Vol. 12(28), pp. 483-492, 25 November, 2018
DOI: 10.5897/JMPR2018.6670
Article Number: 7EDACD659082
ISSN: 1996-0875

http://www.academicjournals.org/JMPR R

Journal of Medicinal Plants Research

*Full Length Research Paper*

# *In vivo* efficacy of *Dialium guineense* fruit pulp on hemeoxygenase-1 and angiotensin converting enzyme in experimental diabetes

**Etah E. Nkanu*, Gabriel Ujong, Victoria Okon and Iya Eze Bassey**

Department of Physiology, Faculty of Basic Medical Sciences, Cross River University of Technology, Calabar, Okuku Campus, Yala, Nigeria.

Received 1 September, 2018; Accepted 17 September, 2018

**The study aims to investigate the effect of aqueous fruit pulp of Dialium guineense on hemeoxygenase, and insulin release, inhibition of angiotensin converting enzyme (ACE) and possible hypoglycemia in streptozotocin-induced diabetic rats. Twenty four male Wistar rats were grouped into control, diabetic (Dm), diabetic + 300 mg/kg body weight Dialium guineese (Dm+ DG) and diabetic + 100 mg/kg metformin (Dm +MET). Apart from the control, other rats were made diabetic by a single dose of 50 mg/kg streptozotocin injected intraperitoneally. Dialium and metformin were administered orally three days after induction of diabetes. Result showed significant ($p<0.01$) increase in serum ACE, blood glucose, and a decrease ($p<0.01$) in HO-1 and insulin in the Dm group. There was also an increase ($p<0.01$) in TC, TG and LDL-c. Tissue peroxidation (Heart and Kidney) was high in the diabetic untreated rats, superoxide dismutase and catalase activity was attenuated. Administration of aqueous fruit pulp and metformin significantly ($p<0.001$) increased HO-1 and insulin secretion, decreased ACE and blood sugar level ($p<0.001$) as well as the TC, TG and LDL-c. Antioxidant activities in the kidney and liver were potentiated. In conclusion, this study showed that Dialium guineese fruit pulp enhances HO-1 and insulin release and inhibits ACE activity. It is hypoglycemic, hypolipidemic and evokes antioxidant activity.**

**Key words:** Hemeoxygenase-1 (HO-1), angiotensin converting enzyme (ACE), insulin, diabetes, Dialium guineese, metformin.

## INTRODUCTION

One major disease that affects man in an alarming rate amongst others is diabetic mellitus. It comes with marked metabolic disorder resulting from different environmental and varied hereditary factors. Commonly associated complications of diabetes mellitus include high toll of morbidity and mortality abnormal insulin secretion or insulin receptor inactivity, hyperglycemia, hyper-cholesterolemia, liver and kidney dysfunction as well as derangement of pancreatic β-cell. It is also associated with profound changes in serum lipid, diabetic

*Corresponding author. E-mail: nkanuee@yahoo.com; nkanuetah@gmail.com. Tel: +2347030299991; 08086127404.

ketoacidosis and culminates in chronic renal failure, neuropathy and coronary heart disease (Arora, 2010). In recognition of all these life threatening factors, concerted efforts to ameliorate the upsurge of the disease have been on course and drugs that can manage diabetes more effectively sorted with vigour.

Research has shown that angiotensin converting enzyme involved in the control of blood pressure plays an essential role in the conversion of inactive angiotensin I to an active angiotensin II which causes vasoconstriction. Studies have also shown that randomized trials using angiotensin converting enzyme inhibitors (ACEI) example, natural ACE inhibitors like polyphenols, flavonoids, xanthines, terpenoids derived from herbs (Kang et el., 2003; Loizzo, et al., 2007) and AT2 receptor blockers significantly decreased the risk of DM (Andraws and Brown, 2007; Abuissa et al.2005), improved insulin sensitivity and glucose metabolism, and reduced plasma glucose in both experimental conditions and in humans with DM (Scheen, 2004).

Similarly, HO-I has been reported to be a key antioxidant enzyme that provents the development of diabetes by abating oxidative stress via suppression of macrophages and acting as an important component in anti-atherogenic activity (Orozco et al., 2007); it also plays a vital role in evoking insulin release (Ndisang et al., 2010; Mosen et al., 2005).

Substantive evidence indicates that factors such as hyperglycemia, free-fatty acids and adipokines that increase oxidative stress contribute to insulin resistance (Evans et al., 2003) even though the exact mechanism by which this occurs is not fully understood. However, some available information has implicated oxidative stress in the development of different forms of insulin resistance (Evans et al., 2003; Vinayagamoorthi et al., 2008).

It is therefore generally believed that elevated oxidative stress may lead to the cascade of events that impairs insulin-signalling (Vinayagamoorthi et al., 2008), and as such strategies that cause reduction in oxidative stress as well as glucose/insulin intolerance may improve glucose metabolism. Today, attention is redirected to the use of medicinal plants to treat most chronic diseases such as diabetes and hypertension (Liu et al., 2003; Ullah et al., 2015) because of its recognized nutritional and medicinal properties.

*Dialium guineense,* otherwise referred to as black velvet tamarind is an indigenous tropical forest fruit tree that belongs to the family leguminosae. The plant is found in many countries in West Africa and is identified by different names. In countries like Sierra Leone, Senegal, and Guinea Bissau, it is called 'Veludo." In Nigeria, Dialium is called by different names depending on the ethnic group. The Igbos call it Icheku, while the Yorubas call it Awin. In Hausa, it is called Tsamiyarkurm (Orwa et al., 2009). The Yakurr ethnic group in Cross River State, Nigeria calls it Okana gben gbenwen. *D. guineense* has been convincingly used in the management of various disease conditions such as severe cough, bronchitis, stomach aches, malaria fever, jaundice, antiulcer, hypertension and hemorrhoids (Lawal et al., 2010).

Phytochemical components identified in the sticky pulp of *D. guineense* include gums, hemicelluloses, mucilage, pectin and tannins. It also contains some level of ascorbic acid, minerals (copper, potassium, calcium, iron, selenium, zinc and magnesium), vitamins like vitamin-A, folic acid, riboflavin, niacin and vitamin C, tartaric acid (an anti-oxidant), carbohydrates in the form of soluble sugars, cellulose, iron and lipids (Nahar et al.,1990; Herzog et al.,1994; Gideon et al., 2012), tannins, alkaloids, saponins, flavonoid, steroids and cardiac glycosides and some phenolic compounds (David et al., 2011; Ezeja et al., 2011). This study was therefore aimed at finding out the possible effect of *Dialium giuneense* fruit pulp consumption on heme oxygenase, insulin and ACE activity in streptozotocin-induced diabetic Wistar rats since the fruit is being consumed locally as a socially and an alternative to Vitamin C.

