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Comparative Effects of Ethanol Leaf and Seed-Extracts of *Persea americana* on Blood Glucose and Oxidative Stress Markers in Alloxan-Induced Diabetic Albino Rats.

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ABSTRACT

The effects of ethanol leaf and seed-extracts of *Persea americana* on blood glucose and oxidative stress markers were investigated in alloxan-induced diabetic male albino rats. Diabetes was induced by single intra-peritoneal injection of alloxan at 100mg/Kg of body weight. A total of 48 diabetic albino rats were placed in three major groups (A, B and C). Animals in groups A and B contained 18 rats each and were further sub-divided into A1, A2, A3, B1, B2 and B3 of 6 rats each. Group C contained 12 rats and was sub-divided into C1 and C2 of 6 rats each while 6 non-diabetic rats were placed in sub-group C3 (normal control). Animals in groups A1, A2 and A3 received 200, 400 and 600mg/Kg body weight of the ethanol leaf-extract respectively while those in groups B1, B2 and B3 received corresponding doses of the seed-extract. Group C1 received 2.5mg/Kg of glibenclamide (standard control) while C2 (negative control) and C3 (normal control) were given 0.6ml of normal saline by oral intubation for two weeks. Blood glucose concentration and oxidative stress markers were determined using standard methods. Treatment with the extracts and standard drug decreased plasma glucose significantly ($P<0.05$). The leaf-extract at 600mg/Kg had comparable efficacy with the standard drug. The leaf-extract, at 200 and 400mg/Kg body weight, was also more effective than same doses of the seed-extract. Activities of superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GSR) and malondialdehyde (MDA) concentration recorded significant ($P<0.05$) decrease in the extract groups. No significant ($P>0.05$) difference on the effect of the leaf and seed-extracts on antioxidant enzymes was recorded while the seed-extract showed greater efficacy in decreasing MDA concentration. Thus, ethanol leaf and seed-extracts of *Persea americana* could decrease plasma glucose concentration and improve oxidative stress indices that were distorted in the diabetic state.

Key words: *Persea americana*, Leaf and seed-extracts, Diabetes, Alloxan and Oxidative stress markers

INTRODUCTION

The study of medical system from the native point of view has been an old practice. In this form of medicine, explanations in terms of disease etiology, symptoms, causes and treatment are investigated [1]. The use of plants in the management of diabetes is becoming popular and many of such plants with antidiabetic activity have been documented. Medicinal plants provide better alternatives as they are less toxic, easily available and affordable [2].

Diabetes mellitus is a chronic disorder characterized by hyperglycemia which often results from defects in insulin secretion, insulin action or both. Diabetes mellitus results in severe disturbances of carbohydrate, lipid and protein metabolism [3]. Hyperglycemia-induced oxidative stress plays vital role in cellular injury usually common with diabetics. High plasma glucose can stimulate free radical production. The weak defense system of the body becomes unable to counteract reactive oxygen species (ROS) generation resulting in imbalance between the ROS and their scavengers [4]. Hyperglycemia causes oxidative stress leading to alterations in major biomolecules and status of plasma antioxidant potentials. Peroxidation of lipids produces highly reactive aldehydes, the most significant of which is malondialdehyde (MDA). Increased levels of free radical scavenging enzymes such as catalase (CAT), superoxide dismutase (SOD) and glutathione reductase (GSR) have also been reported in diabetic state [5]

Persea americana (avocado pear) belongs to the family lauraceae. It is a medium to large tree that grows to about 9-20 m in height. It has wide geographical distribution. It is classified as an evergreen, although some varieties lose their leaves for a short time before flowering. The tree canopy ranges from low, dense, and symmetrical to upright and asymmetrical. The leaves are elliptic, oval or lanceolate. They are often pubescent and reddish when young, becoming smooth, leathery, and dark green when mature. Flowers are yellowish green and 1-1.3 cm in diameter. The many-flowered inflorescences are borne in a pseudoterminal position. The central axis of the inflorescence terminates in a shoot. The fruit is a berry, consisting of a single large seed surrounded by a buttery pulp. Most varieties contain 3-30% oil. The skin is variable in thickness and texture. Fruit color at maturity is green, black, purple, or reddish, depending on the variety. Fruit shape ranges from spherical to pyriform [6].

