

EVALUATION OF ANTI-DIABETIC AND ANTI-LIPIDAEMIC POTENTIAL OF AQUEOUS LEAF EXTRACT OF *MORINGA OLEIFERA* ON STREPTOZOTOCIN DIABETIC-INDUCED ALBINO RATS

¹Omeodu, S. I., *¹Onyegeme-Okerenta, B. M., Nnake, N. B.

¹Department of Biochemistry, University of Port Harcourt, PMB 5323
Port Harcourt, NIGERIA

*Corresponding author E-mail: blessing.onyegeme-okereanta@uniport.edu.ng

ABSTRACT

The present study was designed to evaluate the anti-diabetic and anti-lipidaemic effect of aqueous leaf extract of *Moringa oleifera* compared with daonil (standard drug). Forty (40) albino rats weighing of 200-340g were used and arranged into 5 groups comprising eight (8) rats in each group. Pilot study was carried out to ascertain that streptozotocin at 60mg/kg caused diabetes in the experimental animals. The first group served as the normal control while the remaining groups were induced with diabetes using streptozotocin at 60mg/kg body weight. Group two received only (STZ) and served as the diabetic control and the remaining group were treated using daonil and aqueous *Moringa oleifera* extract. After 5 weeks of treatment, aspartate amino transferase [AST], alanine amino transferase [ALT], alkaline phosphatase [ALP], gamma glutamyl transferase [GGT], Cholesterol [CHOL], Triacylglycerol [TG], Low-density lipoproteins [LDL] and High-density lipoproteins [HDL] and Glucose level were assayed. It was observed that there was a significant ($p < 0.05$) reduction in the concentration of AST, ALT, ALP, CHOL, TG, LDL of the various doses of aqueous *Moringa oleifera* extract when compared with the streptozotocin group (diabetic group) but no significant difference when daonil treated group was compared with diabetic group. Results obtained shows that there was significant difference when compared with daonil treated group only in the concentration of blood glucose and total protein. The study concluded that aqueous extract of *M. oleifera* produced a significant anti-diabetic (hypoglycaemic) and anti-lipidaemic (hypolipidaemic) effect in diabetic rats, impaired liver and impaired pancreas organ/function than daonil (standard drug).

Keywords: *Moringa oleifera*, Daonil, anti-diabetic, anti-lipidaemic, streptozotocin.

INTRODUCTION

Diabetes mellitus is a major and growing public health problem throughout the world, with an estimated worldwide prevalence in 2008 of more than 347 million people and is a heterogeneous disorder with varying prevalence among different ethnic group. This disease is particularly characterized by the excessive accumulation of free glucose in blood resulting from defect in insulin secretion, insulin action or both. This is likely to increase the risk for developing various metabolic disorders including hyperglycaemia which is the major cause of diabetic complication such as retinopathy, nephropathy and neuropathy (Lefebvre 2005; Cade 2008; American Diabetes Association 2012; Paneni *et al.*, 2013). It also increase the risk of hyperlipidaemia, liver – kidney dysfunction and hypertension. Evaluation of mortality pattern by race showed that blacks had a higher mortality rate than whites (Fenelon 2013). Modern drugs including insulin and other biochemical hypoglycaemic agent e.g. tolbutamide, phenformin, troglitazone, rosiglitazone and repaglinide control the blood

glucose level only when they are regularly administered, but these treatment are tedious and have several undesirable side effects and fail to significantly combat the course of diabetic complications (Rang and Dale 1991; Upadhyay *et al.*, 1996). In the last few years there has been an exponential growth in the field of herbal medicines and these drugs are gaining popularity both in developing and developed countries because of their natural origin and less side effect (Prasanna and Ravi, 2013)

Moringa tree leaves (*Moringa oleifera*) is the cultivated specie of the genus Moringa of the family Moringaceae and is a remarkably nutritious vegetable with several antioxidant properties (Paliwal *et al.*, 2011; Bhargave *et al.*, 2015). Several health benefits were reported as a result of supplementation with moringa leaves or their extract or seed. *Moringa oleifera* is described as the miracle tree, tree of life and God's gift to man.