## MATERIALS AND METHODS

### Experimental design

Twenty four male Wistar rats weighing between 170-230 g were used for this study. The animals were randomly selected into four groups of six rats each. Group 1 was the control and received tap water. Group 2, 3 and 4 were injected with 50 mg/Kg body weight of streptozotocin intraperitoneally to render them diabetic. Groups 3 and 4 were then administered 300 mg/kg of *D. guineense* fruit pulp and 100 mg/kg body weight of metformin respectively. Administration of drugs lasted for three weeks. Blood samples were collected by cardiac puncture for biochemical analysis and the tissues removed and used for histological studies and tissue peroxidation.

### Preparation of *Dialium* fruit pulp extract

The fruit pulp of *D. guineense* was purchased from Okuku Market, Yala Local Government Area of Cross River State. *D. guineense fruits were collected and the dark coloured hard coats broken to expose the soft pink pulp of the fruit* .The pulp was peeled from the water proof- like coat and then dried at room temperature by hot air oven (Amstel Hearson Oven, England) to evaporate its water content to a thick orange paste. Dried pulp was blended to powder and used when necessary. Animals received 300 mg/kg body weight of the suspension.

### Induction of diabetes mellitus

The rats except the control group were rendered diabetic on a 12 h fast by a single intraperitoneal injection of 50 mg/kg body weight streptozotocin (SantaCruz Biotechnology, USA) dissolved in 0.01M citrate buffer, at a pH 4.5. All experiments on animals were carried out in absolute compliance with ethical guideline for research, care, and use of laboratory animals. After 3 days of streptozotocin injection, blood glucose concentrations were determined via AccuChek glucometer to confirm diabetes. Blood glucose levels

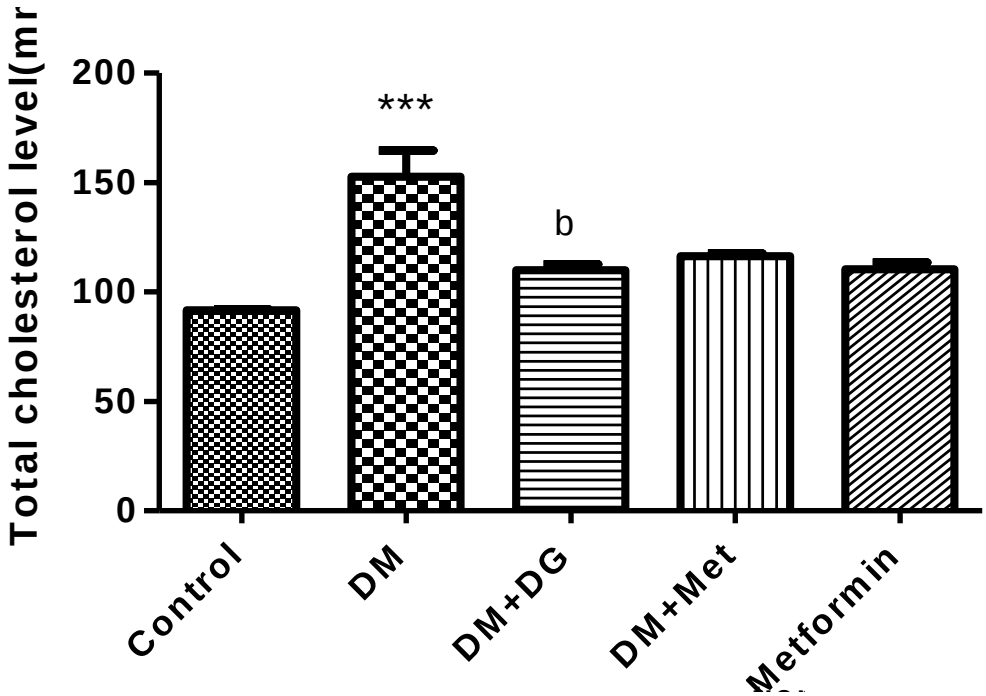


**Figure 1.** Showing effect of daily oral administration of aqueous *D. guineense* fruit pulp on lipid profile in streptozotocin-induced diabetic rats. n=5; ***=p<0.01 vs Control; b=p<0.01 vs Dm.

below 130 mg/dl were not considered.

**Blood collection and analysis**

Blood samples were collected from the animals through cardiac puncture. The blood samples were centrifuged at 3000 (rpm) revolutions for 10 min to obtain serum for lipid profile, insulin, angiotensin converting enzyme and hemeoxygenase-1 analysis. Tissues were homogenized, centrifuged and supernatant used to measure malondealdehyde concentration and antioxidant activity.

**Catalase**

The method of Sinha (1972) was used to estimate catalase activity. The major principle involves reduction of dichromate in acetic acid to chromic acetate when heated in the presence of hydrogen peroxide ($H_2O_2$).The perchromic acid formed is an unstable intermediate. The chromic acetate finally produced is measured using the colorimeter.

**Superoxide dismutase**

The activity of SOD was assayed using the method of Mishra et al. (1972). The ability of superoxide dismutase to inhibit the auto-oxidation of epinephrine at pH 10.2 has been used as the basis of a convenient and sensitive assay for this enzyme.

**Malondialdehyde**

Plasma MDA was estimated by method of Jean et al. (1983). After the reaction of thiobarbituric acid with malondialdehyde, the reaction product was extracted in butanol and was measured.

**Determination of ACE**

ACE activity was determined using the method of Hooper et al. (1987) with modifications. In brief, a pre-incubation mixture contained 100 mM Tris-HCl buffer with 300 mM NaCl and 10 µM $ZnCl_2$, pH 8.3/positive control/test sample of various concentrations and 2 mU of ACE enzyme. The reaction mixture was mixed and pre-incubated at 37°C for 10 min. Following pre-incubation, substrate (N-HippurylL-histidine-L–leucine tetrahydrate) was added to a final concentration of 5 mM. The reaction mixture was mixed and incubated at 37°C for 30 min. The reaction was heated up in boiling in water bath for 4 min. A control reaction was also carried out without the test samples. The reaction mixture was centrifuged at 15,000 rpm for 10 min at 25°C. The supernatant was transferred to HPLC vials and subjected to HPLC analysis.

**Histological studies**

Heart and kidney were removed, dissected and washed immediately on ice cold saline. A portion of these tissues was fixed in 10% neutral formal-saline fixative solution for histological studies. After fixation, tissues were embedded in paraffin. Solid sections were cut at 5 cm and stained with hematoxylin and eosins as described by Strate et al. (2005). The slides were viewed at magnification of X 400 and photomicrographs were taken.