Apart from scientifically investigating the folkloric use of *Persea* a*mericana* leaf and seed - decoctions in the treatment of diabetics in the Eastern Nigeria, there is also need to compare the effects of the two parts of the plant on hyperglycemia-induced oxidative stress with a view to establishing their relative efficacy.

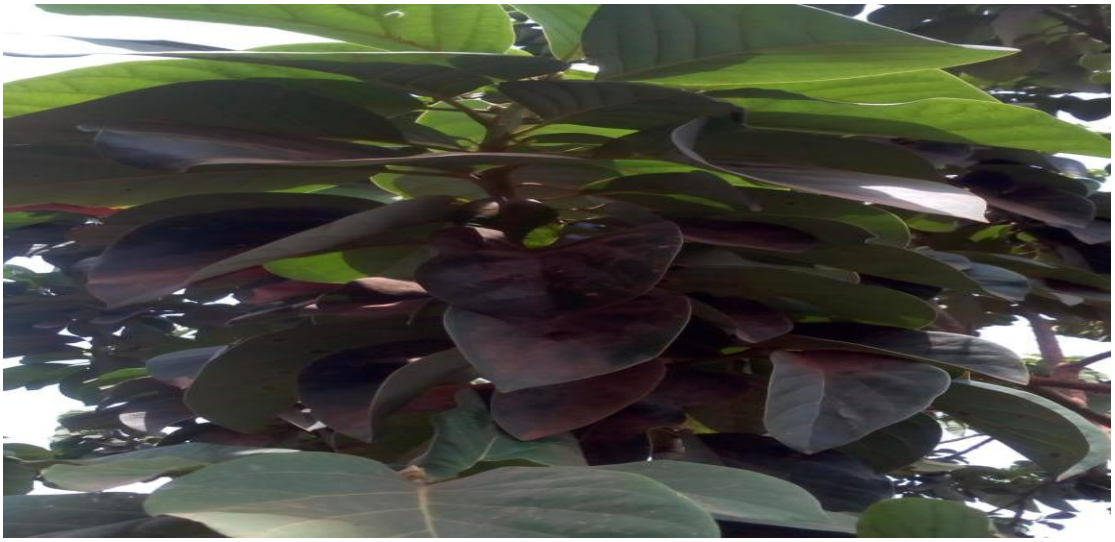

Plate 1. *Persea americana* plant showing the leaves.

Plate 2. *Persea americana* seeds.

MATERIALS AND METHODS

Materials

The fresh leaves and seeds of *Persea americana* were plucked from same plant in Ngbo, Ohaukwu government area of Ebonyi State, Nigeria in the month of January. Adult male albino rats were procured from the animal house of the Pharmacology Department, University of Nigeria, Enugu campus (UNEC), Enugu State.

METHODS

Extraction of Plant Materials

Fresh leaves of *Persea americana* were washed with distilled water and dried under ambient temperature. The dry leaves were pulverized. A mass of 1500g powder was macerated in 3000ml of ethanol for 48 hours and was filtered using muslin cloth. The filtrate was concentrated using rotary evaporator. The fruits were washed and cut with a sharp stainless knife. The seeds were removed and washed with distilled water. The seeds were then cut into slices and air dried under room temperature. The dry seeds were ground with a mechanical blender. A mass of 1500g of the powder was soaked in 3000ml of ethanol for 48 hours. The mixture was filtered using a muslin cloth and filtrate concentrated using rotary evaporator

Induction of Diabetes

A single dose of 100mg/Kg body weight of the alloxan was injected into 48 adult male albino rats in groups A1, A2, A3, B1, B2, B3, C1 and C2 intra-peritoneally. The blood glucose levels of the animals were determined after 72 hours and animals that had blood glucose concentrations ≥ 200mg/dl (11.1mmol/l) were used for the study [7].

