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Cardio-Protection Potential of Avocado Pear Seeds' Extract on Ethylene Glycol-Triggered Toxicity in Wistar Rat

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Abstract

Cardio - protection activity of Avocado pear seeds (APS) extract was investigated, on ethylene glycol-triggered toxicity using Wistar rat model. Designation of rodents was made to accommodate under listed administration; 1st group or control, Group 2 administered 0.1ml/kg b.w ethylene glycol only for seven days, 3rd and 4th groups administered avocado pear seed extract (100mg/kg b.w and 200mg/kg b.w morning and evening respectively) for 21 days and 0.1ml/kg b.w ethylene glycol for 7 days with continuous administration of the treatment dosage and the fifth group treated with 0.2mg/kg b.w folic acid and 0.3mg/kg b.w thiamine (morning and evening) for 21 days and 0.1ml/kg b.w ethylene glycol for 7 days with continuous administration of the treatment dosage. Cardioprotective effects of ethanolic extract of avocado pear seed was evaluated by estimating the activities of Lactate Dehydrogenase (LDH) and Creatine Kinase (CK). The effects of the extracts on biomarkers of oxidative damage (lipid peroxidation) and antioxidant enzymes namely, catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) were also measured in the heart using homogenates of the organ.Histological sections of hearts were also prepared. Ethylene glycol elevated CK, with LDH ($p < 0.05$) as seen in the positive control group. Treatment by ethanolic extracts of avocado pear seeds significantly reduced the effect of ethylene glycol induced biochemical and histopathological alterations; implying the extract reduced toxic effects of ethylene glycol and has potential to stabilize cell membranes and protect the cell /tissues from more damage.

INTRODUCTION

The heart is a muscular organ situated in the centre of the chest behind the sternum consisting of four chambers organized into two pumps whose major function is the pumping and transfer of blood to every part of the body (Moore *et al* 2013). Disease affecting heart and blood vessels is one top ranking source of fatality most especially amongst third world nations, (WHO) and Africa, where close to thirty percent in entire populace has cardiac failure (Boombhi *et al.*, 2016).

Ethylene glycol clear, viscous liquor at room temperature is useful in many consumer products including antifreeze in cooling and heating systems, it is also used in hydraulics, in cosmetics and also to make plastics. (Staples*et al.*,2001;The Merck Index, 1989).Ethylene glycol is a toxic substance that can cause a range of toxicology effects on the human body, one of the main effects is its ability to cause severe cardiovascular effects as reported by Parry and Wallach, 1974;Gordon and Hunter, 1982.
Although modern drugs have proven to be useful for managing heart and blood diseases, many adverse effects limits their use (Monteil *et al.,* 2004). Recently the use of medicinal plants is gaining greater acceptance from the medical and public profession due to their positive contribution and influence on health and qualify of life. So search for indigenous cardioprotective medicine is still ongoing as part of scientific research.

Melgar and others 2018 asserts Avocado pear is a member of the lauracea family and it's a tropical tree that is mostly cultivated for its edible fruit. Seeds of avocado pears possess a plethora of nutritional and bioactive components including phenolics, flavonoids, condensed tanninsetc and also contain high antioxidant properties – by Calderón-Oliver and others in 2016,

In context, this study aimed to evaluate the potential of ethanol extracts of avocado pear seeds in mitigating the effects of ethylene glycol toxicity in Wistar rats as relates /to cardiac integrity.

METHODOLOGY

Fresh avocado pears were purchased from Swali market, Yenagoa Bayelsa Stateand the flesh striped revealing the endocarp (seed). These seeds were then washed, dried and crushed into powder. Twenty-five (25) mature rodents were gotten from pharmacology Department, Niger Delta University, had distilled H_2O and feed in proper laboratory condition; following University Guidelines for Care and Use of Laboratory Animals in Biomedical Research and approved by the Committee on Ethics in Animal Experimentation of the University.

Approximately 400grams of the crushed Avocado pear seed powder was weighed and homogenized with one (1) litre of ethanol in glass jars and allowed to standing for 48 hrs inside container and stirred intermittently. The mixture was sieved using a 110mm Whatman filter paper and the filtrate was then placed in a rotary evaporator and it extracts collected in bottles, for storage in refrigerator till required.