The antioxidant and antidiabetic activity of aqueous extract of moringa leaves indicated potential benefits as a potent antibacterial in streptozotocin induced diabetic albino rats (Jaiswal *et al.*, 2009). It was also a good scavenger for nitric oxide radical and has a potential source of natural antioxidant. Moringa leaves contains chlorogenic and isothiocyanates used in treatment of hypercholesterolemia and hyperglycemia and also as a nutritional supplementation (Lakshmipriya *et al.*, 2016). The study evaluates the anti-diabetic and anti-lipidaemic potential of aqueous leaf extract of moringa as compared with daonil drug tablet (antidiabetic standard drug).

MATERIALS AND METHODS

Chemicals/Reagents

The Reagents used for liver enzyme assays were products of Radox Commercial Kits (United Kingdom). Formalin was used for preservation of the dissected liver.

Experimental Animals

The total number of animals used for this experiment was forty (40) wister albino rats. They were purchased and housed in the pharmacology department animal house at Ofrima, Abuja Park of the University of Port Harcourt, Choba Rivers State. They were left for 2 weeks to acclimatize to the laboratory condition during which they were fed with normal feed (Top Feeds grower's mash) and clean water. They average weight of the rats was 200g and they were marked for easy identification, Three (3) were used for pilot studies to ascertain that the rat could be diabetic by streptozotocin treatment at the dose level used (60mg/kg).

Preparation of *Moringa Oleifera* extract

The leaves of *M. oleifera* were collected from the Botanical garden of the University of Port Harcourt, washed and shade dried at room temperature, after which the leaf powder was prepared using home blender. Powdered *M. oleifera* leaves weighing 50g was soaked in 500ml of distilled H₂O for 24hrs after which it was sieved using a white clean handkerchief and afterwards filtered through a whatmann filter paper No. 1. Then the two concentration (10% and 15%) was prepared. for 10% concentration 10ml of stock was dissolved in 90ml of distilled H₂O then for 15% concentration 15ml of stock was dissolved in 85ml of distilled H₂O. That implies moringa extract at dose of 100mg/kg body weight and 150mg/kg.

Administration of Streptozotocin

Streptozotocin weighing 5.0g was dissolved in 100ml of citrate buffer (0.1M citric buffer standardized with PH meter 4.5 from which a single dose of 60mg/kg body weight of freshly prepared streptozotocin was induced intravenously (single Tail vein infection). The use of appropriate doses of streptozotocin, according to the body weight of the animals, allowed acute or mild diabetes to be established in experimental animals. Diabetes was confirmed by ascertaining the glucose concentration in the blood of the rats 3 – 4days following streptozotocin injection and was found to have increased two to three times above the normal value.

Experimental Design

The acclimatized wistar rats were sorted according to their weight into 5 groups. Group 1 served as the negative control. The animals in this group are non-diabetic and were given distilled water and normal feed throughout the course of this study. Group 2 served as the positive control (Streptozotocin diabetic- induced).The animals in this group were intravenously injected with freshly prepared streptozotocin (60mg/kg body weight) in a 0.1M citric buffer (PH 4.5) not treated with any drug or extract. Group 3 were diabetic rats treated with 0.14mg/kg body weight of daonil. Group 4 were diabetic rats treated with 10% (100mg/kg body weight) of moringa extract. Group 5 were diabetic rats treated with 15% (150mg/kg body weight) of moringa extract. After five days of administration of streptozotocin their blood glucose levels were higher than 200mg/dl which is considered as being diabetic in the fasting state.

Method of Blood and Organ Collection Collection and Analysis of Sample

Blood samples used to check for glucose level were collected from the tip of the tail of the rats. The animals to be sacrificed were first anaesthetized with chloroform (inhalational anaesthesia) followed by cervical dislocation. Each animal was then placed on a dissecting slab and then cut along the thorax down the abdominal region; blood was collected from the jugular vein and dispensed into the Heparin bottle for biochemical assays (ALT, AST, ALP, and GGT). The plasma activities of all the liver enzymes were assayed using Randox kit (Randox laboratories limited, BT 294QY, United Kingdom) and absorbance of test results were read on a double-beam spectrophotometer. Freshly dissected pancreas of 10% moringa extract treated rats and positive control (streptozotocin induced diabetic) rats were cut off with surgical blades and placed in a sample bottle containing 10% formalin for histological examination. The tissues were subjected to standard routine histological procedures as described by Kiernan [2008]. The slides were viewed using the light microscope and histopathological changes and observations were recorded at X400 magnification identifying both the normal and the degenerated pancreatic cells.