Administration of Ethanol Leaf and Seed-Extracts of *Persea americana* and Glibenclamide Solution

A total of 48 diabetic adult male albino rats were placed in three major groups (A, B and C). Groups A and B contained 18 rats each and were further sub-divided into A1, A2, A3 and B1, B2 and B3 of 6 rats each. Group C contained 18 rats and was sub-divided into C1, C2 and C3 of 6 rats each (C3 sub-group did not receive alloxan injection). The leaf and seed-extracts and glibenclamide tablets were separately reconstituted in 0.9% normal saline. Animals in sub-groups A1, A2, and A3, respectively, received 200, 400, and 600mg/kg body weight of *Persea americana* leaf- extract while sub-groups B1, B2, and B3 received 200, 400 and 600mg/kg body weight of *Persea americana* seed-extract respectively twice daily for 14 days. Animals in sub-group C1 received 2.5mg/kg body weight of glibenclamide while groups C2 and C3 received 0.1ml of normal saline twice daily for 14 days.

Collection of Blood Samples

Blood sample collection for daily glucose check was done through the tail veins and finally the blood samples for determination of oxidative stress indices were collected through heart puncture.

Determination of Blood Glucose Level

The blood glucose levels were determined daily using one touch Accu-Check glucometer.

Determination of Oxidative Stress Markers

The activity of Superoxide Dismutase was determined following the method of [8]. Catalase (CAT) activity was determined using the method of [9]. Reduced glutathione was estimated according to the method of [10]. MDA concentration was determined according to the method described by [11].

Statistical Analysis

Data generated were expressed as mean ±SD for the 6 animals in each group and subjected to one way analysis of variance (ANOVA) and finally to Dunnett's Multiple Comparison Test (DMCT). P values < 0.05 were considered statistically significant.

RESULTS

There was a significant (P<0.05) decrease in plasma glucose concentration in animal groups that received ethanol leaf and seed-extracts of *Persea americana.* The leaf-extract at 600mg/kg body weight exhibited high efficacy with the standard drug. At 200 and 400mg/Kg body weights the leaf-extract also showed significantly (P<0.05) higher efficacy compared with the groups that received same doses of the seed-extract (Figure 1). The percentage reductions in plasma glucose concentrations for the leaf and seed-extract groups were 10.6 and 8.22 at 200mg/Kg body weight; 26.66 and 16.33 at 400mg/Kg body weight; and 35.66 and 22.22 at 600mg/Kg body weight respectively as shown in Figure 2.

A significant (P<0.05) decrease in superoxide dismutase (SOD) activity was recorded in groups that received the extracts and standard drug. There was no significant (P>0.05) difference in the SOD activity across all doses of the extract groups (Figure 3). There was also a significant (P<0.05) decrease in the activity of catalase (CAT) in groups that received the extracts and standard drug. There was however, no significant (P>0.05) difference in the activity of the enzyme across the extract groups as shown in Figure 4.

A significant (P<0.05) decrease in the activities of glutathione reductase (GSR) in animal groups that received the ethanol leaf and seed-extracts of *Persea americana* was recorded as presented in Figure 5.

The concentrations of malondialdehyde (MDA) decreased significantly (p<0.05) in animal groups that received the ethanol leaf and seed-extracts. The seed-extract at all the doses produced more significant decrease in MDA concentration. However, the decrease in the MDA concentration was more pronounced at 200 and 400mg/Kg body weight as shown in Figure 6.

