International Food Research Journal 24(5): 2133-2139 (October 2017)
Journal homepage: *http://www.ifrj.upm.edu.my*

Antihyperanaemic and antihyperlipidemic activities of *Artocarpus altilis* fruit based-diet on alloxan-induced diabetic rats

*Ajiboye, B. O., Ojo, O. A., Aganzi, I. Y., Chikezie, G. S., Fadaka, O. A., Jayesimi, K. and Olaoye, O

Department of Chemical Sciences, Biochemistry Programme, Afe Babalola University, Ado-Ekiti, Ekiti State, Nigeria

Article history

Received: 29 July 2016
Received in revised form: 24 September 2016
Accepted: 27 September 2016

Keywords

Artocarpus altilis fruit
Haematology
Lipid profile

Abstract

In West Africa especially in Nigeria uses of *Artocarpus altilis* fruit (breadfruit) have been used traditionally in the management of diabetes mellitus and its complications. This study was designed to investigate the effect of the *Artocarpus altilis* fruit (breadfruit) based-diet on fasting blood glucose level, haematological parameters and serum lipid profiles in alloxan-induced diabetic rats. In this study, the in vitro antioxidant parameters were determined on aqueous extract of the *Artocarpus altilis* fruit, while in the in vivo study thirty-two albino rats *(Rattus norvegicus)* were used and grouped into four: control, diabetic untreated rats, diabetic rats administered with metformin daily and diabetic rats fed with *Artocarpus altilis* fruit based-diet. All rats except those in control group were induced intraperitonially with a single dose of 150 mg/kg of alloxan. The aqueous extract of the *Artocarpus altilis* fruit contained total phenol, total flavonoid, DPPH, FRAP and Fe^{2+} chelation. Administration of alloxan significantly increased ($p<0.05$) fasting blood glucose, LDL and VLDL with significant reduction ($p<0.05$) in all the haematological parameters and HDL. The feeding of the diabetic rats with *Artocarpus altilis* fruit based-diet significantly ameliorated all these effects to normal. Therefore, the results suggest that consumption of *Artocarpus altilis* fruit based-diet by diabetic rats ameliorated antihyperanaemic and antihyperlipidemic complications.

Introduction

Diabetes mellitus is a disorder characterized with hyperglycaemia due to deficiency in insulin secretion and/or insulin action. It is the most common serious metabolic disease in the world, affecting millions of people worldwide (Nelson and Cox, 2010). Diabetes mellitus comprises of three major types (Nelson and Cox, 2010). Type I diabetes also called juvenile onset diabetes is usually detected in childhood. This type of diabetes, the body makes little or no insulin and daily injection of insulin is needed. The exact causes are unknown but genetics, viruses, and autoimmune problems may be involved (Naik, 2011). The symptoms of type I diabetes as reported by Murray *et al.* (2009) are fatigue, increased thirst and urination, nausea, at times vomiting and weight loss despite increased appetite. Type II diabetes, commonest type of diabetes, observed mainly in adulthood, but nowadays young people are increasingly being diagnosed with this disease. In this case the pancreas does not produce enough insulin to maintain the normal blood glucose levels (Alemzadeh and Wyatt, 2010). The occurrence of obesity, sedentary living and failure to exercise exacerbating this type of diabetes, coupled with symptoms such as blurred vision, fatigue, increased appetite, thirst and urination (Alemzadeh and Wyatt, 2010). Gestational diabetes which is the third type of diabetes, with high fasting blood glucose levels, this condition may develops at any time during pregnancy in non-diabetic individuals. Women who have it are at high risk of type II diabetes and cardiovascular disease later in life (Naik, 2011).