The twenty five male Wistar rats were used were divided into 5 groups of 5 Wistar rats each. The design follows the experimental design by Brai *et al.,* (2013) with slight modifications.

Group 1 (normal control): received animal feed and distilled water *ad libitum.*

Group 2 (positive control) received ethylene glycol (0.1ml/kg b/w) for seven days.

Group 3 was treated with ethanolic extracts of avocado pear seed at a dose of [100mg/kg b.w/twice daily]

Group 4 was treated with ethanolic extracts of avocado pear seed at a dose of [200mg/kg b.w/ twice daily].

Group 5 was treated with a reference drug of 0.2mg/kg b.w folic acid and 0.3mg/kg b.w thiamine (both twice daily).

Twice daily is at morning and evening.

All the treatments were first administered for 21 days.

Animals in groups 3 – 5 were given 0.1ml/kg b.w ethylene glycol for 7 days (starting from day 22) as well as the treatment dosage.

After twenty eight days experiment duration, all rodents were euthanized following standard protocol as previously adopted by Sule and his team of researchers and prepared for assays (Sule *et al*, 2017; Erigbali *et al*, 2024; Addy *et al*, 2024). The Lactate Dehydrogenase (LDH) and Creatine Kinase (CK) were determined by the method of King (1965) and by the method of Okinaka *et al.,* 1961, respectively. Oxidative stress and antioxidant investigations were performed by methods established (**Misra & Fridovich, 1972; Cohen *et al,*1970; Paglia &Valentine, 1967) and previously adopted** (Sule *et al*, 2017; Erigbali *et al*, 2024).

ANALYTICAL STATISTIC

The obtained results were statistically analyzed using one-way ANOVA. Data were presented as mean±standard error of mean and significance was declared at $p<0.05$.Statistical level of significance was determined by one-way Analysis of Variance (ANOVA) followed by Tukey post-hoc test.

RESULT

For LDH activity, group 1 (normal control) Wistar rats serum values analyzed, showed a lower value of 17.19±0.77 U/L which was significantly different from 48.99±2.10 U/L obtained from the positive control (group 2), ($P<0.05$).

Treatment of theWistar rats with 100mg/kg b/w (twice daily) avocado pear seed extracts (group 3) and 200mg/kg b/w (twice daily) avocado pear seed extract 4th grp had decreased ($P<0.05$) LDH levels of 25.46±1.09 U/L and 20.00±0.56 U/L respectively as compared to 48.99±2.10 U/L obtained from the positive control (group 2).

Treatment of theWistar rat with 0.2mg/kg Folic acid 2 times every day, then 0.3mg/kg thiamine twice a day (5th group), also showed a lower value of 20.06±0.83 as compared to of 48.99±2.10 U/L obtained from the positive control (group 2).

For Creatine Kinase activity, group 1 (normal control) Wistar rats serum values analyzed, showed a lower value of 13.31±0.48 U/L which was significantly different from 47.55±1.40 U/L obtained from the positive control (group 2), ($P<0.05$).

Treatment of theWistar rats with 100mg/kg b/w (twice daily) avocado pear seed extracts (group 3) and 200mg/kg b/w (twice daily) avocado pear seed extract (4th grp) had depleted Creatine Kinase levels of 26.84±0.46 U/L and 22.98±0.72 U/L respectively as compared to 47.55±1.40 U/L obtained from the positive control (group 2).

Treatment of theWistar rat with 0.2mg/kg Folic acid (2 times a day) then 0.3mg/kg thiamine twice a day (5th group), also showed a lower value of 17.38±0.54 U/L as compared to of 47.55±1.40 U/L obtained from the positive control (group 2).

Table 1: Avocado Pear Seed extract's effect on Plasma Cardiac Integrity Biomarkers (LDH, Creatine Kinase) of Wistar Rats

Groups/Parameters	LDH (U/L)	Creatine kinase (U/L)
1st group 1	17.19 ± 0.77^{b}	13.31 ± 0.48^{b}
2nd group (Positive control)	48.99 ± 2.10^{a}	47.55 ± 1.40^{a}
3rd group (100mg/kg b/w extract (twice daily) + EG)	25.46 ± 1.09^{ab}	26.84 ± 0.46^{ab}
Group 4 (200mg/kg b/w extract (twice daily) + EG)	20.00 ± 0.56^{b}	22.98 ± 0.72^{ab}
Group 5 (Reference drug + EG)	20.06 ± 0.83^{b}	17.38 ± 0.54^{ab}

Result in Mean ± Standard Error of Mean - SEM. Superscript 'a' and 'b', imply significance ($p<0.05$) vs 1st grp and 2 respectively.