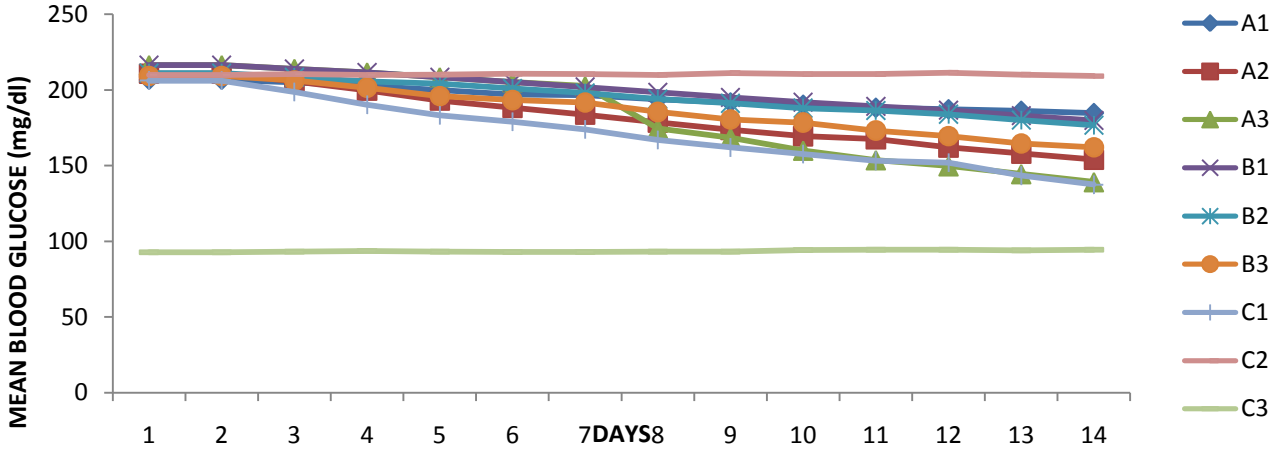

Figure 1: Blood glucose levels in Alloxan-induced Diabetic rats that received Ethanol Leaf and Seed-extracts of *Persea americana*. Values are reported as Mean ± SD (n = 6).

KEY: A1B1 = 200mg/kg; A2B2 = 400mg/kg; A3B3 = 600mg/kg C1, C2 and C3 = Standard, negative and normal controls respectively.

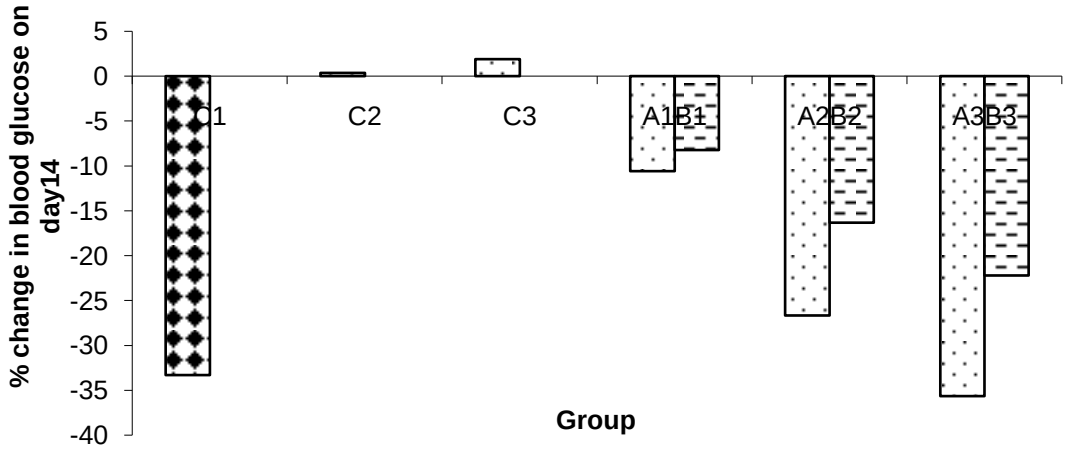

Figure 2: Percentage Change in Blood glucose levels in Alloxan-induced Diabetic rats that received Ethanol Leaf and Seed-extracts of *Persea americana*

KEY: Ethanol leaf-extract Ethanol seed-extract

A1B1 = 200mg/kg; A2B2 = 400mg/kg; A3B3 = 600mg/kg
C1, C2 and C3 = Standard, negative and normal control respectively