Management of diabetes mellitus, focus on making available sufficient amount of insulin in the body system. This relies mainly on dietary measures which include the uses of traditional plant therapies, before insulin therapy was introduced in 1922 (Ekeanyanwu *et al.*, 2012). A number of plants have been acclaimed for antidiabetic properties worldwide as reported by Ekeanyanwu *et al.* (2012). This became more apparent following WHO (1994) recommendations regarding the needs to develop and evaluate better pharmacological agents for improving insulin secretion, enhancing insulin sensitivity, preventing beta cells destruction, promoting beta cells regeneration and interrupting pathways leading to various complications of diabetes (World Health Organization, 1994). These

*Corresponding author.
Email: *bash1428@yahoo.co.uk*

recommendations, together with the cost and side effects of most orthodox hypoglycaemic agents, have stimulated an increasing demand for natural products with antidiabetic activities that have fewer side effects (Adoum *et al*., 2012). The most promising of such products are of plant origin (World Health Organization, 1994). The hypoglycaemic properties of these plants used by traditional medical practitioners may be due to their bioactive constituents (Bako *et al*., 2014). Example of plant that can be used for this purpose is *Artocarpus altilis* fruit, as reported by Ajiboye *et al.* (2016) where they assessed the uses of this plant based-diet on liver and kidney functions indices of alloxan-induced diabetic rats but other complications of diabetes mellitus like aneamia and lipidaemia were not addressed. *Artocarpus altilis* fruit (Parkinson ex FA. zora) belong to a family of moraceae. Therefore, the present study was designed to assess antihyperanaemic and antihyperlipidemic activities of *Artocarpus altilis* fruit based-diet on alloxan-induced diabetic rats.

Materials and Methods

Plant material, authentication and processing

Artocarpus altilis fruit (breadfruits) was purchased from popular market in Ikere, Ekiti State, Nigeria. This was then authenticated at the herbarium of the Department of Plant Science, Ekiti State University, Ado-Ekiti, Nigeria with herbarium voucher number UHAE/127. Thereafter *Artocarpus altilis* fruit flesh was peeled washed and oven dried at 60°C until it's dried. This was then grinded using blender. The powdered sample was then used in this study.

Chemicals

Total cholesterol, High density lipoprotein (HDL)-cholesterol, Low density lipoprotein (LDL)-cholesterol, Very low density lipoprotein (VLDL)-cholesterol and triglycerides assay kits used were product of Randox Laboratories Ltd., Antrim, UK. All other chemicals were of analytical grades and prepared in all-glass apparatus using sterilized distilled water (BDH, UK).

Preparation of aqueous extract of Artocarpus altilis

Twenty gram of the already powdered *Artocarpus altilis* fruit was extracted in 200 ml of distilled water for 24 hours and the mixture was filtered using cheese cloth. The filtrate was dried using freeze dryer (Yakubu *et al*., 2008).

In vitro *analyses*

Total phenolic and flavonoid contents of *Artocarpus altilis* fruit were determined using the methods described by Singleton *et al.* (1999) and Miliauskas *et al*. (2004) respectively. The free radical scavenging ability of the Artocarpus altilis fruit extracts against 1, 1 – diphenyl – 2 – picrylhydrazyl (DPPH) free radical was measured using the method of Szabo *et al.* (2007). In addition, the determination of ferric reducing property and iron chelation assay were carried out the methods of Ali *et al*. (1996) and Puntel *et al.* (2005) respectively.

Experimental animals

Thirty two albino rats *(Rattus norvegicus)* were used in this study. They were obtained from the animal house of the Department of Biochemistry, Afe Babalola University, Ado-Ekiti, Ekiti State.

Induction of diabetes

A single dose of 150 mg/kg of alloxan monohydrate was dissolved in normal saline (0.9%) and induced into the already 12 hours fasted rats. Forty eight hours after the induction, the animals fasting blood glucose level was checked using Accu chek Glucometer. Only the animals with fasting blood glucose level ≥ 250 mg/dl was used in this study (Eyo *et al.*, 2011).

Animal's grouping

The animals were grouped into four (4): Group 1: Normal control placed on yam flour based-diet, Group 2: Diabetic untreated rats placed on yam flour based-diet, Group 3: Diabetic rats placed on yam flour based-diet and administered (14.2mg/kg) metformin daily, and Group 4: Diabetic rats placed on *Artocarpus altilis* fruit based-diet

Formulation of experimental diets

The composition of the experimental diet was as already described by Ajiboye *et al.* (2016).

Collection of blood

At the end of the experimental period, animals were anaesthetized with diethyl ether. The blood of each animal was collected through cardiac puncturing method and collected into EDTA and plain sample tubes for haematological and serum analysis respectively. The serum was obtained by centrifuging the blood at 3000 revolution per minutes for 15 minutes. The serum was then collected using a Pasteur's pipette and stored in a freezer at -5°C until required for further analysis.