**Statistical analysis**

All data obtained in this study were expressed as mean ± standard error of mean. Collected data were analyzed using ANOVA (analysis of variance) followed by Bonferronis multiple comparison post hoc tests to compare the level of significance between control

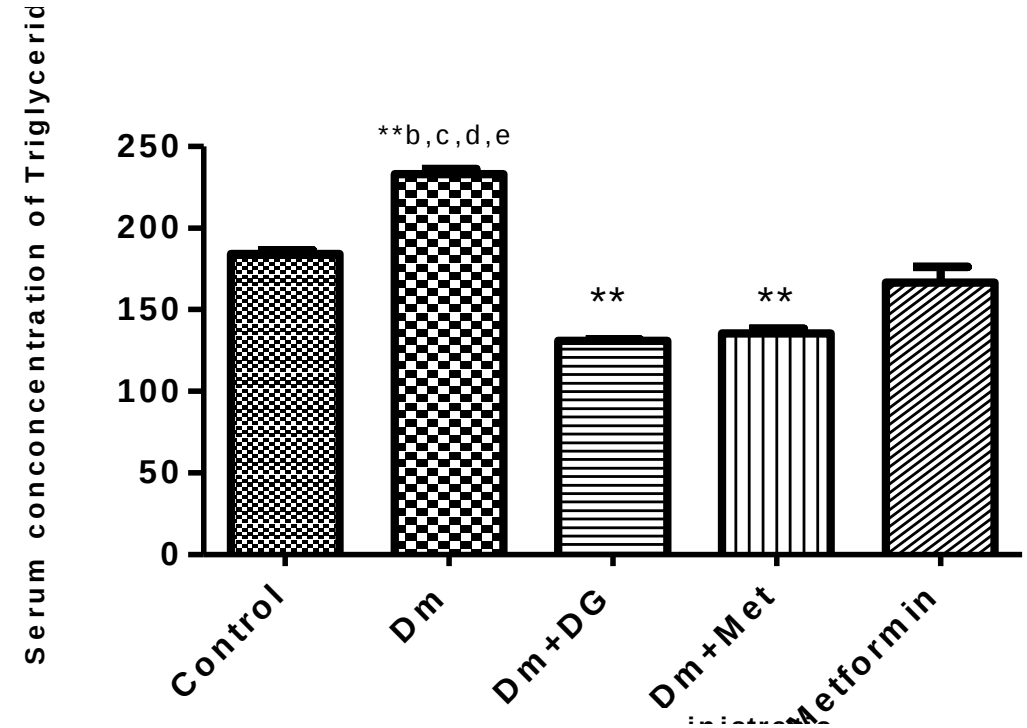


**Figure 2.** Showing effect of daily oral administration of aqueous *D. guineense* fruit pulp on triglyceride concentration in streptozotocin-induced diabetic rats. n=5; **=p<0.01 vs Control; b=p<0.01 vs Dm; c=p<0.01 vs Dm+DG;d=p<0.01 vs Dm Vs Met; e=p<0.01 vs Metformin.

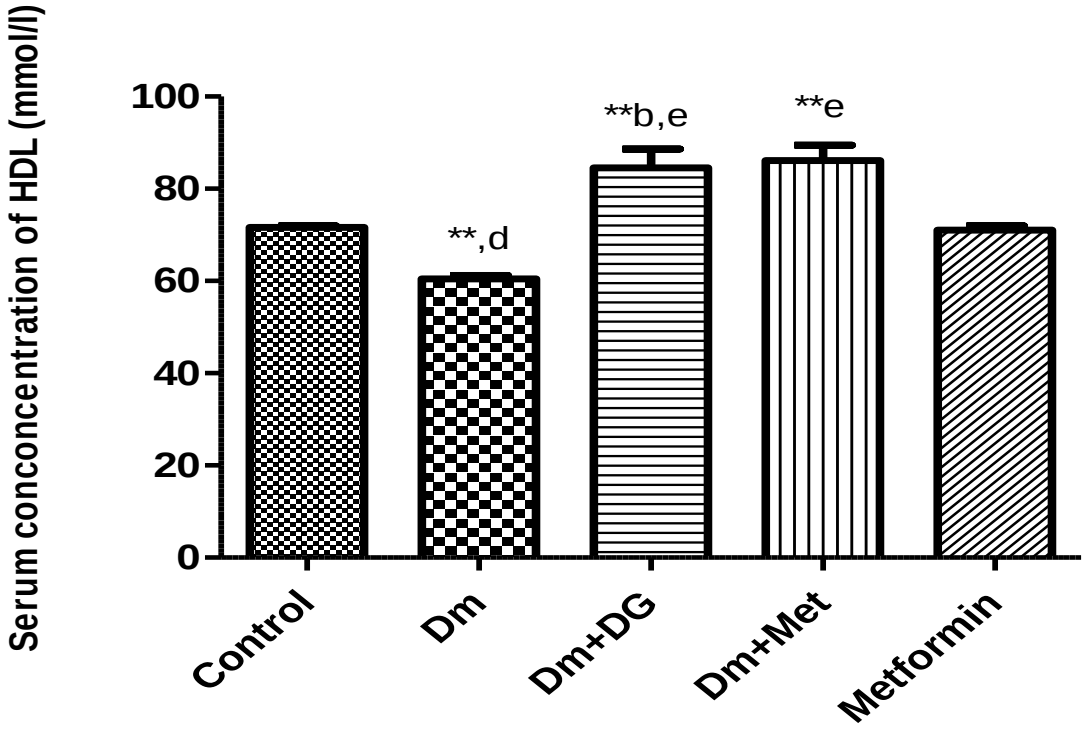


**Figure 3.** Showing effect of daily oral administration of aqueous *D. guineense* fruit pulp on high densitylipoprotein (HDL) concentration in n=5; **=p<0.01 vs Control; b=p<0.01 vs Dm; c=p<0.01 vs Dm+DG;d=p<0.01 vs Dm Vs Met; e=p<0.01 vs Metformin.