ANTIOXIDANT ANALYSIS

In table 2 below, serum values for antioxidant enzymes was analyzed on the Heart homogenates of theWistar rats.

For SOD activity, group 2 (positive control) Wistar rats serum values analyzed showed a lower value of 3.40±0.27U/mg which is significantly different from of 9.11±0.19 U/mg obtained in the normal control (group 1).

Treatment of the Wistar rats with 100mg/kg b/w (twice daily) avocado pear seed extract (group 3) and 200mg/kg b/w (twice daily) avocado pear seed extract (group 4), showed increased SOD; 5.40±0.17U/mg and 5.36±0.08U/mg respectively as compared to 3.40±0.27U/mg obtained from the positive control (group 2).

Treatment of the Wistar rats with 0.2mg/kg Folic acid 2 times a day then 0.3mg/kg thiamine (twice daily (group 5), also showed increased SOD quantity - 5.28±0.03U/mg relative to 3.40±0.27U/mg obtained from the positive control (group 2).

For CAT activity, group 2 (positive control) Wistar rats serum values analyzed showed a lower value of 2.27±0.11 U/mg which is significantly different from 5.17±0.13U/mg obtained in the normal control (group 1).

Treatment of theWistar rats with 100mg/kg (2 times a day) avocado pear seed extract (group 3) and 200mg/kg b/w (twice daily) avocado pear seed extract (group 4), showed a significantly ($P<0.05$) increased CAT levels of 2.99±0.38U/mg and 4.26±0.21U/mg respectively as compared to 2.27±0.11U/mg obtained in the positive control (group 2).

Treatment of theWistarrats with 0.2mg/kg Folic acid (twice daily) and 0.3mg/kg b/w thiamine (twice a day (5th group), also showed a significantly ($P<0.05$) increased CAT levels of 4.26±0.14U/mg as compared to the value 2.27±0.11U/mg obtained in the positive control (group 2).

For GPx activity, group 2 (positive control) Wistar rats serum values analyzed showed a lower significant (P<0.05) values of 2.91±0.21U/mg which is different from the higher significant value of 8.72±0.34U/mg in the (normal control) group 1.
Treatment of theWistar rats with 100mg/kg b/w (two times day) avocado pear seed extract (group 3) and 200mg/kg b/w (twice daily) avocado pear seed extract (group 4), showed a significantly (P<0.05) increased GPx levels of 4.07±0.06U/mg and 6.16±0.12U/mg respectively as compared to 2.91±0.21U/mg obtained from the positive control (group 2).
Treatment of theWistar with 0.2mg/kg b/w Folic acid (twice daily) and 0.3mg/kg b/w thiamine (twice daily (group 5), also showed a significantly (P<0.05) increased GPx levels of 6.05±0.07U/mg as compared to the positive control (group 2) of 2.91±0.21U/mg.
For MDA activity, group 2 (positive control) Wistar rats serum analyzed, showed a higher value of 4.94±0.07U/mg which is significantly different from 1.91±0.05U/mg in the normal control (group 1).
Treatment of theWistar rats with 100mg/kg b/w (twice daily) avocado pear seed extract (group 3) and 200mg/kg b/w avocado pear seed extract (group 4), showed a significantly (P<0.05) decreased MDA levels of 2.00±0.05U/mg and 1.95±0.03U/mg respectively as compared to 4.94±0.07U/mg obtained from the positive control (group 2).
Treatment of theWistar with 0.2mg/kg b/w Folic acid (twice daily) and 0.3mg/kg b/w thiamine (twice daily (group 5), also showed a significantly (P<0.05) reduced MDA levels of 1.94±0.03 U/mg as compared to the value 4.94±0.07U/mg obtained from positive control (group 2).

