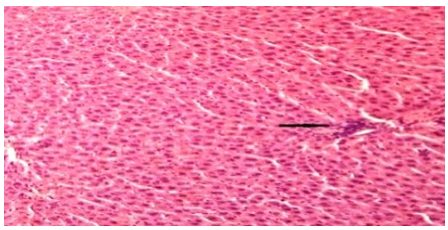
Fig. 9**:** Haematology (white cell parameters) for seed oil

Administration of *D.edulis* petroleum ether seed oil extracts did not induce changes in total and differential WBC count of the extracts at the different graded doses(1,000mg/kg, 1,500mg/kg, 2,000mg/kg, 5,000mg/kg). There are no significant changes in the levels of WBC, LY, MO, GR, PLT, MPV, PDW when compared to the control group ($p>0.05$) as shown in Fig. 9 above.The introduction of foreign body by a toxicant increases the WBC values. If WBC is elevated, it is due to the stimulation of immune defence system (Kashinath, 1990). Similarly, literatures have shown that increased concentration of antigen in the body results in high values of WBC (Schalm *et al.*,1975). These findings show that the seed oil does not stimulate changes in total and differential WBC count.

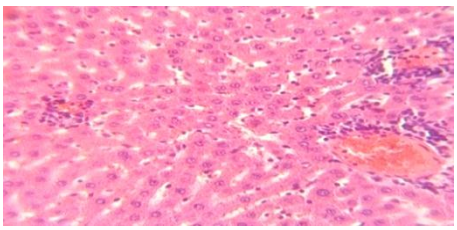
Histology of the Liver (Seed Oil)



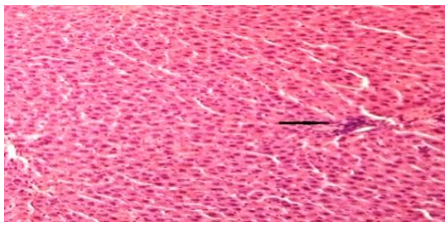
(a)



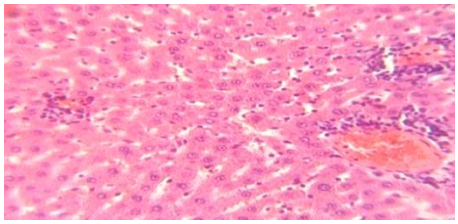
(b)



(c)



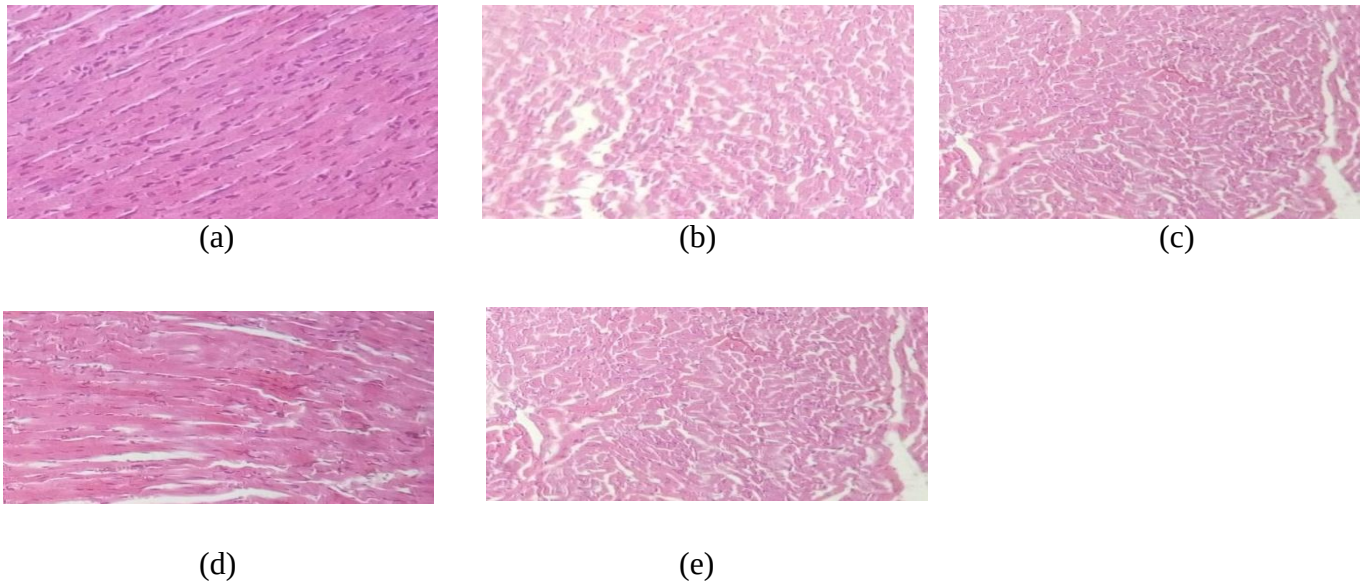
(d)