Statistical Analysis

The results of the study were reported as mean \pm standard error of mean (SEM) of triplicate determinations. Data were analysed using one way analysis of variance (ANOVA) to test for differences between treatment groups and differences were considered significant at $p < 0.05$ that is, at 95% confidence level.

Result

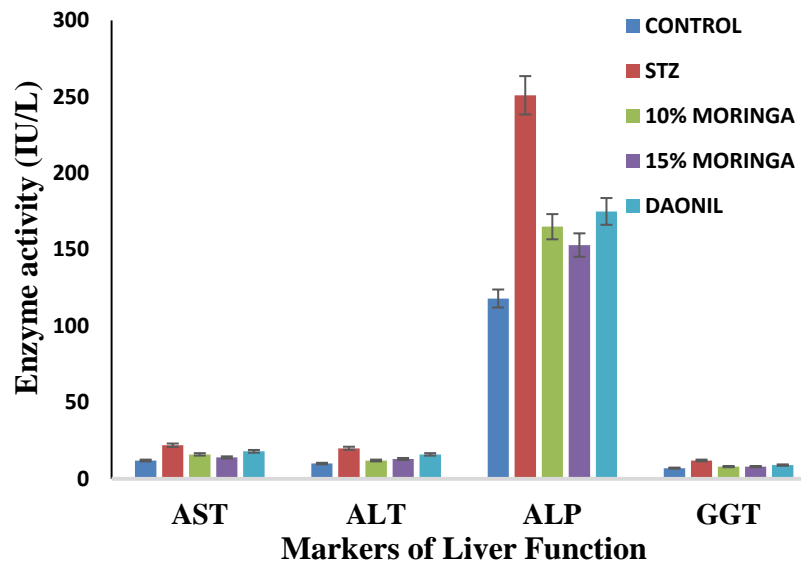


Figure 1: Effect of aqueous leaf extracts of *M. Oleifera* on markers of liver function of streptozotocin – Induced Diabetic albino rats

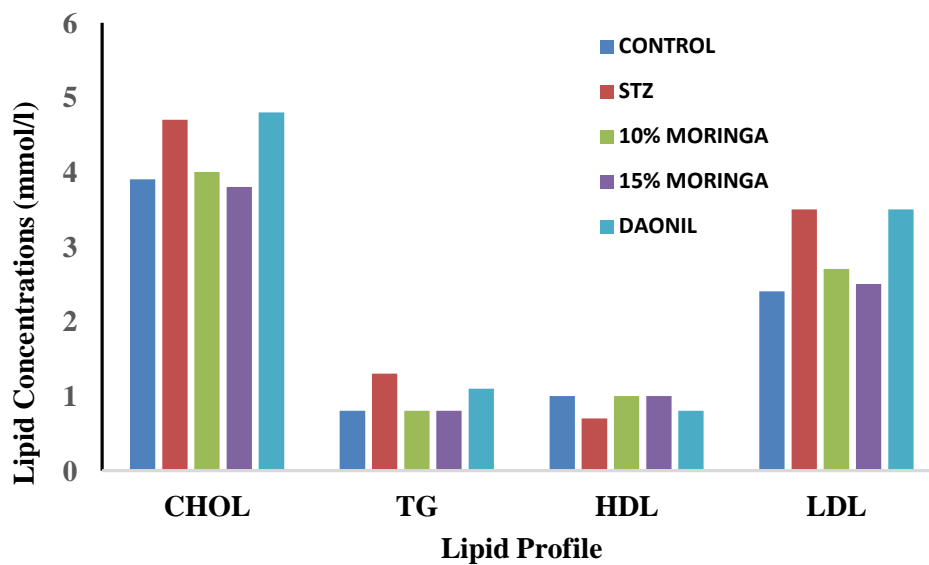


Figure 2: Effect of aqueous leaf extracts of *M. Oleifera* on Lipid Profile of streptozotocin – Induced Diabetic albino rats