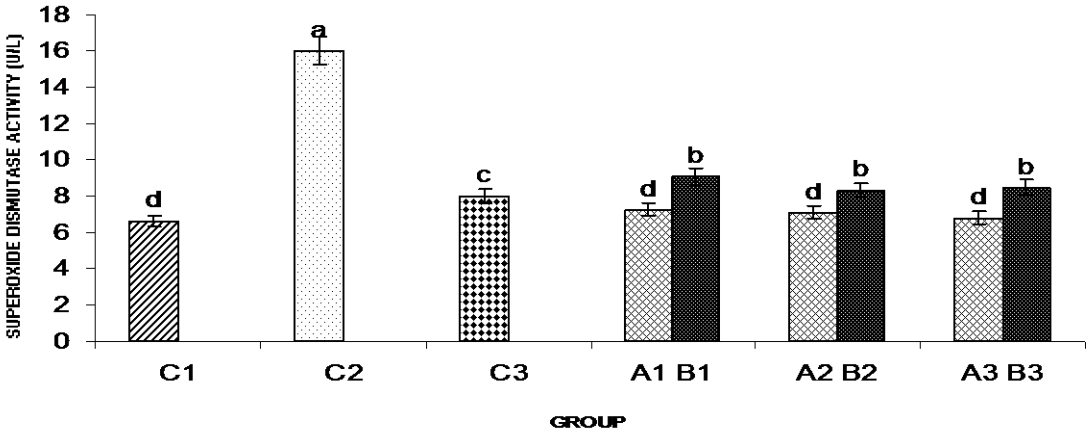

Figure 3: Superoxide Dismutase Activities in Alloxan-induced Diabetic Rats that received Ethanol Leaf and Seed - Extracts of *Persea americana*. Values are reported as Mean ± SD (n=6). Bars with different alphabets are significantly different at P<0.05.

KEY: Ethanol leaf-extract Ethanol seed-extract

A1B1 = 200mg/kg; A2 B2 = 400mg/kg; A3 B3 = 600mg/kg
C1, C2 and C3 = Standard, negative and normal control respectively.

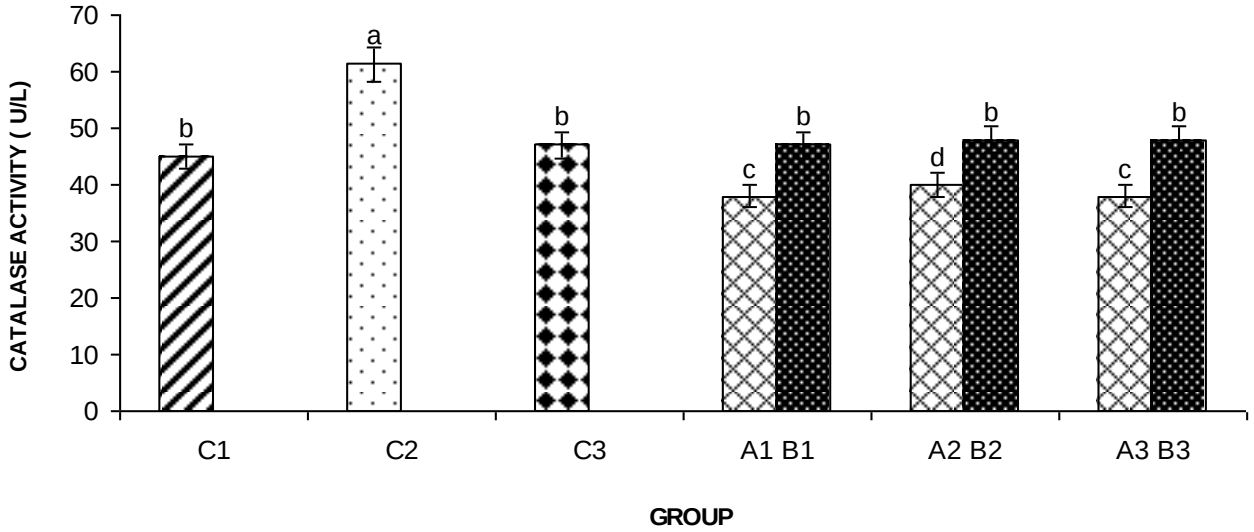

Figure 4: Catalase Activities in Alloxan-induced Diabetic rats that received Ethanol Leaf and Seed - Extracts of *Persea americana*. Values are reported as Mean ± SD (n=6). Bars with different alphabets are significantly different at P<0.05.

KEY: Ethanol leaf-extract Ethanol seed-extract

A1B1 = 200mg/kg; A2 B2 = 400mg/kg; A3 B3 = 600mg/kg

C1, C2 and C3 = Standard, negative and normal controls respectively.

