Asian Journal of Research in Biochemistry

4(4): 1-10, 2019; Article no.AJRB.47339

Biochemical Indices and Haematological Studies of Ethyl Acetate Extract of *Persea americana* Leaf in Albino Rats

J. A. Mashi[1*], A. M. Sa'id[1], F. Bello[2], H. M. Yakasai[1], B. Bello[3] and R. I. Idris[1]

[1]Bayero University, Kano, Nigeria.
[2]Kebbi State University of Science and Technology, Kebbi, Nigeria.
[3]Mudibo Adama University of Technology, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Author JAM designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors HMY, FB, BB and AMS managed the analyses of the study. Author RII managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJRB/2019/v4i430074
Editor(s):
(1) Dr. V. Spirina Liudmila Professor, Department of Biochemistry and Molecular Biology , Siberian State Medical University, Russia.
(2) Dr. Héctor Manuel Mora Montes, Departamento de Biología, División de Ciencias Naturales y Exactas, Universidad de Guanajuato, Guanajuato, México.
Reviewers:
(1) Muhammad Shahzad Aslam, Pakistan International Human Right Organization, Pakistan.
(2) Souravh Bais, Adina Institute of Pharmaceutical Sciences, Sagar, India.
(3) Uchendu, Mbah Okuwa, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.
(4) Md Riaj Mahamud, University of Oklahoma HSC, USA.
Complete Peer review History: http://www.sdiarticle3.com/review-history/47339

Original Research Article

Received 15 January 2019
Accepted 25 March 2019
Published 22 May 2019

ABSTRACT

The aim of this present study was to evaluate the effect of ethyl acetate extract of *P. americana* (avocado) on liver and kidney function, lipid profile as well as haematological parameters in albino rats. A total of 20 albino rats were used for this experiment and they were divided into four groups of 5 (A-D) rats each. Group A served as normal control, group B-D served as experimental groups administered with 100, 200 and 400 mg/kg body weight of ethyl acetate extract of *Persea americana* leaf per day for 4 weeks respectively. This study was conducted in the Department of Biochemistry, Bayero University, Kano, in the month of May, 2018. Liver function test (colorimetric method), kidney indices and lipid profile (spectrophotometry method), and hematological examination (SYSMEX XE-2000) were analysed. Administration of ethyl acetate extract did not

**Corresponding author: E-mail: jmahmed.bch@buk.edu.ng;*

produced significant effect on liver and kidney indices in all the treated groups. The extract significantly (P=.05) decrease total cholesterol, triglyceride, low density lipoprotein and very low density lipoprotein in all the treated groups. Haematological parameters analysed were not significantly affected in all the treated groups. Thus, ethyl acetate extract of *P. americana* leaf possesses hypolipidemic potentials and relatively safe for kidney, liver and hematological indices but extremely high doses may not be advisable.

Keywords: P. americana; liver function test; kidney function test; lipid profile; haematological parameters.

1. INTRODUCTION

Many medicinal plants used in ethnomedical practices in Nigeria are known or little known to scientific world [1]. Many important drugs used in medicine today are directly or indirectly derived from plants [2]. Plants and herbs have been a tremendous source of food and folk remedies for mankind and have served as starting material for the development of new synthetic drugs. They have the ability to synthesize a wide variety of chemical compounds which are used to perform biological functions and to defend against attack from predators such as insects, fungi, yeasts, bacteria, virus and other pathogens [3]. 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 [4,5]. *Persea americana* belongs to the family *Lauraceae*, unflatteringly known in the past as alligator pear, midshipman's butter and vegetable butter, it is one of those plants currently used by indigenous persons for it nutritional value and to manage health problems. It is well known in many parts of the tropical world including Nigeria [6] and is commonly called avocado or pear. The fruit tree can attain a height up to 20 m, with large spreading and flat topped crown. The plant is reported to possess antidiabetic, antihyperlipidemic potentials [2,7], antioxidant [8,9], cancer risk reduction [10], wound healing [11], hepatoprotection [12], analgesic and anti-inflammatory [13], anticonvulsant [14], vasorelaxant and blood pressure reducing [15,16]. The use of traditional medicines as substitutes to orthodox medicines has been on the increase [17]. The reasons, which have given rise to this trend, include the cheapness, availability and accessibility of these natural medicines. Besides, there has been the erroneous belief that these medicines are free from adverse effects [18,19]. On the other hand they have been rejected because many of the acclaimed medicinal values have not been scientifically evaluated and their safety profiles uncertain [19]. It is, therefore, pertinent that safety assessments should be conducted on natural products for which certain medicinal uses have been scientifically validated. It was recommended by the World Health Organization to evaluate herbal medicine, because orthodox medicines are not safe [20]. It is, therefore, relevant that safety assessments should be conducted on ethyl acetate extract of *P. americana* leaf for which certain medicinal uses have been scientifically proven.