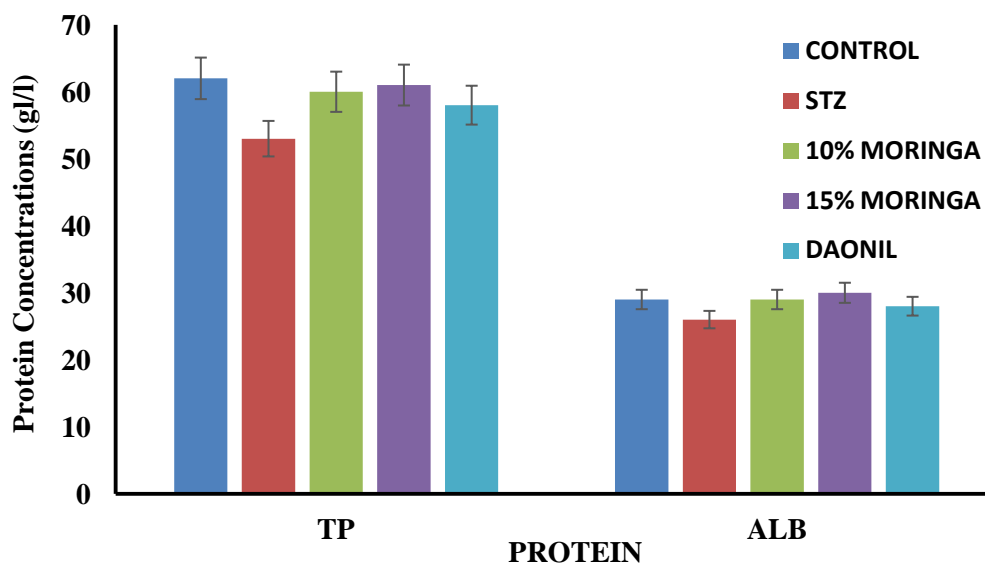


Figure 3: Effect of aqueous leaf extracts of *M. Oleifera* on Total Protein and Albumin of streptozotocin – Induced Diabetic albino rats

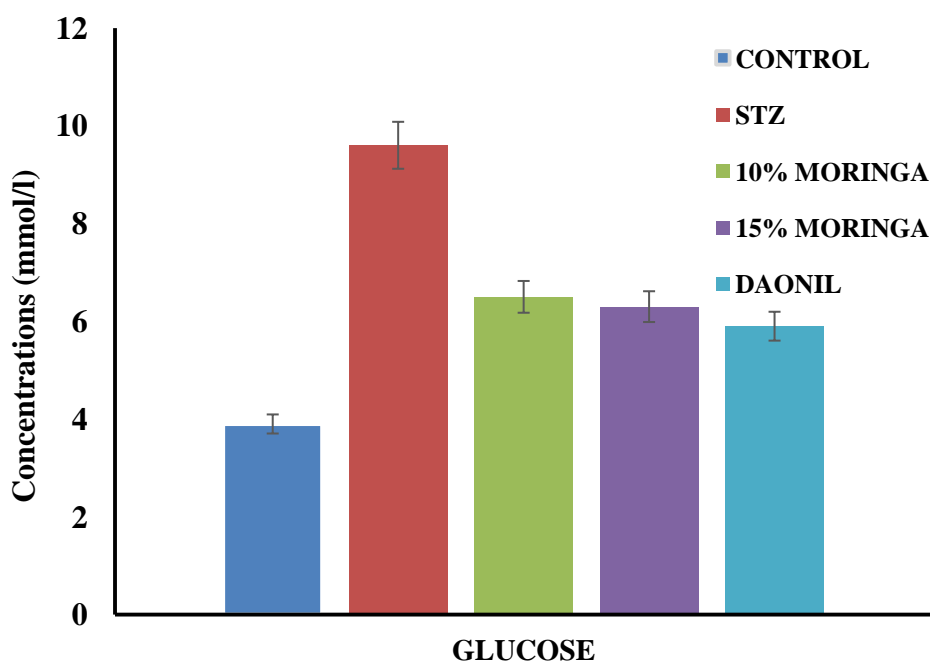
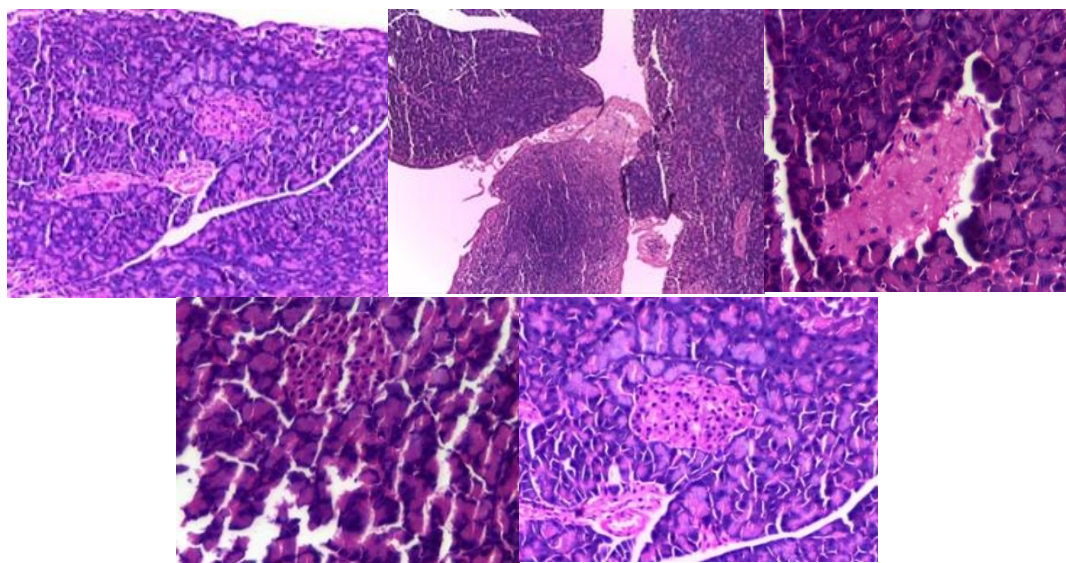