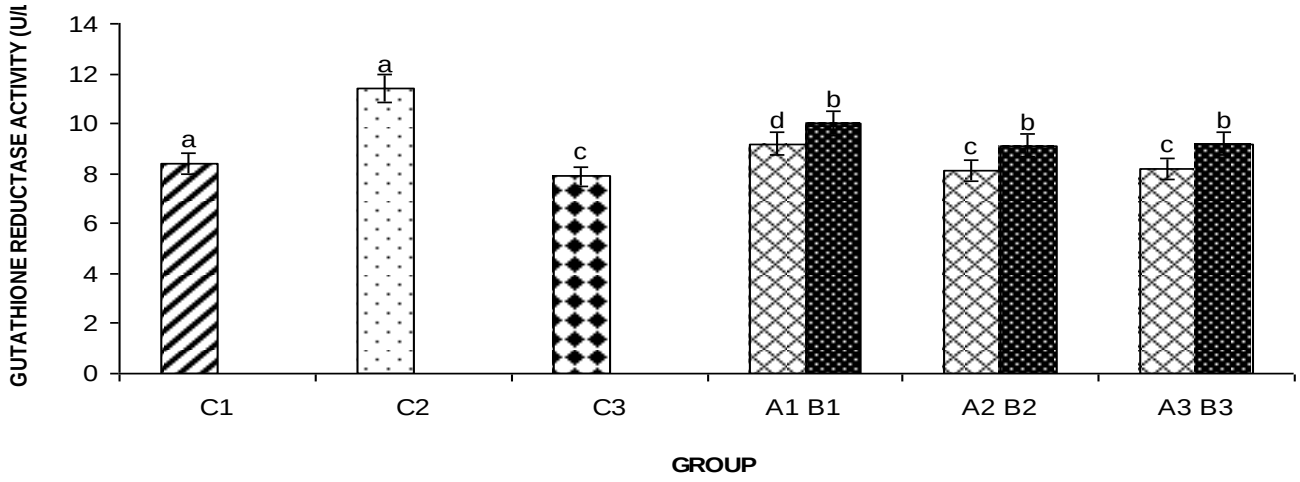

Figure 5: Glutathione Reductase Activities in Alloxan-induced Diabetic rats that received Ethanol Leaf and Seed - Extracts of *Persea americana*. Values are reported as Mean ± SD (n=6). Bars with different alphabets are significantly different at P<0.05.

KEY: Ethanol leaf-extract Ethanol seed-extract

A1B1 = 200mg/kg; A2 B2 = 400mg/kg; A3 B3 = 600mg/kg
C1, C2 and C3 = Standard, negative and normal control respectively.

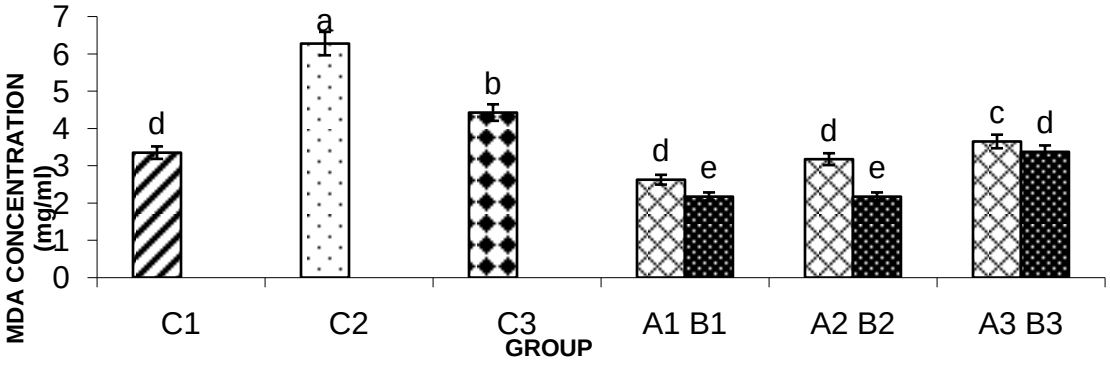

Figure 6: Malondialdehyde concentrations in Alloxan-induced Diabetic rats that received Ethanol Leaf and Seed - Extracts of *Persea americana*. Values are reported as Mean ± SD (n=6). Bars with different alphabets are significantly different at P<0.05.

KEY: Ethanol leaf-extract Ethanol seed-extract

A1B1 = 200mg/kg; A2 B2 = 400mg/kg; A3 B3=600mg/kg

C1, C2 and C3 = Standard, negative and normal controls respectively.