2. MATERIALS AND METHODS

2.1 Sample Collection and Identification

The leaf of *Persea americana* were collected from Jos, Plateau state of Nigeria. The plant part was authenticated by a Botanist at Plant Science Department, B.U.K. with accession number BUKHAN 0305 and was deposited in Bayero University, Kano herbarium for reference.

2.2 Extraction of Plant Material

Soxhlet extraction method was used for the extraction of the plant materials. The sample (200 g) was chopped into small pieces and then shades dried and ground into powdered form (120 g). Ethyl acetate was used as the extraction solvent and later concentrated in vacuo using rotary evaporator at 40℃ resulting to a total extraction yield 35.5% (29.6 g).

2.3 Experimental Animals

Experimental rats (80-100 g) of same sex (males) were obtained from the Physiology department, Faculty of Basic Medical Sciences, Bayero University, Kano. The animals were kept

in cages and clean drinking water provided *ad libitum* while they were fed with standard commercial pelleted feed (Vital Feed® Nigeria). The temperatures varied between 27-30℃ and relative humidity of about 55%-60% with 12-h light-dark cycle and adequate ventilation maintained in the animal house. This study was conducted in the Department of Biochemistry, Bayero University, Kano, in the month of May, 2018. Ethical conditions governing the conducts of experiments with life animals as stipulated were strictly observed. Also, the experimental protocol was approved by the College of Health Science ethical committee with a reference number 0653.

2.4 Experimental Design

A total of 20 rats were used for this experiment and they were divided into four groups of 5 rats each.

Group 1- Normal rats
Group 2- Treated with ethyl acetate extract of *P. americana* leaf (100 mg/kg body weight).
Group 3- Treated with ethyl acetate extract of *P. americana* leaf (200 mg/kg body weight).
Group 4- Treated with ethyl acetate extract of *P. americana* leaf (400 mg/kg body weight).

The sub-chronic toxicity study lasted for a period of four weeks. The various doses (100, 200 and 400 mg/kg body weight) were administered to group 2, 3 and 4 respectively of normal rats. At the end of 4 weeks treatment the rats were sacrificed under anesthesia and blood samples collected were centrifuged at 3000 rpm for 10 minutes and the serum obtained was used for liver function test (Alanine amino transferase, Aspartate amino transferase, Alkaline phosphatase, Albumin, Total protein and Globulin), kidney function indices [creatinine, urea, electrolytes (Na^+, K^+, Cl^- and HCO_3)] and lipid profile (Cholesterol, Triglyceride, High density lipoproteins, Low density lipoproteins and very low density lipoproteins). The hematological parameters (White blood cells, Red blood cells, Haemoglobin, Packed cell volume, Mean corpuscular volume, Mean corpuscular haemoglobin, Mean corpuscular haemoglobin concentration and Platelets) were determined using the blood samples.

2.5 Assays

Liver function test were assayed using the methods of; serum alanine and aspartate amino transferase activity determination by Reitman and Frankel, [21], serum alkaline phosphatase activity determination by Rec, [22], serum albumin concentration determination by Grant, [23], serum total protein and serum globulin concentration determination by Tietz, [24]. Kidney function test were assayed using the methods of; serum urea concentration determination by Weatherburn, [25], serum creatinine concentration determination by Bartels and Bohmer, [26], serum potassium concentration determination by Henry, [27], serum sodium concentration determination by Kraut and Madias, [28], serum chloride concentration determination by White, [29] and serum bicarbonate concentration determination by Forrester et al. [30].

Lipid profile was analysed using the methods of; serum total cholesterol concentration determination by Lothar, [31], triglycerides concentration and serum HDL-cholesterol concentration determination by Jacobs et al. [32], serum LDL and VLDL-cholesterol concentration determination by Friedewald et al. [33]. Haematological parameters were analyzed using SYSMEX XE-200 (QBC Autoread Plus, UK).

2.6 Statistical Analysis

Statistical package for social sciences (SPSS) version 17 software was used for all calculations and statistical analysis. Analyses were performed using student t-test at 95% confidence level with P=.05 being significant. Results were presented as mean ± standard deviation.