Figure 4: Effect of aqueous leaf extracts of *M. Oleifera* on Glucose level of streptozotocin – Induced Diabetic albino rats

Histological analysis was carried out on the pancreas of experimental animals. Plate 1A- E showed the findings of the analysis observed in the various group



- Plate 1: Photomicrograph of the pancreas of various groups. A: Control group showing normal pancreatic islet; and acini with numerous cell
B: STZ-induced diabetic control showing cytolysis of islet cells leading to decrease in cell mass.
C: Group treated with Daonil drug showing infiltration of pancreas by mononuclear inflammatory cells forming lymphoid follicles consistent with pancreatitis.
D: Group treated with 10% aqueous leaf extract of *M. oleifera*; showing recovering pancreatic islet cells and normal pancreatic acini.
E: Group treated with 15% aqueous leaf extract of *M. oleifera*; showing normal pancreatic Islet and acini.

DISCUSSION AND CONCLUSION

The present study assessed the effect of treatment with aqueous leaf extract of *M. oleifera* on the glucose level, liver enzymes, lipid profiles, Total protein and albumin in STZ-induced diabetic rats when compared with standard drug (daonil- a synthetic hypoglycemic agent). From the glucose result, it was observed that there was a significant ($p < 0.05$) increase of glucose level in the untreated diabetic animals but significantly decreased when compared with Daonil treated animals but most effective and efficient in the animal treated with individual doses of moringa extract. The result obtained was in consonance with that of Ndong *et al.*, (2007) that moringa leaves significantly decreased blood glucose concentration in wister rats and Goto – Kakizaki (GK) rats, modelled type 2 diabetes. Another study indicated that the extract of moringa leaves is effective in lowering blood sugar levels within 3 hours after ingestion (Mittal *et al.*, 2007). As a mechanistic model for antidiabetic activity of moringa, it has been indicated that dark chocolate polyphenols (Grassi *et al.*, 2005) and other polyphenols (AL – Awwadi *et al.*, 2004; Moharram *et al.*, 2003) are responsible for its hypoglycemic activity.

Another study confirmed that *M. oleifera* leaves contain many powerful antioxidant phytochemicals, especially quercetin and kaempferol. Quercetin, a strong antioxidant flavonoid revealed a protective effective against streptozotocin induced diabetes in rats by

intraperitoneal injection of quercetin 15mg/kg body weight for 3 days prior to streptozotocin administration (Coskun *et al.*, 2005) and protected an insulin secreting cell line (INS-I) against oxidative damage (Youl *et al.*, 2010). It also exhibited hypoglycemic properties in diabetic rats (Shetty *et al.*, 2004).