Table 2: Avocado Pear Seed extract's effect on Antioxidant and Oxidative Stress (SOD, CAT, GPx and MDA) in the Heart of Wistar Rats

Groups/Parameters	SOD (U/mg)	CAT(U/mg)	GPx(U/mg)	MDA(U/mg)
Group 1(Normal control)	9.11 ± 0.19^{b}	5.17 ± 0.13^{b}	8.72 ± 0.34^{b}	1.91 ± 0.05^{b}
Group 2 (Positive control)	3.40 ± 0.27^{a}	2.27 ± 0.11^{a}	2.91 ± 0.21^{a}	4.94 ± 0.07^{a}
Group 3 (100mg/kg b/w extract (twice daily) + EG)	5.40 ± 0.17^{ab}	2.99 ± 0.38^{ab}	4.07 ± 0.06^{ab}	$2.00\pm0.05a^{b}$
Group 4 (200mg/kg b/w extract (twice daily) + EG)	5.36 ± 0.08^{ab}	4.26 ± 0.21^{ab}	6.16 ± 0.12^{ab}	1.95 ± 0.03^{b}
Group 5 (Reference drug + EG)	5.28 ± 0.03^{ab}	4.26 ± 0.14^{ab}	6.05 ± 0.07^{ab}	1.94 ± 0.03^{b}

Result in Mean ± SEM (the Standard Error of Mean). Superscript 'a'and 'b', implying significance (p<0.05) vs grp 1, and 2 respectively.

HISTOLOGY

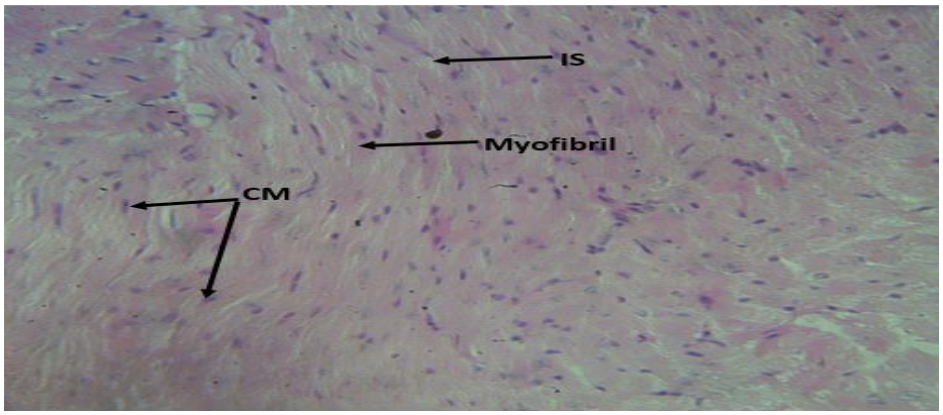

Plate 1: Normal Control (Group 1)
Photomicrograph (H&E X400) of the normal myocardium architecture: layers striated cardiac myocytes (CM) arranged in a spiral fashion interspersed with interstitium (IS).
Diagnosis: Normal myocardium.

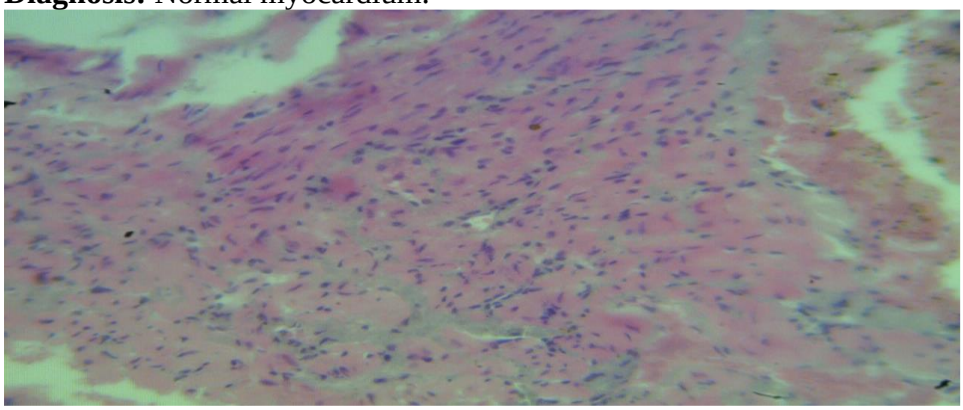

Plate 2: 0.1ml/kg b/w ethylene glycol Positive control (Group 2)
Photomicrograph (H&E X400) of the cardiac muscle showing mild and diffused granulation appearance of striated cardiac myocytes (arrows).
Diagnosis: mild myocardium granulation.