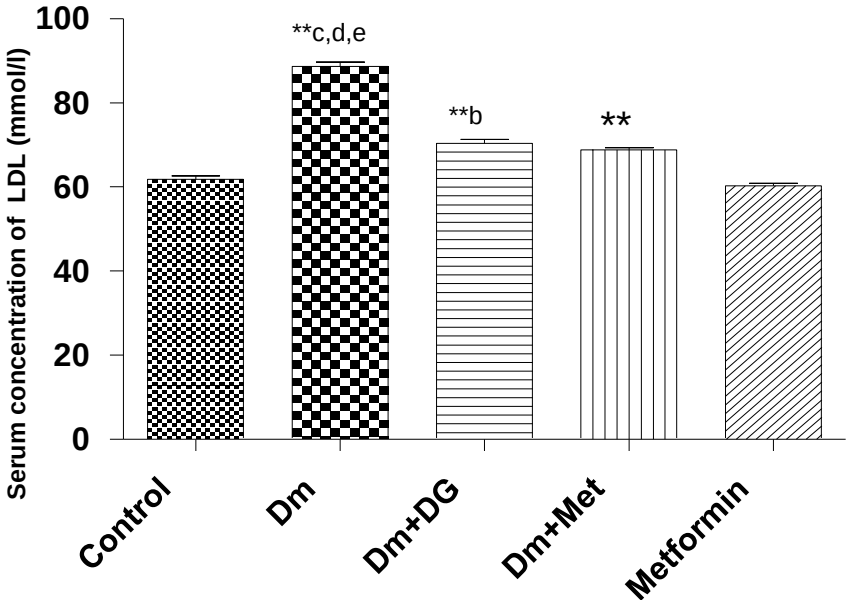


**Figure 4.** Showing effect of daily oral administration of aqueous *D. guineense* fruit pulp on high densitylipoprotein (HDL) concentration in n=5; **=p<0.01 vs Control; b=p<0.01 vs Dm; c=p<0.01 vs Dm+DG.

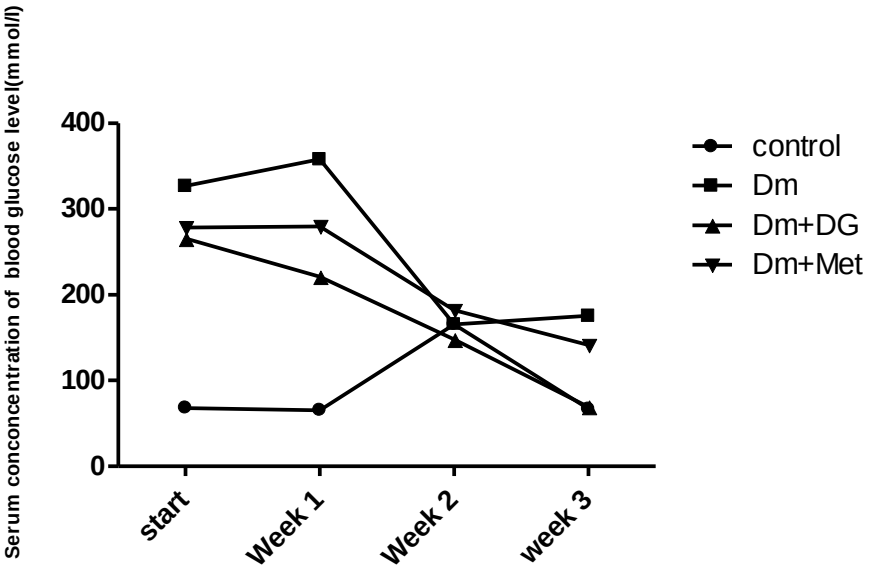


**Figure 5.** Showing effect of daily oral administration of aqueous *D. guineense* fruit pulp on blood glucose concentration in streptozotocin-induced diabetic rats. Values are expressed in Mean ± SEM n=5; **=p<0.01 vs Control.

and experimental groups. A value of p<0.05 was considered significant. All analysis was performed using the graph pad version 5 statistical software program.

## RESULTS

The results of this study are presented in Figures 1-9 and Plates 1 and 2.

## DISCUSSION

World over, research on ways to ameliorate developing cases of diabetes mellitus has been intensified. This diseases which is life threatening is said to be associated

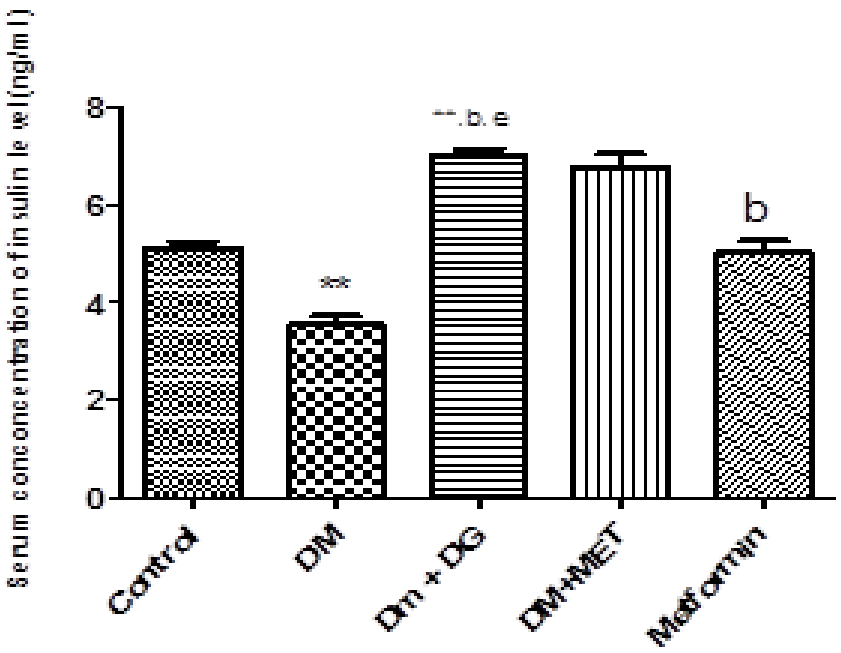


**Figure 6.** Showing effect of daily oral administration of aqueous *Dialium guineense* fruit pulp on insulin concentration in streptozotocin-induced diabetic rats. Values are expressed in Mean ± SEM n=5; **=p<0.01 vs Control; b=p< 0.01 vs Dm, e=p<0.01 vs Met.