3. RESULTS

3.1 Liver Function Test

Result of sub-chronic toxicity study showed no significant difference (P=.05) in AST and ALP activities in all the treated groups when compared with normal control. However, significant increase (P=.05) was observed in serum level of ALT of the entire treated group when compared with normal control (Fig. 1). There was a significant decrease (P=.05) in serum total protein and globulin in all the treated groups compared with the normal control (Fig. 2). The level of serum albumin was found to

increase significantly (P=.05) in all the treated groups compared with normal control.

3.2 Kidney Function Test

Kidney function tests carried out include serum urea, creatinine, sodium, potassium, chloride and bicarbonate (Figs. 3 and 4). No significant difference (P=.05) was observed in serum urea, creatinine, sodium, chloride and bicarbonate in all the groups when compared with normal control group. Serum Potassium level was found to decrease significantly in group 2 (100 mg/kg b.w.) When compared with normal and groups 3 and 4 (200 and 400 mg/kg b.w.).

3.3 Lipid Profile

The levels of serum total cholesterol, triglyceride and low density lipoprotein in the treated groups were found to decrease significantly (P=.05) when compared with normal control in a dose dependent pattern within the treated groups. No significant difference was observed (P=.05) in serum high density lipoprotein and very low density lipoprotein levels (Fig. 5).

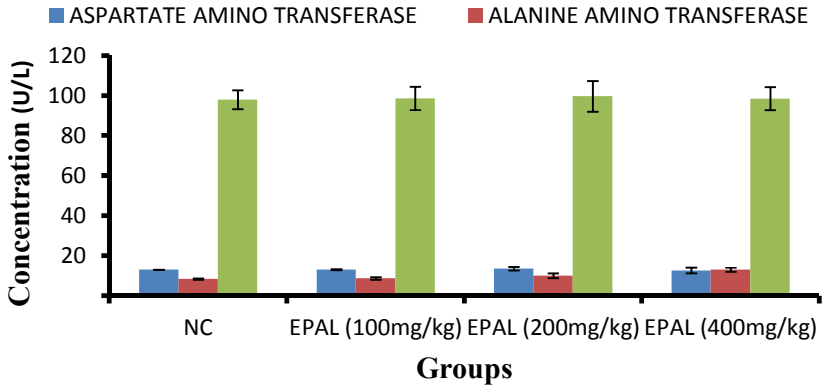

Fig. 1. Serum levels of aspartate amino transferase, alanine amino transferase and alkaline phosphatase of rats administered with ethyl acetate extract of *P. americana* leaf (100, 200 and 400 mg/kg b.w.) for 4 weeks

Results are presented as Mean ± SD, n=5. Values with different superscripts are significantly different (P<0.05) with respect to normal control, NC= Normal control, EPAL= Ethyl acetate extract of P. americana leaf

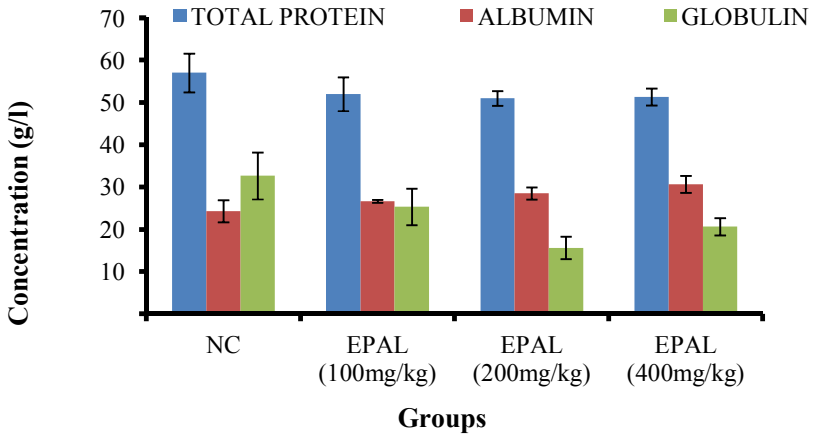

Fig. 2. Serum levels of total protein, albumin and globulin of rats administered with ethyl acetate extract of *P. americana* leaf (100, 200 and 400 mg/kg b.w.) for 4 weeks

Results are presented as Mean ± SD, n=5. Values with the different superscripts are significantly different (P<0.05) with respect to normal control, NC=Normal control, EPAL=Ethyl acetate extract of P. americana leaf