(e)

Fig. 10:(a)Photomicrograph of liver ofalbino rat (Control); (b)Photomicrograph of liver of Albino rat administered with 1,000 mg/kg body weight of extract of *D. edulis* seed oil. The arrow indicate insignificant periportal inflammation with lymphocytes aggregating;(c) .Photomicrograph of liver of Albino rat administered with 1,500 mg/kg body weight of extract of *D. edulis* seed oil. Periportal and lobular inflammation seen near centre of field;(d) Photomicrograph of liver of Albino rat administered with 2,000 mg/kg body weight of extract of *D. edulis* seed oil. Mild periportal and lobular inflammationobserved; (e) Photomicrograph of liver of Albino rat administered with 5,000 mg/kg body weight of extract of *D. edulis* seed oil. Periportal (arrow) and lobular (star) inflammation observed(H& E x100)

Histology of the Heart (Seed Oil)

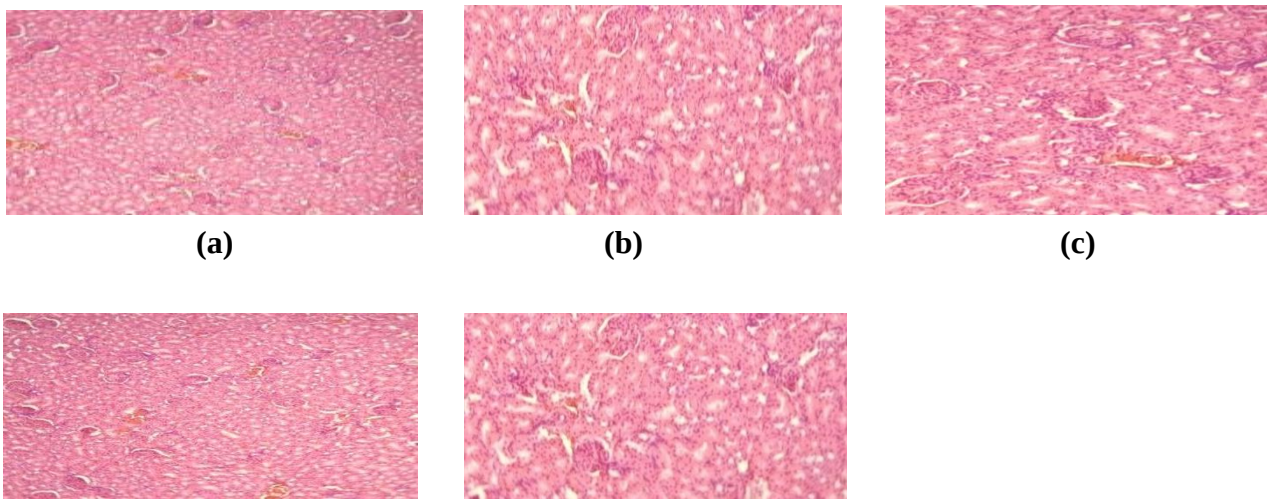


(a) (b) (c)

(d) (e)

Fig. 11:(a) Photomicrograph of heart of albino rat (Control); (b)Photomicrograph of heart of Albino rat administered with 1,000 mg/kg body weight of extract of *D. edulis* seed oil. Myocardial fibres as seen in transverse section;(c) Photomicrograph of heart of Albino rat administered with 1,500 mg/kg body weight of extract of *D. edulis* seed oil. Normal myocardial fibres are seen; (d) Photomicrograph of heart of Albino rat administered with 2,000 mg/kg body weight of extract of *D. edulis* seed oil. Myocardial fibres as seen in transverse section; (e) Photomicrograph of heart of Albino rat administered with 5,000 mg/kg body weight of extract of *D. edulis* seed oil. Normal myocardial fibres are seen. (H& E x100)