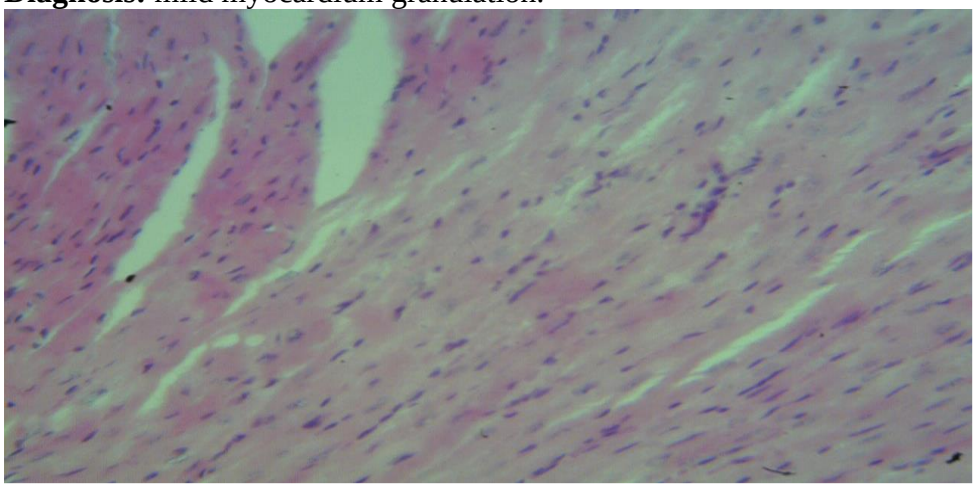

Plate 3: 100mg/kg b/w extracts (twice daily) Group 3

Photomicrograph (H&E X400) of the cardiac muscle showing mild interstitial disruption of the layers of striated cardiac myocytes (arrows).
Diagnosis: mild distortion of the cardiac muscle.

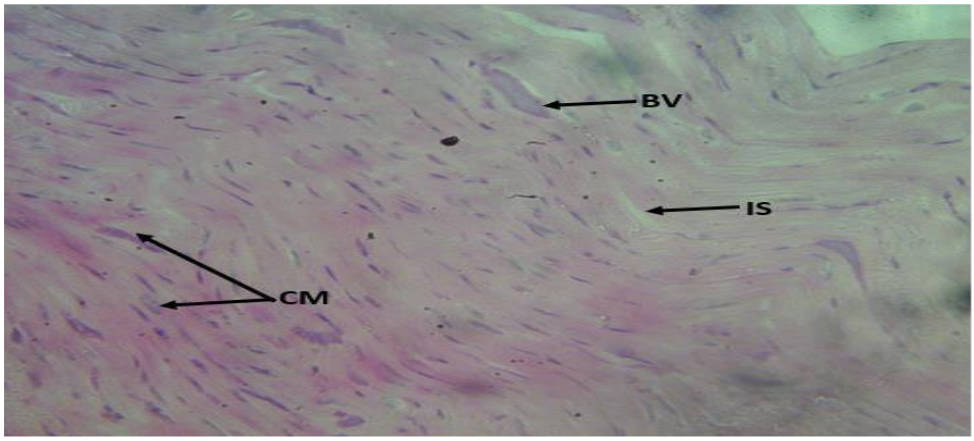

Plate 4: 200mg/kg b/w extracts (twice daily) (Group 4)
Photomicrograph (H&E X400) of the cardiac muscle showing wavy layers of striated cardiac myocytes (CM), blood vessels (BV) and interstitium (IS).
Diagnosis: Normal myocardium.