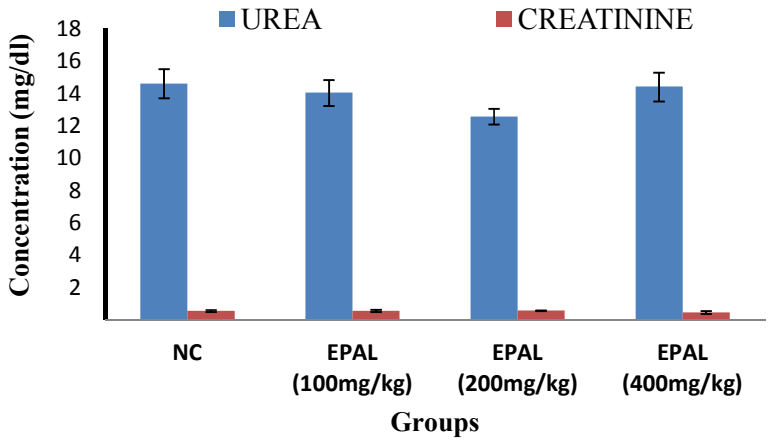

Fig. 3. Serum levels of urea and creatinine levels of rats administered with ethyl acetate extract of *P. americana* leaf (100, 200 and 400 mg/kg b.w.) for a period of 4 weeks

Results are presented as Mean ± SD, n=5. Values with the different superscripts are significantly different (P< 0.05) with respect to normal control, NC= Normal control, EPAL= Ethyl acetate extract of P. americana

3.4 Haematological Parameters

There was no significant difference (P=.05) between the normal control and the tested groups in all the haematological parameters assessed. These include, white blood cell, red blood cell, haemoglobin, packed cell volume, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular heamoglobin concentration and platelets (Table 1).

4. DISCUSSION

Toxicity studies are essential tools in assessing the bioaccumulative effects of xenobiotics in biological systems. Assessment of liver and kidney function is very important in evaluating toxicity of modern and traditional medicines since these organs play major roles in metabolism of xenobiotics in the body. Elevated activities of liver enzymes are often diagnostic of underlying cellular injuries [34,35]. From the biochemical result of this study, AST, ALT and ALP activities were not affected in all the groups administered with the extract. Increase in serum concentrations of these marker enzymes can be as a result of increase in hepatocytes proliferation or regeneration [36]. Chemicals often cause subclinical injury to the liver, which manifests only as abnormal liver enzyme tests. Certain medicinal agents, when taken in overdoses and sometimes even when introduced within therapeutic ranges may injure the organ [37]. Studies conducted by [38] showed that a large intake of tannins may cause liver damage. Reduction in total protein and globulin are indications of diminished synthetic function of the liver or might be due to impaired hepatocellular function [39,40,41,42]. Other factors which can cause elevation of these markers are nutritional status, catabolism, hormonal factors, urinary and gastrointestinal losses [42]. Ethyl acetate extract of *P. americana* was found to contain appreciable amount of saponins and tannins [8,43] which may act by damaging cell membranes causing leakage of cellular materials, ultimately leading to cell death [44,45].

Kidney function was evaluated by means of serum urea, creatinine and blood electrolyte concentrations. Urea is formed in the liver and is mainly excreted by the kidney, it is useful in evaluating kidney function in conjunction with creatinine which originates from the muscle and is filtered by the kidney. The serum urea and creatinine levels of treated groups in this study were not significantly changed. Increased blood urea and creatinine is a good indicator of compromised kidney function. These results suggest that the extract may not have altered the kidney function. In a similar study by [40] serum creatinine, urea and electrolytes such as sodium and calcium were not affected after *P. americana* extract treatment.