DISCUSSION AND CONCLUSION

Alloxan administration to the animals resulted in increased blood glucose concentration as shown in Figure 1. Alloxan has been shown to selectively destroy the beta cells of the pancreas by generation of reactive oxygen species (ROS) which ultimately causes beta cell necrosis and depletion of insulin biosynthesis and secretion [12]. Alloxan selectively destroys the beta cells of the pancreas by generation of reactive oxygen species (ROS) which ultimately causes beta cell necrosis and depletion of insulin biosynthesis and secretion [13]. A similar result was reported by [14] who opined that alloxan causes massive reduction in insulin release leading to several metabolic alterations such as hyperglycemia, hypercholesterolemia and elevated levels of alkaline phosphatase and liver transaminases. [15] also recorded elevation in blood glucose level in alloxanised mice.

Oral administration of ethanol leaf and seed-extracts of *Persea americana* to the alloxan-induced diabetic rats twice daily for two weeks resulted in a significant ($P<0.05$) decrease in the plasma glucose concentration compared with the positive control group (Figure 1). The results also showed that the leaf-extract at 200, 400 and 600 mg/kg body weight were more effective than corresponding doses of the seed-extract. The leaf-extract particularly at 600 mg/Kg body weight exhibited efficacy comparable to that of the standard drug (glibenclamide). The decrease in plasma glucose concentration for the leaf and seed- extracts at 200 mg/Kg body weight were 10.6% and 8.22%, 400 mg/Kg body weight were 26.66% and 16.33% and 600 mg/Kg body weight were 35.66% and 22.22% respectively as shown in Figure 2. The ability of the plant extracts to decrease plasma glucose concentration may be due to the presence of alkaloids, flavonoids, saponins, tannins, terpenoids, antioxidant vitamins and essential minerals present in the samples. The ethanol leaf-extract of *Allium sativum* significantly ($p<0.05$) and dose-dependently reduced plasma glucose levels in alloxan-induced diabetic albino rats [13]. A report by [16] showed that ethanol leaf-extract of *Talfaira occidentalis* also decreased plasma glucose concentration.

Administration of alloxan to the albino rats significantly ($P<0.05$) raised the activities of tissue superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GSR) and concentration of malondialdehyde (MDA) as seen in the positive control group (Figures 3, 4, 5 and 6). The ethanol leaf and seed-extracts of *Persea americana* significantly ($P<0.05$) decreased the activities of superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GSR) and concentration of malondialdehyde (MDA) (Figures 3, 4, 5 and 6). [17] also reported increase in antioxidant enzymes in streptozotocin- induced diabetic rats following the administration of *Moringa oleifera* leaf- extract. [18] reported that administration of extracts of *Cassia auricuata* leaves and flowers on alloxan-induced diabetic rats increased the activities of antioxidant enzymes and decreased the MDA level in the treated groups. A similar observation was made by [19] in streptozotocin – induced diabetic rats treated with *Nigella sativa* seed extract.

In conclusion, oral administration of ethanol leaf and seed-extracts of *Persea americana* to the alloxan-induced diabetic rats resulted in a significant ($P<0.05$) decrease in the plasma glucose concentration. It also significantly ($P<0.05$) decreased the activities of superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GSR) and concentration of malondialdehyde (MDA). Ethanol leaf and seed-extracts of *Persea Americana* could serve as potent antidiabetic and antioxidant agents.