Histology of the Kidney (Seed Oil)



(a) **(b)** **(c)**

(d) (e)

Fig. 12:(a) Photomicrograph of kidney of albino rat (Control); (b)Photomicrograph of kidney of Albino rat administered with 5,000 mg/kg body weight of extract of *D. edulis* seed oil. Normal kidney with normal glomeruli; (c) Photomicrograph of kidney of albino rat administered with 1,000 mg/kg body weight of extract of *D. edulis* seed oil. Normal kidney with normal glomeruli; (d)Photomicrograph of kidney of albino rat administered with 1,500mg/kg body weight of extract of *D. edulis* seed oil. Normal kidney with normal glomeruli; (e) Photomicrograph of kidney of albino rat administered with 2,000 mg/kg body weight of extract of *D. edulis* seed oil. Normal kidney with normal glomeruli (H& E x100).

The observedcellular degeneration, hepatocellular necrosis, tubular epithelial cell necrosis and diffuse tubular lumina observed in the liver tissue of albino rats fed with *D. edulis* seed oil, cannot be verified from previous studies as it has not been previously documented. Also, the present result of biochemical alteration is not insured by histopatological examination in the liver of the fed albino rats, which reveal degenerative and necrotic changes.However, this is not severe enough to alter the liver function as manifested in the activity of the liver enzyme, which shows normal liver function. The kidney and heart do not manifest serious abnormal histopathological changes as shown above.

CONCLUSION AND RECOMMENDATION

There has been tremendous increase in the use of functional foods and, or nutraceuticals due to their beneficial effects on human health.At acute toxicity and sub-chronic studies, no animal death is observed even at doses of 10,000mg/kg and 5,000mg/kg of the oil respectively. This indicates that the oil is safe orally. These findings also suggest that petroleum ether extracted seed oil of *D. edulis*does not impair the liver function,and heart and kidney tissues are not compromised. The extract does not cause hypernatremia, hyperkalemia; it does not elevate plasma lipid cholesterol, HDL-cholesterol, LDL-cholesterol, and triglycerides. Thus, haematological parameters are not adversely affected. The African pear can be used as an alternative source of oil and its consumption should be with caution due to its lipid peroxidation assessment as manifested in TBARS of the liver tissue of albino rats fed with them.

REFERENCES

Ajibensin, K.K., Ekpo B.A and Bala N.D., (2002) Antimicrobial activity of the leaves of combretum micranthum and C. Racemosum. *GlobaJ. Med.Sci.* 1:13-17.

Ajibesin, K.K., (2011) *Dacryodes edulis* (G. Don) H.J. Lam: A review on its medicinal, phytochemical and economical properties. *Res. J. Med. Plant*, 5: 32-41

Awvioro, O .G. (2002). Histochemistry and Tissue Pathology *Principles Techniques* 9:154-162.

Arisi A.C.M, Cornic G, Jouanin L, and Foyer C.H. (1998*)* Over-expression of iron superoxide dismutase in transformed poplar modifies the regulation of photosynthesis at low CO_2 partial pressures or following exposure to the prooxidant herbicide methyl viologen. *Plant Physiol*.,117*:*565*–574.*

Buege J, and Aust, S .D. (1978).MicrosomalLipidPeroxidation.In:Methods in Enzymology.Colowick, S. P., Kaplan, N.O. (eds). Academic Press, New York. pp.302-311

Chawla,R.(1999). Serumtotalproteinandalbumin-globulinratio.InthousandInterpretations)*India.Pract.Clin.Biochem.*pp.106-118.

Cohen, G., Dembiec, D. and Marcus, J. (1970) Measurement of catalase activity in tissuesextracts. *Anal. Biochem*., 34, 30-38.