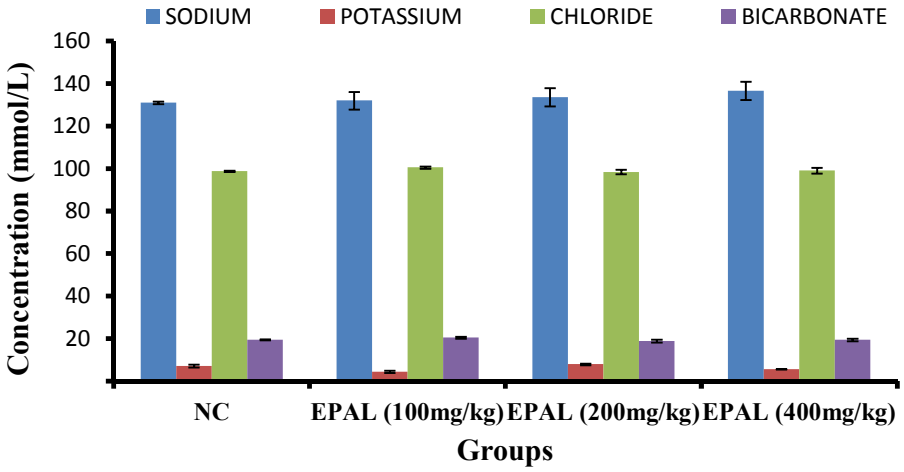

Fig. 4. Serum levels of sodium, potassium, chloride and bicarbonate of rats administered with ethyl acetate extract of *P. americana* leaf (100, 200 and 400 mg/kg b.w.) for 4 weeks

Results are presented as mean ± SD, n=5. Values with the different superscripts are significantly different (P<0.05) with respect to normal control, NC= Normal control, EPAL= Ethyl acetate extract of P. americana

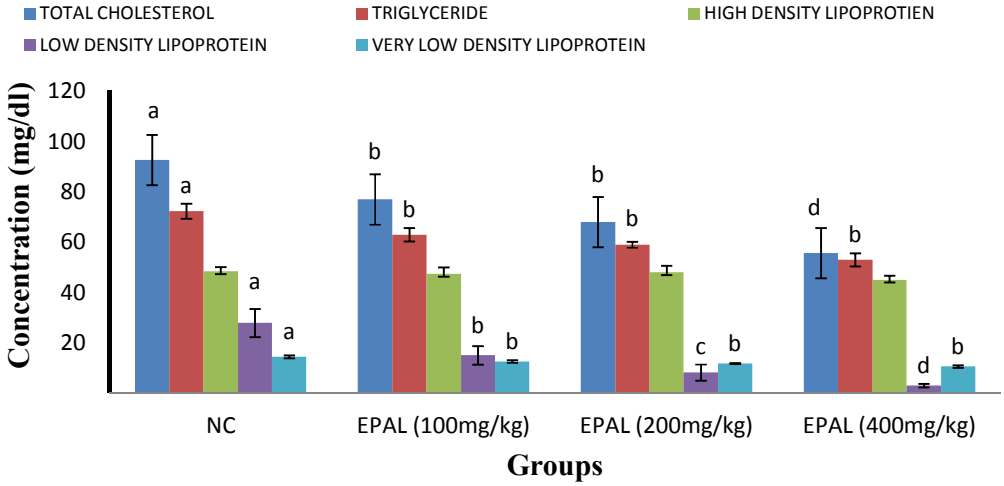

Fig. 5. Levels of serum total cholesterol, triglyceride, high density lipoprotein, low density lipoprotein and very low density lipoprotein of rats administered with ethyl acetate extract of *P. americana* leaf (100, 200 and 400 mg/kg b.w.) for 4 week

Results are presented as Mean ± SD, n=5. Values with the different superscripts (a,b,c or d) are significantly different (P<0.05) with respect to normal control, NC= Normal control, EPAL= Ethyl acetate extract of P. americana leaf

In this study, the administration of graded doses of EPAL significantly reduced serum levels of TC, TG, LDL and VLDL in treated rats. Saponins are known anti-nutritional factors, which lower cholesterol by binding with cholesterol in the intestinal lumen, preventing its absorption, and/or by binding with bile acids, causing a reduction in the enterohepatic circulation of bile acids and increase its faecal excretion [46,47]. Increased bile acid excretion is offset by enhanced bile acid synthesis from cholesterol in the liver and consequent lowering of the plasma cholesterol; hence saponins have been reported to have hypocholesterolaemic effect [48]. Thus, the observed hypolipidemic effect of EPAL may be linked to the synergistic actions of phytochemicals like saponins and polyphenolic compounds contained in the plant extract.