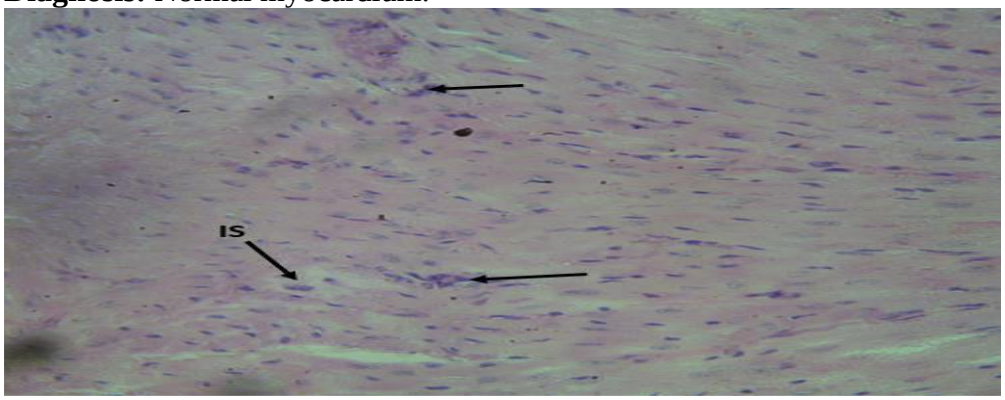

Plate 5: 0.3mg/kg thiamine and 0.2 mg/kg Folic acid (twice daily) (Group 5)
Photomicrograph (H&E X400) of the cardiac tissue with minimal mononuclear infiltration and interstitial oedema (arrows).
Diagnosis: minimal inflammation of cardiac tissue.

DISCUSSION

This study assessed the cardioprotective potential of ethanolic extracts of avocado pear seed against ethylene glycol induced toxicity in Wistar rats. Ethylene glycol is a toxic substance that has been proven to cause various cardiovascular defects including; high and low blood pressure, irregular heart rhythm, and cardiopulmonary failures (Parry and Wallach, 1974;Gordon and Hunter, 1982).
Results reveal that serum values of LDH and CK analyzed showed a significant value increase in the positive control group. However, treatment with ethanolic extracts of avocado pear seed significantly decreased the heart enzymes CK, LDH; corroborating report that Avocado fruits

extracts has preservative health benefit for cardiovascular system (Gouegni & Abubakar, 2013; Chatterjea & Shinde, 2002). Reduction in antioxidants actions by administration of avocado pear seed extract implies avocado pear seed may be beneficial for membrane integrity.

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Homogenate values of SOD, CAT and GPx analyzed showed a significant decrease in the value of the positive control (group 2) that was administered ethylene glycol only.This shows that ethylene glycol as a toxicant significantly reduces antioxidant enzymes corroborating study carried out by Jurczyk *et al.*, (2002) which reported that long term intoxication with ethylene glycol leads to a constant generation of free radicals and gradual exhaustion of the antioxidative system. However, treatments of the Wistar rats with 100mg/kg b/w (twice daily) avocado pear seed extract and 200mg/kg (twice daily) avocado pear seed extract, and also treatment with the reference drug of with 0.2mg/kg Folic acid (two times a day) then 0.3mg/kg thiamine (twice a day) showed a significantly ($P<0.05$) increased in the enzymes levels. MDA activity studied showed a significant increase in the Wistar rats administered only ethylene glycol (group 2) and treatment with ethanolic extracts of avocado pear seed significantly decreased the values of MDA to relatively near normal value range.
Histological evidence also supported the findings. Histopathologic photomicrographs in plates 1 to 5 and it also showed that treatment with avocado pear seed extracts preserved organ /tissue.

CONCLUSION

Avocado pear seed is potentially cardio protective, in face of toxin-exposed heart; a property that may be associated with the bioactive antioxidant activities exhibited.

REFERENCES

Addy, P.S., Ergbaki P.P., & Sule O.J. (2024). Avocado Pear Seed Extract Attenuated Liver Tissue Toxicity Induced by Ethylene Glycol in Experimental Wistar Rats Model. Journal of Biology and Genetic Research , Vol 10 No. 1 page 1 – 13.

Boombhi, J.H., Menanga, A., Nkoke, C., Hamadou, B., Kingue, S. (2016). Variabilité sinusale chez un sujet en Insuffisance cardiaque congestive à Yaoundé. *Health sciences and disease.* 17(1): 1-5.

Brai, B.I.C., Adisa, R.A., Odetola, A.A.(2014). Hepatoprotective properties of aqueous leaf extract of Persea Americana, Mill (Lauraceae)'Avocado'against CCl 4-induced damage in rats. *African Journal of Traditional, Complementary and Alternative Medicines,* 11(2), 237-244. Doi: 10.4314/ajtcam.v11i2.2.