Haematological parameters determination

The following haematological parameters were analysed using automated hematologic analyzer: haemoglobin, packed cell volume, red blood cells, white blood cells, neutrophils, lymphocytes, mean cell volume, mean cell haemoglobin and mean cell haemoglobin concentrations by employing the method of Dacie and Lewis (1991).

Serum lipid profile

The concentration of total cholesterol was carried out using the method described by Fredrickson *et al.* (1967). Tietz (1990) method was used in determining the concentrations of triacylglycerol, HDL-cholesterol, LDL-cholesterol and VLDL-cholesterol.

Statistical analysis

The statistical evaluation of data was performed by SPSS version 20, with one way analysis of variance (ANOVA) and Dunett'sposthoc test for multiple comparison. The data were expressed as mean of five replicates ± standard error of mean (S.E.M) and values were considered statistically significant at $p<0.05$.

Results

Table 1 shows the present of total phenol, flavonoid and some *in vitro* antioxidant parameters such as; 1,1-diphenyl-2-picrylhydrazyl (DPPH), ferric reducing antioxidant properties (FRAP) and Fe^{2+} chelation in aqueous extract of *Artocarpus altilis* fruit. The blood glucose concentration was markedly increased in the diabetic rats before treatment; suggesting a diabetic state as shown in Table 2. At the end of the three weeks duration of the treatment, a significant reduction ($p<0.05$) was observed in group D when compared to diabetic untreated rats (Group B), whereas there were no significant difference ($p>0.05$) in control rats (Group A) when compared to group D.

Table 3 shows the effect of *Artocarpus altilis* fruit based-diet on haematological parameters in alloxan-induced diabetic rats. It shows that there was a significant decrease in all the haematological parameters in diabetic untreated rats (Group B) when compared to control (Group A), diabetic rats administered with metformin (Group C) and diabetic rats fed with *Artocarpus altilis* fruit based-diet (Group D).

The effect of *Artocarpus altilis* fruit based-diet on serum lipid profile parameters of alloxan-induced diabetic rats is presented in Table 4. Group B (diabetic untreated rats) shows a significant increase ($p<0.05$) in cholesterol, triglycerides, VLDL and LDL concentrations when compared to the control (Group A), diabetic rats administered with metformin (Group B) and diabetic rats fed with Artocarpus altilis fruit based-diet (Group D). Group B also shows a significant decrease ($p<0.05$) in HDL (good cholesterol) concentrations when compared to groups A, C and D.

Table 1. Phenolic contents and *In vitro* antioxidant parameters of aqueous extract of *Artocarpus altilis* fruit (mg/100g)

Parameters	Quantity
Total Phenol	8.68±0.01
Total Flavonoid	2.90±0.02
Ferric reducing antioxidant properties	1.30±0.01
1,1-diphenyl-2-picrylhydrazyl	89.20±0.04
Fe^{2+} Chelation	14.24±0.02

Each values is a mean of three determination ± SEM

Discussion

In this study, the presence of phenol and flavonoids alongside with ferric reducing antioxidant property (FRAP), 1,1-diphenyl-2-picrylhydrazyl (DPPH) and Fe^{2+} Chelation (Table 1) in the aqueous extract of *Artocarpus altilis* fruit. These concord with Wiley *et al.* (2011) that phenolics compounds and in vitro antioxidant parameters may be used to assess the antioxidant nature of a plant or animal samples in scavenging free radical. Gharib *et al.* (2013) reported that antioxidants reduce oxidative injury to cells by ameliorating the affected organs biomarkers caused by reactive oxygen species (ROS). This is because antioxidants can serve as free radical scavengers and singlet oxygen reducer (Gharib *et al.*, 2013), which makes antioxidant rich food highly helpful to diabetes mellitus patients

Diabetes mellitus is characterized by elevated level of oxidative stress, decreased level of antioxidant defences, haematology parameters and serum lipid profile abnormalities (Wali *et al.*, 2013). Alloxan induces diabetes by damaging the insulin secreting cells of the pancreas leading to hyperglycaemia. The observation in this study correlates with the earlier research finding, in that the blood glucose levels significantly increased in