Table 1. Haematological Parameters of Rats administered with ethyl acetate Extract of *P. americana* leaf (100, 200 and 400 mg/kg b.w.) for 4 weeks

GROUPS	RBC(10^3/µl)	WBC(10^6/µl)	Hb(g/dl)	PCV(%)	MCV(FL)	MCHC(g/dl)	MCH(pg)	PLT(10^3/µl)
Normal Control	6.45 ± 0.10[a]	8.23 ± 0.20[b]	7.60 ± 0.36[c]	36.67 ± 1.86[d]	55.30 ± 0.06[e]	21.80 ± 0.23[f]	11.23 ± 0.35[g]	346.67 ± 3.22[h]
EE (100mg/kg)	6.47 ± 0.14[a]	8.73 ± 0.23[b]	6.97 ± 0.27[c]	37.67 ± 1.86[d]	58.07 ± 0.54[e]	22.93 ± 0.17[f]	11.83 ± 0.19[g]	336.67 ± 3.93[h]
EE (200mg/kg)	6.73 ± 0.04[a]	8.53 ± 0.35[b]	8.03 ± 0.35[c]	36.67 ± 0.88[d]	55.20 ± 0.47[e]	23.33 ± 0.98[f]	11.40 ± 0.42[g]	349.00 ± 8.50[h]
EE (400mg/kg)	6.63 ± 0.22[a]	8.77 ± 0.09[b]	7.47 ± 0.35[c]	37.67 ± 2.40[d]	53.00 ± 0.78[e]	22.23 ± 0.73[f]	11.60 ± 0.40[g]	357.33 ± 4.10[h]

Results are presented as Mean ± SD, n=5. Values with the different superscripts in the same column are significantly different (p< 0.05) with respect to normal control, EE= Ethyl acetate extract of P. americana leaf

Fig. 6. Image of *P. americana* (avocado) tree

The haematopoietic system is one of the most sensitive targets of toxic compounds and is an important index of physiological and pathological status in man and animals as it investigate the extent of damage to the blood [37]. It provides vital information regarding the status of bone marrow activity and intravascular effects such as haemolysis and anaemia [49]. Assessment of the haematological indices showed that the extract did not cause any significant effect on WBC, RBC, PCV, Hb, PLT, MCV, MCH and MCHC. The results on the haematological parameters indicate normal haemopoiesis and absence of anaemia confirming the non-toxic nature of the extract.

5. CONCLUSION

The ethyl acetate leaf extract administered to experimental rat models did not produced significant effect on liver and kidney indices in all the experimentally treated groups. But it significantly decreases the total cholesterol, triglyceride, low density lipoprotein and very low density lipoprotein in all the treated groups. Haematological indices analysed were not significantly affected in all the treated groups. Thus, ethyl acetate extract of *P. americana* leaf possesses hypolipidemic potentials due to the presence of active phytochemicals and relatively safe for kidney, liver and hematological indices at the doses administered.

ETHICAL APPROVAL

All authors hereby declare that; principle of laboratory animals care (NHI publication number 829 revised 1985) were followed, as well as all experiment have been examined and approved by the appropriate ethic committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Mashi JA, Atiku MK, Bala M, Sa'id AM, Idris RI, Babandi A, Babagana K, et al. Hypoglycaemic and hypolipidemic properties of ethyl acetate fraction of *P. americana* leaf in alloxan-induced diabetic rats. IJBBMB; 2018. In Press. DOI: 10.11648/j.xxxx.2018xxxx.xx
2. Mashi JA, Atiku. MK, Shehu D, Sa'id AM, Idris RI, Dangambo MA, Babagana K, Ya'u M, Babandi A. et al. Comparative study of different solvents extract of *Persea americana* leaf on alloxan induced hyperglycemic rats. Asian J. Biol. Sci. 2019a;12:67-72.
3. Tapsell LC, Hemphill I, Cobiac L. Health benefits of herbs and spices: The past, the present, the future. Med J Aust. 2006;185(4):S4–24.
4. Lai PK., Roy J. Antimicrobial and chemopreventive properties of herbs and spices. Curr Med Chem. 2004;11(11): 1451–1460.
5. Tene Tcheghebe O, Nyamen LD, Ngouafong Tatong F, Seukep AJ. Ethnobotanical uses, phytochemical and pharmacological profiles, and toxicity of *Persea americana* mill. An overview. 2016;3:213-221.
6. Oluwole S, Yusuf K, Fajana O, Olaniyan D. Qualitative studies on proximate analysis and characterization of oil from *Persea americana* (Avocado Pear). Journal of Natural Sciences Research. 2013;3(2):68-73.
7. Brai BI, Odetola AA, Agomo PU. Hypoglycemic and hypocholesterolemic potential of *Persea americana* leaf extracts. J Med Food. 2007;10:356–360.
8. Mashi JA, Sa'id AM, Idris RI, Aminu I, Muhammad AA, Inuwa IM. Phytochemical, radical scavenging and oxidative stress potentials of ethyl acetate extract of *P. americana* leaf in alloxan-induced hyperglycemic rats. Journal of Applied Sciences; 2019b. In Press: 95063-JAS-ANSI.
9. Mahadeva Rao US. Phytochemical screening and *in vitro* antioxidant and anti-diabetic potentials of *Persea americana*

mill. (*Lauraceae*) fruit extract. Universal Journal of Pharmaceutical Research. 2018;3(5):38-45.