Thus, dried leaves or aqueous extract of *M. oleifera* were more effective compared to the reference drug and this may be because leaves might have some direct effect by increasing the tissue utilization of glucose (Gray *et al.*, 2000) by inhibiting hepatic gluconeogenesis or absorption of glucose into the muscles and adipose tissue.

There was a significant increase in the activity of all liver enzymes (ALT, AST and ALP) of untreated STZ- induced diabetic rats when compared with that of other groups which showed a significant decrease in the level of liver enzyme. After administration of extract, it was interesting to note that there was a significant ($p < 0.05$) reduction in the concentration values of AST, ALP and ALT of the treated diabetic rats with 10% and 15% aqueous extract of *M. oleifera* when compared with untreated STZ-induced diabetic rats but there was no significant difference in the liver enzymes concentration when untreated STZ induced diabetic rats were compared with standard drug (daonil). Hence, aqueous extract of *M. oleifera* was more effective on liver functions enzymes than reference drugs. This observation agrees with the findings of Rej (1989) in which liver enzymes were used as markers for liver damage. Aminotransferases (ALT and AST) are produced in the liver and are good markers for damage to liver cells but not necessarily the severity of the damage (Rej 1989). They are normally present at low levels in the blood so if the liver cells are damaged it would be expected that some of the enzymes leak into the blood and increase in level.

Elevated alanine transaminase and aspartate transaminase have traditionally been considered a “hepatocellular” pattern concerning for ischemic, viral, or toxic hepatitis. Elevations in these levels pose a diagnostic dilemma in patients without a clinical picture consistent with liver disease. On the other hand, elevated alkaline phosphatase historically represents a “cholestatic” pattern concerning for gallbladder and biliary tract disease (Tetangco *et al.*, 2016). Findings from this research may indicate damage to the liver cells of STZ-induced diabetic rats since there is a significant difference between the concentration of ALT, AST, and ALP when the untreated diabetic control group was compared with the other experimental groups. *M. oleifera* leaf has a repairing effect on the liver due to their nutritional properties such as the presence of essential amino acid like methionine and cysteine (Oliveira *et al.*, 1999) and thus boosting the total proteins and albumin level (Ekam *et al.*, 2012).

There was significant reduction ($p < 0.05$) in cholesterol, triglyceride, LDL – Cholesterol and significant increase ($p < 0.05$) in HDL-Cholesterol levels when treated diabetic rats with varied doses of aqueous extract of *M. oleifera* were compared with untreated STZ-induced albino rats but there was no significant difference ($p > 0.05$) when daonil treated diabetic rats were compared with STZ-induced diabetic albino rats. HDL – C increase is a desirable event, since it indicates a possible role in reducing the incidence of atherosclerosis. This showed that the extract of moringa has hypolipidaemic properties and therefore can serve as a very good therapeutic agent in control of atherosclerosis and other lipid metabolism disease. Phenolic compounds inhibit the formation of cholesterol micelles (Vermeer *et al.*, 2008). Thus consumption of this plant may help in the management of hyperglycaemia and hyperlipidemia.

Albumin is used as an indicator of liver impairment, reduced absorption of protein loss (Sacher and Mcpherson, 2000). Albumin apart from being a useful indicator of the integrity of glomerular membrane is also important in determining the severity of disease (Adedapo *et al.*, 2005). Decreased albumin may be due to primarily reduction in synthesis by the liver and secondarily due to reduced protein intake which further confirms hepatic damage (Luskova *et al.*, 2002). Therefore, it is interesting to note that aqueous leave extract of *M. oleifera* significantly increased the albumin level of diabetic animals when compared with untreated STZ-induced albino rat. But there was no significant difference in daonil treated diabetic rats. Similarly, there was a significant increase in the total protein level of all the treated experimental groups but it is interesting to note that aqueous leave extract of moringa is more effective and efficient in increasing protein level when compared with standard drug (daonil). Photomicrographs of pancreatic cells showed that moringa extract at 10% (100mg/kg body weight) and 15% (150mg/kg body weight) may be effective in its capacity to restore pancreatic cells that were hitherto damaged by streptozotocin. This findings agrees with Onyegeme-Okerenta and Essien (2015) which showed that aqueous leaf extract of *Millittia aboensis* has anti-diabetic and anti-lipidemic properties and can be used to treat diabetes induced by alloxan.