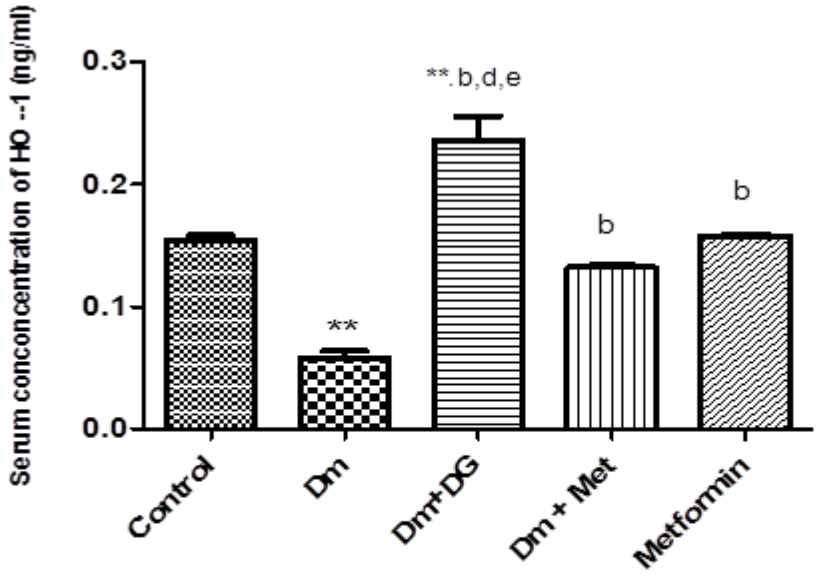


**Figure 7.** Showing effect of daily oral administration of aqueous *D. guineense* fruit pulp on insulin concentration in streptozotocin-induced diabetic rats. Values are expressed in Mean ± SEM n=5; **=p<0.01 vs Control; b=p< 0.01 vs Dm, d=p<0.01 vs Dm+ Met e=p<0.01 vs Met.

with increased oxidative stress, hypercholesterolemia and inflammatory activity with a resultant increase in high incidence of liver and kidney damage. The aim of this study therefore, was to investigate the effect of aqueous fruit pulp of *Dialium guineense* on hemoxygenase-1, insulin and angiotensin converting enzyme in

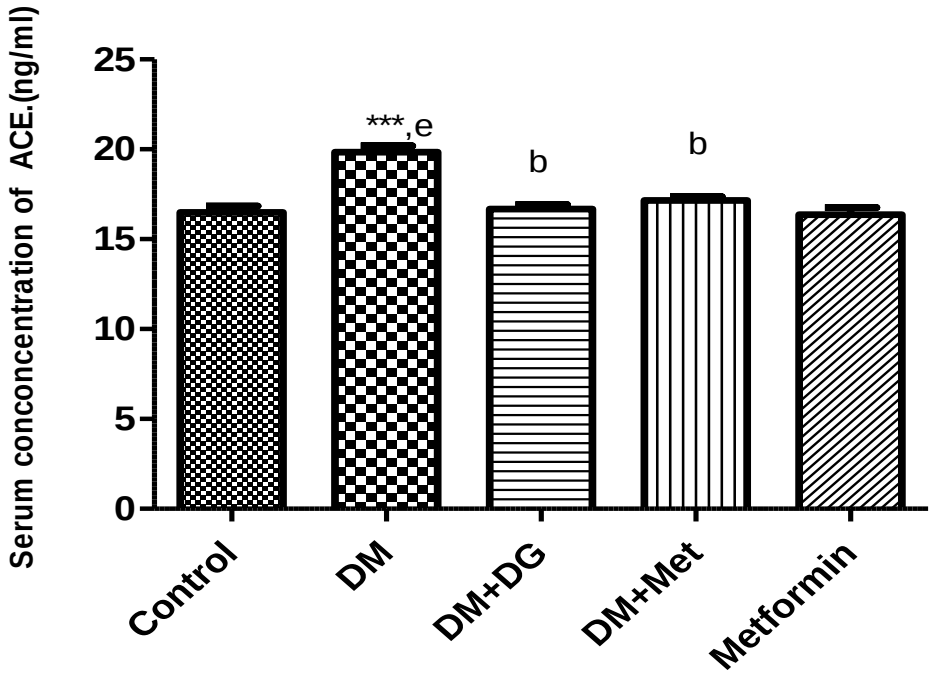


**Figure 8.** Showing effect of daily oral administration of aqueous *D. guineense* fruit pulp on insulin concentration in streptozotocin-induced diabetic rats.Values are expressed in Mean ± SEM n=5; **=p<0.01 vs Control;b=p< 0.01 vs Dm, e=p<0.01 vs Met.

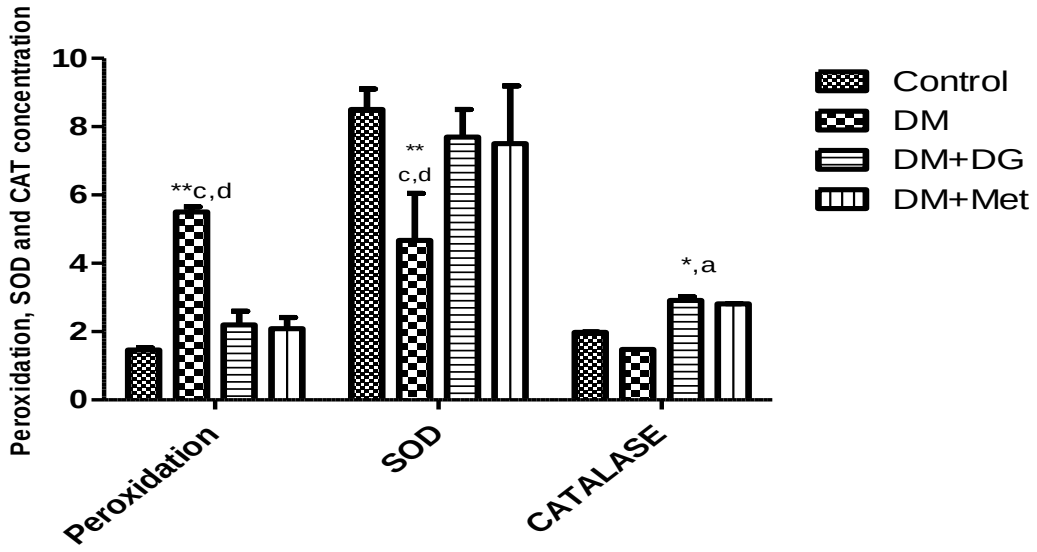


**Figure 9.** Showing effect of daily oral administration of aqueous *D. guineense* fruit pulp on lipid peroxidation, superoxide dismutase and catalase activity inthe Kidney in streptozotocin-induced diabetic rats. Values are expressed in Mean ± SEM n=5; **=p<0.01 vs Control;c=p< 0.01 vs Dm+DG, d=p<0.01 vsDM+ Met.

streptozotocin-induced diabetic rats, all of which are pointers to the etiology of cardiovascular and coronary heart disease. Streptozotocin used in this study has been reported to be specific in cytotoxicity, lipotoxicity, generation of hydroxyl free radicals, hyperglycemia and inflammation that may cause lipid peroxidation in