10. Lu QY, Arteaga JR, Zhang Q, Huerta S, Go VL, Heber D. Inhibition of prostate cancer cell growth by an avocado extract: Role of lipid-soluble bioactive substances. J Nutr Biochem. 2005;16:23–30.
11. Nayak BS, Raju SS, Chalapathi Rao AV. Wound healing activity of *Persea americana* (avocado) fruit: A preclinical study on rats. J Wound Care. 2008;17: 123–126.
12. Kawagishi H, Fukumoto Y, Hatakeyama M. Liver injury suppressing compounds from avocado (*Persea americana*). Journal of Agriculture and Food Chemistry. 2001;32(4):128-131.
13. Adeyemi OO, Okpo SO, Ogunti OO. Analgesic and anti-inflammatory effects of the aqueous extract of leaves of *Persea americana* Mill (*Lauraceae*). Fitoterapia. 2002;73:375–380.
14. Odetola AA, Akinloye O, Egunjobi C, Adekunle WA, Ayoola AO. Possible anti-diabetic and anti-hyperlipidemic effect of fermented *Parkia biglobosa* extract in alloxan-induced diabetic rats. Clinical and Experimental Pharmacology and Physiology. 2006;33(4):808-812.
15. Owolabi MA, Jaja SI, Coker HA. Vasorelaxant action of aqueous extract of the leaves of *Persea americana* on isolated thoracic rat aorta. Fitoterapia. 2005;76:567–573.
16. Ojewole JA, Kamadyaapa DR, Gondwe MM, Moodley K, Musabayane CT. Cardiovascular effects of *Persea americana* Mill (*Lauraceae*) (avocado) aqueous leaf extract in experimental animals. Cardiovasc J Afr. 2007;18:69–76.
17. Ozolua RI, Anaka ON, Okpo SO, Idogun SE. Acute and sub-acute toxicological assessment of the aqueous seed extract of *Persea americana* Mill (Lauraceae) in rats. Afr J Tradit Complement Altern Med. 2009;6(4):573–578.
18. Larrey D. Liver involvement in the course of phytotherapy. Presse Med. 1994;23: 691–693.
19. Ernst E. The efficacy of herbal medicine – an overview. Fundam Clin Pharmacol. 2005;19:405–409.
20. Jagdish CN, Lalit SC. Antihyperglycemic and antihyperlipidemic activity of *Breynia vitis-idaea*. Pharmacognosy Journal. 2016;8(3):396-404.
21. Reitman S, Frankel SA. Colorimetric method for the determination of serum glutamic oxaloacetate and glutamic pyruvic transaminase. Amer. J. Clin. Patho. 1957;28:56-58.
22. Rec GSCC. Colorimetric method for serum alkaline phosphatase determination. J. Clin. Biochem. 1972;10(2):182.
23. Grant GH. Amino acids and proteins; Fundamentals of clinical chemistry. Tietz N.W. Editor, 3rd Edition, WB Saunders Company Philadelphia USA. 2014;328-329.
24. Tietz NW. Clinical guide to laboratory tests. Second Edition W.B. Saunders Company, Philadelphia, USA. 1987;554-556.
25. Weatherburn MW. Determination of serum urea. Analytical Chemistry. 1967;39:971.
26. Bartels H, Bohmer MA. Colorimetric method for determination of serum creatinine. Clinica Chimica Acta, 1972;37: 193.
27. Henry JB. Clinical diagnosis and management by laboratory methods. 20th ed. Philadelphia, PA.: W. B. Saunders Company. 2001;186-193.
28. Kraut JA, Madias NE. Serum anion gap: Its uses and limitations in clinical medicine. Clinical Journal of the American Society of Nephrology. 2007;2(1):162-174.
29. White WL. Chemistry for technologist. 3rd Edition. The C.V. Mosby Co., St. Louis. 1970;182.
30. Forrester RI, Wataji, IJ, Silverman DA, Pierre KJ. Enzymatic method for determination of CO_2 in serum. Journal of Clinical Chemistry. 1976;22:243.
31. Lothar T. Clinical laboratory diagnostic. 1st Edition TH-Books Verlagsgesellschaft mbh, Frankfurt/ Main, Germany. 1998;169.
32. Jacobs D, Kasten BL, De Mott WR, Wolfson WL. Laboratory and test handbook. Lexi-company Inc: Hudson (Cleveland). 1990;219.
33. Friedewald WT, Levy IR, Fredrickson DS. Estimation of this concentration of low-density lipoprotein cholesterol in plasms, without use of the preparative ultracentrifuge. Clinical Chemistry. 1972; 18(6):206-228.
34. Karthikeyan S, Gobianand K, Pradeep K, Mohan CV, Balasubramanian MP. Biochemical changes in serum, lung, heart and spleen tissues of mice exposed to sub-acute toxic inhalation of mosquito repellent mat vapour. Journal of Environ-mental Biology. 2006;27(5):355-358.