REFERENCES

1. Ofuegbe, S. O. and Adedapo, A. A. (2015). Ethnomedicinal Survey of some Plants used for the Treatment of Diabetes in Ibadan, Nigeria. *Asian Journal of Medical Sciences*, **6**(5): 1-5.
2. Noor, A., Bansal, V. S. and Vijayalakshmi, M. A. (2013). Current Update on Antidiabetic Biomolecules from key Traditional Indian Medicinal Plants. *Current Science*, **104**(60): 721-727.
3. ADA (2011). American Diabetes Association. Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care*, **34**(1): 62-69
4. Pandey, K. B., Mishra, N. and Rizvi, S. I. (2010). Protein Oxidation Biomarkers in Plasma of Type 2 Diabetic Patients. *Clinical Biochemistry*, **43**(4-5): 508-511
5. Calabrese, V., Cornelius, C. and Leso, V. (2012). Oxidative Stress, Glutathione Status, Sirtuin and Cellular Response in Type 2 Diabetes. *Biochimica et Biophysica Acta*, **1822**(5): 729-736
6. Ding, H., Chin, Y. W., Kinghorn, A. D and Ambrosio, S. M. (2007). Chemopreventive Characteristics of Avocado Fruit. *Semi Cancer Biology*, **17**: 386–394.
7. Bournaventure, C.O., Theophine, C. O., Elijah, P. J., Collins, A.O. and Edwin, O. A. (2015). Comparative Antidiabetic Effects of Metformin , Glibenclamide and Repaglinide in alloxan-induced Diabetic Rats. *African Journal of Pharmacy and Pharmacology.* **9**(44): 1026-1036
8. Kakar, P., Das, B. and Viswanathay (1984). A Modified Spectrophotometric Assay of Superoxide Dismutase. *Indian Journal of Biochemistry and Biophysiology*, **21**: 130-2
9. Moron, M.S., Depierre, J.W. and Mannervik, B. (1979). Levels of Glutathione, Glutathione Reductase and Glutathione-S-transferase Activity in Rat Lungs and Liver. *Biochemistry Biophysiology,* **582**: 67-78.
10. Luck, H. (1971). Catalase. Methods of Enzymatic Analysis. Bergmeyer H. U. Edition, Academic Press, New York. 885 - 893.
11. Ohkawa, H., Ohishi, N. and Yagi, K. (1979). Assay for Lipid Peroxidation in Animal Tissues by Thiobarbituric Acid Reaction. *Annals of Biochemistry,* **95**: 351-358.
12. Wu, J. and Yan, L. J (2015). Streptozotocin-induced Type 1 Diabetes in rodents as a Model for Studying Mitochondrial Mechanism of diabetic beta Cell Glucotoxicity. *Diabetes Metabolic Syndrom Obesity,* **8**: 181-188
13. Vivek, K. S., Suresh, K., Hitesh, J.P. and Shivakumar, H. (2010). Hypogycemic Activity of *Ficus glomerata* in Alloxan-induced Diabetic Rats. *International Journal of Pharmaceautical Sciences Review and Research,* **1**(2): 18-22
14. Nidhi, S. and Veena, G. (2009). Antihyperglycemic and Antioxidative Potential of hydroalcoholic Extract of *Butea monospermia* Lam Flowers in Alloxan-induced Diabetic Mice. *Indian Journal of Experimental Biology,* **47**: 571-576
15. Offor, C.E., Ugwu Okechukwu, P.C. and U. Alum E.U. (2014). The Anti-Diabetic Effect of Ethanol Leaf-Extract of *Allium sativum* on Albino Rats. *International Journal of Pharmacy and Medical Sciences,* **4** (1): 01-03.
16. Esayin, O. A., Ebong, P. Eyong, U. E.; Emem, U and Oladoja, o. (2010). Comparative Hypoglycemic Effects of ethanolic and Aqueous Extracts of Leaf and Seeds of *Talfaira*

occidentalis. *Turkish Journal of Pharmacological Science*, **7**(1): 29-34

17. Ram, N. J and Gyan, C. J. (2016). Antidiabetic and Antioxidant Potential of Hydroalcoholic Extract of *Moringa oleifera* Leaves in Streptozotocin-induced Diabetic Rats. *European Journal of Pharmaceautical and Medical Research,* **3(**9): 438-450
18. Kalivani, A., Umamaheshwari, A., Vinayagam, A. and Kalaivivani, K. (2008). Antihyperglycemic and Antioxidant Properties of *Cassia auriculata* Leaves and Flowers on Alloxan-induced Diabetic Rats. *Pharmacology online,* **1**: 204-217
19. Hadded, A. E., Madeha, N.A. and Amal, S. B. (2017). The Anti-diabetic Activity of *Nigella sativun* and Propolis on Streptozotocin-induced Rats. *Evidence–Based Complementary and Alternative Medicine,* **54**: 39-42.