## KIDNEY HISTOLOGY

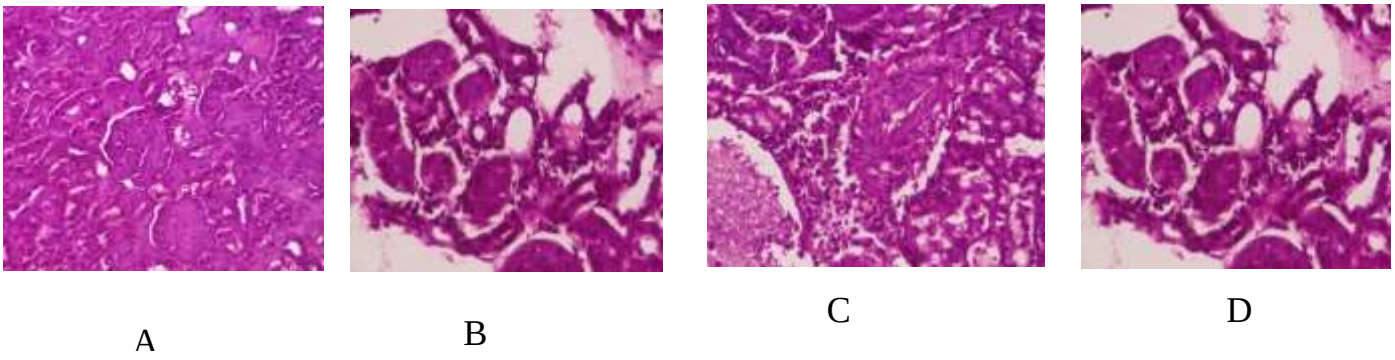


**Plate 1.** Photomicrographs of renal tissues in (A)**control** showing normal glomeruli, bowman capsules and tubules. No significant lesion seen. (B) **Diabetic untreated** showing moderate perivascular inflammation and peritubular inflammation (C) **Diabetic treated with Dialium guineense** showing show normal glomeruli, bowman capsule and tubules. No significant lesion seen.(D) **Diabetic treated with Metformin**: showing moderate perivascular inflammation and mild peritubular inflammation. H&E X400.

## HEART

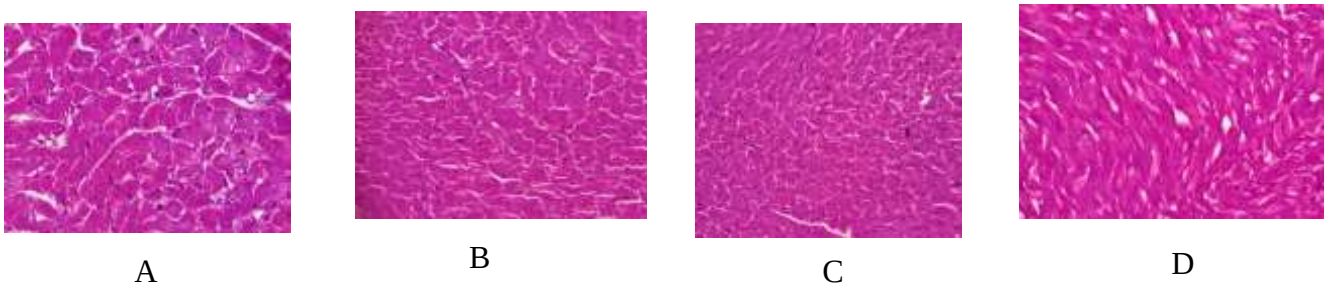


**Plate 2.** Photomicrographs of cardiac tissue showing no inflammation of myocardium and infiltration of adipocytes in all groups. H &E X400.

pancreatic β-cells resulting in decreased endogenous insulin release (Donath et al., 2009).

In our study, results obtained showed an appreciable decrease in the level of insulin and hemoxygenase-I (HO-1) but with a concomitant increase in blood glucose, TC, LDL-c and angiotensin converting enzyme (ACE) levels in the diabetic untreated group. Similar results have been reported by Erman et al. (1998) and Ustundag et al. *(*1998). Usually, with hyperglycemia, the pancreatic β-cells become easily destroyed by redox imbalance between free radical production and scavenging processes causing lipid peroxidation, β-cells dysfunction (Lenzen 2008b; Donath, 1999; Djordjevic et al., 2004) and decreased insulin secretion (Robertson et al., 2003).

Treatment of the diabetic group with aqueous fruit pulp of *D. guineese* and a standard antidiabetic drug, metformin, respectively, resulted in a significantly ($p<0.01$) decreased blood glucose level and ACE but interestingly, increased serum hemoxygenase-1 (HO-1) and insulin concentration. HO-I and its derivative like carbon monoxide, ferritin and biliverdin have been reported to be key antioxidant enzymes that prevent the development of diabetes by abating oxidative stress via suppression of macrophages and acting as an important component in antiatherogenic activity (Orozco *et al.,* 2007); they play a vital role in evoking pancreatic beta-cell insulin release and improve glucose metabolism thus reducing hyperglycemia (Ndisang et al., 2010; Mosen et al., 2005; Ndisang et al., 2014).

The mechanism by which *D. guineneese* exerts hypoglycemia and induces HO-I release may not have been unconnected with the reported presence of such components as flavonoid, vitamin C and tanins (Lever et al., 1979; Arogba et al., 2006). There are numerous natural HMOX1 inducers originating from plants, including polyphenols that exert positive effect on diabetic subjects (Bonifaz et al., 2009) Flavonoids is reported to contain quercetin and rutin both of which prevent oxidative stress by scavenging free radicals (Larocca et al., 1995., Cox et al., 2000). Previous studies have shown that quercetin and rutin particularly lower blood glucose level in rats (Vessal et al., 2003), preserve pancreatic

beta cell integrity (Coskun et al., 2005), increase insulin secretion and prevent liver injury (Kobori et al., 2009).

Tanins and phenolic compounds on the other hand, available in several plant derivatives including *D. guineense* are reported to be anti-ACE and act as specific inhibitors of the enzyme (Liu et al., 2006). ACE activity in both experimental animals and humans is usually characteristically high and is required for the conversion of inactive angiotensin-I(AT-I) to the potent and pro-oxidative angiotensin II(AT2). ACE is associated with increased superoxide production and impaired endothelium function that may lead to cardiovascular problems. Our study has shown that hyperglycemia predisposes to hypercholesterolemia and elevated triglyceride and LDL-cholesterol as earlier demonstrated by Arora et al. *(*2010). This factors are associated with enhanced AT receptor expression (Andraws and Brown, 2007; Abuissa et al., 2005) and are often remote cause of cardiovascular disease and atherosclerosis. The study of these risk factors is nevertheless imperative because they are intervening with the management of diabetes mellitus.