Aqueous leave extract of *M. oleifera* had favourable effect in bringing down the severity of hyperglycaemia and hyperlipidaemia observed in diabetic rats. The extract might have a mixture of biomolecules with hydroxyl groups that prevent the abstraction of hydrogen atom from the double bond of lipid bilayer thereby preventing the damage of lipid membrane. This was not exactly the same with untreated animals which showed pancreatic cytolysis of islet cells leading to decrease cell mass as ailments progresses.

REFERENCES

- Adedapo, A.A., Adegbayibi, A.Y and Emikpe B.O. (2005). some clinicopathological changes associated with the aqueous extract of the leaves of the phyllanthus amarus in rats. *Phytotherapy Research*, 19:971-976.
- Al-Awwadi, N., Azay, J., Poucheret, P., Cassanas, G., Krosniak, M., Auger, G., Gasc, F., Rouanet, G. C. and Teissedre, P. L. (2004). Antidiabetic activity of red wine polyphenolic extract, ethanol, or both in streptozotocin treated rats. *Journal of Agricultural Food Chemistry*. 52: 1008-1016.
- American Diabetes Association (2012). "Diagnosis and classification of diabetes mellitus," *Diabetes Care*. 35(1): S64–S71.
- Bhargave, A., Pandey, I., Nama, K.S. and Pandey, M. (2015). Moringa oleifera Lam. – Sanjana (Horseradish Tree) – A Miracle food plant with multipurpose uses in Rajasthan-India-An overview. *International Journal of Pure and Applied Bioscience*. 3(6): 237-248
- Cade, W. T. (2008). Diabetes-related microvascular and macrovascular diseases in the physical therapy setting. *Physical Therapy*. 88(11): 1322–1335,
- Coskun, O., Kanter, M., Kormaz, A. and Oter, S. (2005). Quercetin, a flavonoid antioxidant, prevents and protects streptozotocin induced oxidative stress and beta cell damage in rat pancreas. *Pharmacological Research*, 51 (2): 117 -123.
- Ekam, V. S., Johnson, J. T., Dasofunjo, K., Odey, M. O, Anyahara, S. E. (2012). Total protein, albumin and globulin levels following the administration of activity directed fractions of Vernonia amygalina during acetaminophen induced hepatotoxicity in wistar rats. *Annals of Biological Research*. 3(12): 5590-5594.
- Eula Plana Tetangco, Natasha Shah, Hafiz Muhammad Sharjeel Arshad, and Hareth