Doumas B.T., *et al*.(1987) A candidate reference method for determination of total protein in serum. I. Development and validation. *Clin Chem*; 27: 1642-1650

Ennouri, M., H. Fetoui, M. Hammami, E. Bourret, A. Attia and N. Zeghal, (2007). Effects of diet supplementation with cactus pear seeds and oil on serum and liver lipid parameters in rats. *Food Chem*. 101: 248-253.

Fridovich I. (1986). Biological effects of the superoxide radical.*Arch Biochem Biophys24:1–11.*

Gaiva,M.H., R.C. Couto, L.M. Oyama, G.E.C. Couto V.L.F.Silveira, E.B. Ribeiro and C.M. Nascimento (2003). Diets Rich in Polyunsaturated Fatty Acids:

Ghasi S, Nwobodo E and Ofili J O (2000) Hypocholesterolemic effects of crude extract of leaf of *Moringa oleifera* Lam in high fat diet fed wistar rats. *Journal of Ethnopharmacology* 69(1):21-2

Hewitt, N. J., Lloyd, S., Hayden, M., Butler, R., Sakai,Y., Springer, R., Facket t, A., Li, A. P. (2002).

Correlation between troglitazone cytotoxicity and drug metabolic enzyme activities in cryo-preserved human hepatocytes. *Chem. Biol. Interact* . 142(1–2):73–82.

Kashinath, K.T., 1990. Hypolipidemic effect of disulphide in rats fed high lipids diet and /or ethanol. Ph. D. Thesis, University of Bangalore

Kengue J.C. and Nyangatou J. (1990).Problem of preserving the germination power of the seed Africa pear (*Darcyodes edulis*) *Fruit* 45 (4): 409-412

Ma M, Eaton JW. 1992. Multicellular oxidant defense in unicellular organisms. *Proc Natl Acad Sci USA* 89: 7924–7928.

Misra, H. P.,and Fridovich, I (1989). The role of superoxide anion in the autooxidation of epinephrine and simple assay for superoxide dismutase. *J. Biol. Chem*. 247, 3170-3175

Mittler R (2002) Oxidative stress, antioxidants and stress tolerance. *Trends Plant Sci* 7**:** 405– 410

Nwaichi E. O. and, Igbinobaro O. (2012) "Effects of Some Selected Spices on Some Biochemical Profile of Wister Albino Rats", *American Journal of Environmental Engineering*, Vol. 2 No. 1, pp. 8-11.

Obasi N.B. and Okolie N.P.(1993). Nutritional constituents of the seeds of Africa pear *Dacryodes edulis.Food Chem*. 46:297-299

Okutucu, B., Dincer, A., Habib, O. and Zihnioglu, F. (2007) Comparison of five methods for determination of total plasma protein concentration, *J. Biochem. Bioph. Meth*., 70, 709–711,

Pereira MD, Eleutherio ECA, Panek AD. 2001. Acquisition of tolerance against oxidative damage in *Saccharomyces cerevisiae. BMC Microbiology* 1:11–20.

Saka, W.,Akigbe, R.E.,Popoola, O.T, and Oyekunle,O.S. (2012) Changes in Serum Electrolytes, Urea, and Creatinine in *Aloe Vera*-treated Rats *J Young Pharm*4(2); 78- 81

Scandalios J.G. 1993. Oxygen stress and superoxide dismutases. Plant Physiol 101:7–12.

Schalm, O. W., Jain, N.C and Carrol, E.J. (1975). Veterinary Haematology, 3rd Edn., Lea and Febiger, Philadelphia, pp. 197 - 199

Sehrawat A, Sultana S. (2006) Tamarix gallica ameliorates thioacetamide-induced hepatic oxidative stress and hyperproliferative response in Wistar rats. *J Enzyme Inhib Med Chem*. 21(2):215-23.

Sims, G.K. (2006) Using the Berthelot Method for Nitrite and Nitrate Analysis. Soil Sci. Soc. *Am. J.* 70 (3): 1038.

Vasudha K .C, Nirmal Kumar A, Venkatesh T (2006). Studies on the age dependent changes in serum adenosine deaminase activity and its changes in hepatitis. *Indian J Clin Biochem*., 21: 116 - 120.

Yakubu M.T., Akanji MA., and Oladiji AT., (2007). Haematological parameters of male albino rats following chronic administration of aqueous extracts of fedogia agrestis stem. *Pharmacognosy magazine* 3: 34