The registered decrease in ACE level due to oral administration of *Dialium* translates to a decrease in serum $AT_2$ level since ACE is needed to convert inactive $AT_1$ to the potent and active $AT_2$ whose effect is to cause systemic vasoconstriction and raise blood pressure. This decrease in ACE concentration is suggestive of a possible ACE inhibition by the fruit pulp. Studies have shown that randomized trials using angiotensin converting enzyme inhibitors (ACEI) and AT2 receptor blockers significantly decrease the risk of DM (Andraws and Brown, 2007; Abuissa et al., 2005), improve insulin sensitivity and glucose metabolism, and reduce plasma glucose in both experimental conditions and in humans with DM (Scheen, 2004).

Our results therefore strongly indicate that Dialium guineense fruit pulp contains agents that promote ACE inhibition. Indeed, some reports have shown that flavonoid, one of the components present in the fruit pulp of Dialium guineense presents an anti-atherogenic effect due to its inhibition of ACE *in vitro* (Loizzo et al., 2007). The reparative effect of the fruit pulp and maintenance of tissue integrity was further observed in the improved heart and kidney morphology. The antioxidant effect of Dialium guineense was further demonstrated by its ability to significantly ($p<0.01$) reduce lipid peroxidation in the liver and kidney by promoting superoxide dismutase and catalase enzyme activity. Many studies have shown that oxidative stress becomes apparent in diabetic subjects (Ceriello, 2000; Waggiallah and Alzohairy, 2011).

Consistent with this view, our data provide further evidence that there is presence of oxidative stress with an alteration in antioxidant enzyme activities and increased lipid peroxidation (MDA levels) in diabetic condition. The reduction in serum SOD activity is thought to be as a result of excessive autoxidation and progressive glycation of enzymatic proteins. The reversal effect of Dialium fruit pulp on this unpleasant activity makes it convincing that it holds to an extent promising therapeutic properties.

## Conclusion

The results of this study clearly demonstrated that *Dialium guineense* fruit pulp has a good antioxidant potential and decreases tissue lipid peroxidation, blood glucose level, induces hemeoxygenase and insulin release and acts as ACE inhibitor.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

## ACKNOWLEDGMENT

The authors of this research study wish to appreciate the technical assistance of Dr. Mrs Iya Eze of the Department of Medical Laboratory Science, University of Calabar and Dr. Sikirullai Olatunde Jeje Department of Physiology, Federal University of Technology, P.M.B 704, Akure, Nigeria.

## REFERENCES

Abuissa H, Jones PG, Marso SP, O'Keefe JH (2005). Angiotensin converting enzyme inhibitors or angiotensin receptor blockers for prevention of type 2 diabetes: Meta-analysis of randomized clinical trials. Journal of the American College of Cardiology 46(5):821-826.

Andraws R, Brown DL (2007). Effect of inhibition of the renin angiotensin system on development of type 2 diabetes mellitus (meta analysis of randomized trials). The American Journal of Cardiology 99(7):1006-1012.

Arogba SS, Ajoboro AA, Odukwe IJ (2006). A physic-chemical study of Nigeria velvet tarmarind (dialium guineense) fruit. Journal of the Science of Food and Agriculture 66:533-534.

Arora R, Vig AP, Arora S (2013). Lipid peroxidation: A possible marker for diabetes. Journal of Diabetes and Metabolism 11:1-6.

Bonifaz V, Shan Y, Lambrecht RW, Donohue SE, Moschenross D, Bonkovsky HL (2009). Effects of silymarin on hepatitis C virus and haem oxygenase-1 gene expression in human hepatoma cells. Liver International 29(3):366-373.

Ceriello A (1997). Acute hyperglycaemia and oxidative stress generation. Diabetic Medicine 14(S3):S45-S49.

Coskun O, Kanter M, Korkmaz A, Oter S (2005). Quercetin, a flavonoid antioxidant, prevents and protects streptozotocin-induced oxidative stress and β-cell damage in rat páncreas. Pharmacology Research 51(2):117-23.

Cox D, Whichelow MJ, Prevost TA (2000). Antioxidant effects of flavonoids. Public Health Nutrition 3:19-29.

David AA, Olaniyi AT, Mayowa AO, Olayinka AA, Anthony OI (2011). Anti-vibro and preliminary phytochemical characteristics of crude methanolic extracts of the leaves of *Dialium guineense* (Willd). Journal of Medicinal Plants Research 5(11):2398-2404.

Djordjevic A, Spasic S, Jovanovic-Galovic A (2004). Oxidative stress in diabetic pregnancy: SOD, CAT and GSH-Px activity and lipid peroxidation products. The Journal of Maternal-Fetal and Neonatal Medicine 16(6):367-372.