- Raddawi. (2016). Markedly Elevated Liver Enzymes in Cholelithiasis in the absence of Hepatocellular Disease: Case Series and Literature Review. *Journal of Investigative Medicine*. 4(2):1-2.
- Fenelon Andrew (2013). An examination of black/white differences in the rate of age-related mortality increase. *Demographic Research*. 29(17): 441-472.
- Grassi, D., Lippi, C., Necozione, S., Desideri, G., Ferri, C. (2005). Short-term administration of dark chocolate is followed by a significant increase in insulin sensitivity and a decrease in blood pressure in healthy persons. *The American Journal of Clinical Nutrition*. 81:611-614.
- Gray, A. M., Abdel-Wahab, Y. H., Flatt, P. R. (2000). The traditional plant treatment, *Sabucus nigra* (Elder) exhibits insulin like and insulin releasing actions in vitro. *Journal of Nutrition*. 130:15–20.
- Jaiswal, D., Kumar, R. P., Kumar, A. V., Mehta, S., Watal, G. (2009). Effect of *Moringa oleifera* Lam. leaves aqueous extract therapy on hyperglycemic rats. *Journal of Ethnopharmacology*. 123:392-396.
- Kiernan, J. A. (2008). *Histological and Histochemical Methods: Theory and Practice*. 4th Edn, Scion, Bloxham, UK.
- Lakshmipriya Gopalakrishnan, Kruthi Doriya and Devarai Santhosh Kumar (2016). *Moringa oleifera*: A review on nutritive importance and its medicinal application. *Food Science and Human Wellness*. 5(2): 49–56
- Lefebvre, P. (2005). Diabetes yesterday, today and tomorrow. The action of the International Diabetes Federation. *Revue Medicale de Liege*. 60:273–277.
- Luskova, V., Svoboda, M. and Kolarova, J. (2002). The effect of Diazinon on blood plasma biochemistry in carp (*Cyprinus carpio* L). *Acta Veterinaria Brunensis* 71: 117-123.
- Mittal, M., Mittal, P. and Agarwal, A. C. (2007). Pharmacognostical and phytochemical investigation of antidiabetic activity of *Moringa oleifera* lam leaf. *Indian Journal of Pharmacology*. 6:70-72.
- Moharram, F. A., Marzouk, M. S., El-Toumy, S. A., Ahmed, A. A. and Aboutabl, E. A. (2003). Polyphenols of *Melaleuca quinquenervia* leaves pharmacological studies of grandinin. *Phytotherapy Research*. 17:767-773.
- Ndong, M., Uehara, M., Katsumata, S. and Suzuki, K. (2007). Effects of oral administration of *Moringa oleifera* Lam on glucose tolerance in Gotokakizaki and wistar rats. *Journal of Clinical Biochemical Nutrition*. 40: 229-233.
- Oliveira, J.T.A, Silveira, S.B., Vasconcelos, I.M., Cavada, B.S, and Moreira, R.A. (1999). Compositional and nutritional attributes of seeds from the multiple purpose tree *Moringa oleifera* Lamarck. *Journal of the Science of Food and Agriculture*. 79: 815-820.
- Onyegeme-Okerenta B. M. and Essien Eka Basse. (2015). Evaluation of anti-diabetic and anti-lipidemic activities of aqueous leaf extract of *Millettia aboensis* and its effect on pancreatic histology of alloxan-induced diabetic rats. *Advances in Biochemistry*, 3(2): 24-29.
- Paliwal, Ritu, Sharma, Veena, Pracheta and Sharma, S.H., (2011b). Hepatoprotective and antioxidant potential of *Moringa oleifera* Lam. pods against DMBA-induced hepatocarcinogenesis in male mice. *International Journal of Drug Development and Research*. 3(2): 128-138.
- Paneni, F., Beckman, J. A., Creager, M. A. and Cosentino, F. (2013). “Diabetes and vascular disease: pathophysiology, clinical consequences, and medical therapy: part I,” *European Heart Journal*. 34(31): L2436–2443.
- Prasanna, K. K. and Ravi, T. M. (2013). Effect of *moringa oleifera* on blood glucose, ldl

- levels in types ii diabetic obese people. *Innovative Journal of Medical and Health Science*. 1(3):23-25.
- Rang, H. P., Dale, M. M. (1991). The endocrine system pharmacology, (2nd ed.) Longman Group Ltd, United Kingdom. 504–508.
- Rej, R. (1989). Aminotransferase in Disease. *Clinical Laboratory Medicine*. 9(4): 667- 87.
- Sacher, R. A. and McPherson, R. A. (2000). Widmann's clinical interpretation of laboratory test. F.A. Davis Company. Washington D.C.
- Shetty, A.K., R. Rashmi, M.G.R. Rajan, K. Sambaiah and P.V. Alimath. (2004). Antidiabetic influence of quercetin in streptozotocin-induced diabetic rats. *Nutrition Research*. 24: 373-381.
- Upadhyay, O. P., Singh, R. M., Dutta, K. (1996). Studies on antidiabetic medicinal plants used in Indian folk-lore. *Aryavaidyan*. 9(3): 159–167.
- Vermeer, M. A., Mulder, T. P. and Molhuizen, H. O. (2008). The aflavins from black tea, especially the aflavin- 3-gallate, reduce the incorporation of cholesterol into mixed micelles. *Journal of Agricultural Food Chemistry*. 56: 12031-12036.
- Youl, E., Bardy, G., Magous, R., Cros, G., Sejalon, F., Virsolvy, A., Richard, S., Quignard, J. F., Gross, R., Petit, P., Bataille, D. and Oiry, C. (2010). Quercetin potentiates insulin secretion and protects INS-1 pancreatic β -cells against oxidative damage via the ERK1/2 pathway. *British Journal of Pharmacology*. 161(4): 799–814.