35. Mosa RA. Some bioactivity of Triterpenes from stem bark of *Protorhus longifolia* and their derivatives. Planta Med. 2014;6(2): 50-58.
36. Mathew Folaranmi Olaniyan. Effect of liquid extract of pear avocado leaf (*Persea americana*) on plasma levels of aminotransferases, cholesterol and total bile acids in hypertensive patients. American Journal of Medicine and Medical Sciences. 2014;4(3):87-91.
37. Omodamiro OD, Jimoh MA, Ewa IC. Hepatoprotective and haemopoeitic activity of ethanol extract of *Persea americana* seed in paracetamol induced toxicity in wistar albino rat. Human Journals. 2016;5(3):149-165.
38. Yamasaki T, Sato M, Mori T, Mohammed AM, Fujii K, Tsukioka J. Toxicity of tannins towards the free-living nematode *Caenorhabditis elegans* and the brine shrimp (*Artemia salina*). Journal of Natural Toxin. 2000;11(3):165-171.
39. Haeri MR, Izaddoost M, Ardekani MR, Nobar MR, White KN. The effect of fenugreek 4-hydroxyisoleucine on liver function biomarkers and glucose in diabetic and fructose-fed rats. Phytother Res. 2009;23(1):61–64.
40. Olorunnisola OS, Bradley G, Afolayan AJ. Acute and sub-chronic toxicity studies of methanolic extract of *Tulbaghia violacea* rhizomes in wistar rats. African Journal of Biotechnology. 2009;11(83):14934-14940.
41. Yakubu MT, Bilbis LS, Lawal M, Akanji MA. Effect of repeated administration of sildenafil citrate on selected enzyme activities of liver and kidney of male albino rats. Niger. J. Pure Appl. Sci. 2003;18: 1395-4000.
42. Ilmie MU, Jaafar H, Mansor SM, Abdullah JM. Subchronic toxicity study of standardized methanolic extract of *Mitragyna speciosa Korth* in Sprague-Dawley Rats. Front. Neurosci. 2015;9:189.
43. Rahman A, Zareen S, Choudhary MI, Akhatar MN, Khan SN. A- Glucosidase inhibitory activity of triterpenoids from *Cichorium intybus*. Journal of Natural Products. 2008;71:910-913.
44. Aliyu MS, Lawal U, Tijjani MB, Doko MH, Garba I, Kokya HA. Phytochemical and antibacterial properties of leaf extracts of *Ipomoea asarifolia*. Journal of Ethno-pharmacology. 2011;19:236–240.
45. Sreeshma LS, Nair BR. Brine shrimp lethality assay in two species of *Biophytum Dc* (*Oxalidaceae*). Innovare Academic Sciences. 2014;6(4):5-7.
46. Nimenibo-uadia R. Effect of aqueous extract of *Canavalia ensiformis* seeds on hyperlipidemic and hyperketonaemia in alloxan-induced diabetic rats. Biokemistri. 2003;15(2):7-15.
47. Gouegni EF, Abubakar H. Phytochemical, toxicological, biochemical and haematological studies on Avocado (*Persea americana*) in experimental animals. NIFOJ. 2013;31(1):64-69.
48. James DB, Owolabi OA, Ibrahim AB, Folorunsho DF, Bwalla I, Akanta F. Changes in lipid profile of aqueous and ethanolic extract of *Blighia sapida* in rats. Asian Journal of Medical Sciences. 2010;2(4):177-180.
49. Adeneye AA, Ajagbonna OP, Adeleke TI, Bello SO. Preliminary toxicity and phytochemical studies of the stem bark aqueous extract of *Musanga cecropioides* in rats. Journal of Ethnopharmacology. 2006;105(4):374-379.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle3.com/review-history/47339