Donath MY, Boni-Schnetzler M, Ellingsgaard H, Ehses JA (2009). Islet

inflammation impairs the pancreatic B-celI in type 2 diabetes. Physiology 24(6):325-331.
Donath MY, Gross DJ, Cerasi E, Kaiser N (1999). Hyperglycemia-induced b-cell apoptosis in pancreatic islets of *Psammomys obesus* during development of diabetes. Diabetes 48(4):738-744**.**
Erman A, Van Dyk DJ, Chen-gal B, Iskera D, Giler S, Rosenfeld JB, Boner G (1993). Angiotensin converting enzyme activity in the serum, lung and kidney of diabetic rats. European Journal of Clinical Investigation 23(10):615-620.
Evans JL, Goldfine ID, Maddux BA, Grodsky GM (2003). Are oxidative Stress-activated signaling pathways mediators of insulin resistance and β-cell dysfunction. Diabetes 52(1):1-8.
Ezeja MI, Omeh YS, Ezeigbo II, Ekechukwu AJ (2011). Evaluation of the analgesic activity of the methanolic stem bark extract of *Dialium guineense* (Wild). Annals of Medical and Health Sciences Research 1(1):55-62.
Gideon I.O, Raphael A (2012). Phytochemical analysis and in vivo anti-diarrhoeal potentials of *Dialiumguineense*(wild)stem bark extract. Journal of Intercultural Ethnopharmacology 1(2):105-110.
Herzog F, Farah Z, Amado R (1994). Composition and consumption of gathered fruits in the V-Babule, Cote D'Ivoire. Ecology of Food and Nutrition 32(3-4):181-196.
Jakus V (2000). The role of free radicals, oxidative stress and -*+antioxidant systems in diabetic vascular disease. Bratislavske lekarske listy 101(10):541-551.
Jean CD, Maryse T, Marie JF (1983). Plasma malondialdehyde levels during myocardial infarction. Clinica Chimica Acta 129(3):319-322.
Kang DG, Kim YC, Sohn EJ, Lee YM, Lee AS, Yin MH, Lee HS (2003). Hypotensive effect of butein via inhibition of angiotensin converting enzyme. Biological and Pharmaceutical Bulletin 26(9):1345-1347.
Kobori M, Masumoto S, Akimoto Y, and Takahashi Y (2009). Dietary quercetin alleviates diabetic symptomsand reduces streptozotocininduced disturbance of hepatic gene expression in mice. Molecular Nutrition and Food Research 53(7):859-868.
Larocca LM, Teofili L, Sica S, Pierelli L, Menichella G (1995). Quercetin inhibits the growth of leukemic progenitors and induces the expression of transforming growth factor-B1 in these cells. Blood 85(12):3654-3661
Lawal IO, Nzokwe NE, Igboanugo ABI, Adio AF, Awosan EA, Nwogwugwu JO, Faleye B, Olatunji BP, Adesoga AA (2010). Ethnomedicinal information on collation and identification of some medicinal plants in research institutes of South-West Nigeria. African Journal of Pharmacy and Pharmacology 4(1):001-007.
Lenzen S (2008b).Oxidative stress:vulnerable beta cells. Biochemical society Transactions 36(3):343-347
Lever M, Vandon-berghe DA, Merilend F, Vlictinck A, Lammense E (1979). Screening of higher plants biological activity/antimicrobial activity. Plants Medica 36:311-321.
Liu JC, Hsu FL, Tsai JC, Chan P, Liu JY, Thomas GN, Tomlinson B, Lo MY, Lin JY (2003). Anti hypertensive effect of tannin isolated from traditional Chinese herbs as non-specific inhibitors of angiotensin converting enzyme. Life Sciences 73(12):1543-1555.
Liu J, Pang Y, Chang T, Bounelis P, Chatham JC, Marchase RB (2006). Increased hexosamine biosynthesis and protein O-GlcNAc levels associated with myocardial protection against calcium paradox and ischemia. Journal of Molecular and Cellular Cardiology 40(2):303-312.
Loizzo MR, Said A, Tundis R, Rashed K, Statti GA, Hufner A, and Menichini F (2007). Inhibition of angiotensin converting enzyme (ACE) by flavonoids isolated from *Ailanthus excelsa* (Roxb) (Simaroubaceae). Phytotherapy research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives 21(1):32-36.
Mishra HP, Fridovich I (1972). The role of superoxide anion in the auto-oxidation of epinephrine and simple assay for superoxide dismutase. Journal of Biological chemistry 247(10):3170-3175.
Mosen H, salehi A, Ail P (2005). Defective glucose-stimulated insulin release in diabetic Goto-Kakizaka (rat coincides with reduced activity of the isletscarbon monoxide signaling pathway. Endocrinology 146(3):1553-1558.
Nahar N, Rahman S, Mosiihuzzaman M (1990). Analysis of carbohydrates in seven edible fruit of Bengladesh. Journal of Science of Food and Agriculture 51:185-192.
Ndisang JF, Jadhav A (2010). The heme oxygenase system attenuates pancreatic lesions and improves insulin sensitivity and glucose metabolism in deoxycorticosterone acetate hypertension. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology 298(1):R211-R223.
Ndisang JF, Tiwari S (2014). The heme oxygenase system and type-1 diabetes. Current Pharmaceutical Design 20(9):1328-1337.
Orozco JD, Kapturczak MU, Banajas B, Wang X, Weinstein MM, Wong J, Dehane J. Bolissetty S, Shaposhnik Z, Shih DM, Agarwal A, Lusis AJ, Araujo JA (2007). Hemeoxygenase -1 expression in macrophages plays a beneficial role in atherosclerosis. Circulation Research 100(12).
Orwa C, Mutua A, Kindt R, Jamnadass R, Anthony S (2009). Agro forest tree database:atree reference and selection guide version 4.0. World Agroforestry Centre ICRAF, Nairobi, KE (http:www.worldagroforestry.org/sites/treedatabases.asp).
Rang HP, Dale MM, Ritters JM (1991). The endocrine pancrease and the control of blood glucose. Pharmacology 4 p.
Robertson RP, Harmon J, Tran PO, Tanaka Y, Takahashi H (2003). Glucose toxicity in beta cells: type 2 diabetes, good radicals gone bad, and the glutathione connection. Diabetes 52(3):581-587.
Scheen AJ (2004). Orevention of type 2 diabetes mellitus through inhibition of the renin-angiotensin system. Drugs 64(22):2537-2565.
Sinha KA (1972). Colorimetric assay of catalase. Analytical biochemistry 47(2):389-394.
Strate T O, Mann H, Kleighans S, Rusani C, Schneider E, Yekebas MS, Freitag T, Bloechle C, Izbicki JR (2005). Micro circulatory function and tissue damage is improved after the therapeutic injection of bovine hemoglobin in server acute rodent pancreatitis. Pancreas 30(3):254-259.
Ullah MF, Bhat SH, Abu-Duhier F (2015). Antidiabetic potential of hydo-alcoholic extract of moringapergrina leaves; implication as functional food for prophylactic intervention in prediabetic stage. Journal of Food Biochemistry 39(4):360-367.
Üstundag B, Çay M, Özercan İH (1998). Angiotensin-converting enzyme activity in the serum, lung, liver and kidney in streptozotocin-induced diabetic rats and diabetic nephropathy. Turkish Journal of Medical Sciences 28(3):231-238.
Vessal M, Hemmati M, Vasei M (2003). Antidiabetic effects of quercetin in streptozocin induced diabetic rats. Antidiabetic effects of quercetin in streptozocin induced diabetic rats. Comparative Biochemistry and Physiology Part C.
Vinayagamoorthi R, Bobby Z, Sridhar MG (2008). Antioxidants preserve redox balance and inhibit c-Jun-N-terminal kinase pathway while improving insulin signaling in fat-fed rats: evidence for the role of oxidative stress on IRS-1 serine phosphorylation and insulin resistance. Journal of endocrinology 197(2):287-296.
Waggiallah H, Alzohairy M (2011). The effect of oxidative stress on human red cells glutathione peroxidase, glutathione reductase level, and prevalence of anemia among diabetics. North American Journal of Medical Sciences 3(7):344.