Calder´on-Oliver, M., Escalona-Buendía, H. B., Medina-Campos, O. N., Pedraza-Chaverri, J., Pedroza-Islas, R. and Ponce-Alquicira, E. (2016). Optimization of the antioxidant and antimicrobial response of the combined effect of nisin and avocado byproducts.*LWT - Food Science and Technology*, 65, 46–52.

Chatterjea, M. N. and Shinde, R. (2002). Serum enzymes in heart diseases. *Text book of Medical Biochemistry 5th ed. Jappe Brothers, New Delhi*.Pp, 555-557.

Cohen, G., Dembiec, D. and Marcus J. (1970). Measurement of Catalase Activity in Tissue Extracts. *Analytical Biochemistry* 34, 30-38.

Erigbali P.P., Arhoghro E. M., Sule O.J., & Nordee J.V. (2024) Antioxidant Effect and Nephro Protective Potential of Terminalia Catappa Leaf on Kidneys of Wistar Rats Exposed to Augumentin – induced Damage. *European Journal of Biomedical and Pharmaceutical Sciences. Volume 11, Issuue1: pp. 116 – 121*

Gordon, H. L. and Hunter, J. M. (1982). Ethylene glycol poisoning. A case report. *Anaesthesia*, 37(3), 332-8.

Gouegni E.F. and Abubakar H. (2013). Phytochemical, toxicological, biochemical and haematological studies on avocado (Persea americana) in experimental animals. *Nigerian Food Journal* 31:64–69

Jurczyk, A., Gałecki, P., Jankowska, B., Meissner, E., Szram, S., Fijałkowski, P., Błaszczyk, J. and Kędziora, J. (2002). Effect of ethylene glycol on antioxidative enzymes and lipid peroxidation activity in erythrocytes. *Archives of Forensic Medicine*, 12, 1-37.

King, J. (1965). In Practical Clinical Enzymology. Van D. ed., Nostrand Co, London, pp: 83-93.

Melgar, B., Dias, M. I., Ciric, A., Sokovic, M., Garcia-Castello, E. M. and Rodriguez-Lopez, A. D. (2018). Bioactive characterization of Persea americana Mill. byproducts: A rich source of inherent antioxidants. *Industrial Crops and Products*, 111, 212–218.

Misra, H.P. and Fridovich, I. (1972). The role of Superoxide Anion in the Autoxidation of Epinephrine and a simple Asaay for Superoxide Dismutase. *Journal of Biological Chemistry*, 247,3170-3175.

Monteil, C., Mulder, P. and Thuillez, C. (2004).Oxydant et insuffisance cardiaque: une cible thérapeutique utopique? *Médecine Thérapeutique de Cardiologie.* 2: 78-85.

Moore K.L., Dalley A.F., Agur A.M.R. (2013). Clinically Oriented Anatomy. 5th ed. Lippincott and Wilkins; Philadelphia, PA, USA.

Okinaka, S.H., Kumogai, S. and Ebashi, S. (1961). Archives of Neurology. 4, 520-525.

Paglia, D.E., and Valentine, W.N. (1967) Journal of Laboratory and Clinical Medicine 70, 158-169.

Parry, M. F. and Wallach, R. (1974). Ethylene glycol poisoning. *American Journal of Medicine*, 57(1), 143-50.

Staples, C.A., Williams, J. B., Craig, G.R. and Roberts, K.M (2001). Fate, effects and potential environmental risks of ethylene glycol: a review. *Chemosphere*, 43, 377–383.

Sule O. J., Arhoghro E. M., & Erigbali P. (2017) Nephro-Protective and Hepato-Protective Property of Commelina Diffusa Leaf Extract in Doxorubicin-induced Albino Rats.

World Journal of Pharmacy and Pharmaceutical Sciences, Volume 6, Issue 10, 51-62
The Merck index : an encyclopedia of chemicals, drugs, and biologicals (1989).

Tremocoldi, M. A., Rosalen, P. L., Franchin, M., Massarioli, A.P, Denny, C., Dainto, E.R., Paschoal, J.A.R., Melo, P.Sand Alencar, S.M. (2018). *Exploration of Avocado by-products as Natural Sources of Bioactive Compounds, PLoS One,* 13(2), e0192577.

World Health Organization. (2017). The top 10 causes of death. WHO Media Centre: the top 10 causes of death.