Table 2. *Artocarpus altilis* fruit based-diet on fasting blood glucose level (mg/dl) of alloxan-induced diabetic rats

Groups	Initial	After 48hours of Alloxan induction	Final glucose level At the 3rd week
A	84.01 ± 2.10^a	82.02 ± 1.12^a	83.12 ± 0.03^a
B	81.01 ± 1.00^a	286.00 ± 0.06^b	304.00 ± 2.14^c
C	78.21 ± 0.20^b	338.00 ± 0.04^c	75.16 ± 2.14^b
D	75.10 ± 0.05^c	380.01 ± 1.02^d	82.10 ± 0.20^a

Column values with different superscripts are significantly (p<0.05) different
Each values is a mean of eight determination ± SEM
Legend: A = Control rats fed with yam flour based-diet, B = Diabetic untreated rats fed with yam flour based-diet, C = Diabetic rats administered with metformin daily and fed with yam flour based-diet, D = Diabetic rats fed with *Artocarpus altilis* fruit-based diet

Table 3. *Artocarpus altilis* fruit based-diet on haematological parameters of alloxan-induced diabetic rats

Groups	PCV (%)	HGB (g/d)	P (u/l)	WBC (mm³)	N (%)	L (%)	M (%)	E (%)	RBC (x 10^{11}/l)
A	36.00 ± 0.11^a	16.00 ± 0.05^a	280.00 ± 4.10^a	5.60 ± 2.10^a	52.00 ± 1.30^a	42.01 ± 1.10^a	8.00 ± 0.01^a	6.10 ± 0.01^a	5.40 ± 0.02^a
B	29.00 ± 0.05^b	13.00 ± 0.01^b	150.00 ± 1.20^b	4.80 ± 1.20^c	44.00 ± 2.10^d	28.00 ± 1.01^d	3.01 ± 0.02^d	2.00 ± 0.02^d	3.60 ± 0.04^b
C	35.00 ± 0.12^a	16.00 ± 0.03^a	260.00 ± 3.10^a	5.20 ± 2.10^a	57.01 ± 0.01^b	45.00 ± 1.01^b	10.00 ± 0.01^b	8.01 ± 0.03^b	4.90 ± 1.10^a
D	36.00 ± 0.03^a	17.00 ± 0.02^a	250.00 ± 5.10^a	6.06 ± 3.10^b	60.01 ± 1.10^c	48.00 ± 1.20^c	12.01 ± 0.03^c	10.00 ± 0.10^c	5.20 ± 0.04^a

Column values with different superscripts are significantly (p<0.05) different
Each values is a mean of eight determination ± SEM
Legend: A = Control rats fed with yam flour based-diet, B = Diabetic untreated rats fed with yam flour based-diet, C = Diabetic rats administered with metformin daily and fed with yam flour based-diet, D = Diabetic rats fed with *Artocarpus altilis* fruit-based diet, PCV = Packed cell volume, HGB = Haemoglobin, P = Platelets, WBC = White blood cell, N = Neutrophils, L = Lymphocytes, M = Monocyte, E = Eosinophils, RBC = Red blood cell

diabetic untreated rats (Table 2) (Rajagopal *et al.*, 2008). The normoglycaemic activity in diabetic rats fed with *Artocarpus altilis* fruit- based diet may be attributed to free radical scavenging of the fruit.

Blood and blood components play vital role in maintenance of homeostasis. However, alteration in blood components may lead to severe diseases or disorders. Haematology is the study of the numbers, morphology of the cellular elements of the blood and the uses of these results in the diagnosis and monitoring of disease (Merck, 2012). Haematological studies are useful in the diagnosis of many diseases as well as investigation of the extent of damage to blood (Togun *et al.*, 2007). The significant increase in all the haematological parameters in diabetic rats fed with *Artocarpus altilis* fruit based-diet (Table 3) demonstrated the anti-anemic activity of the diet and good blood compositions for the animals, probably due to oxidative stress suppressor of the diet (Isaac *et al.*, 2013).

Hyperglycaemia in diabetic patients is associated with alterations in glucose and lipid metabolism (Ajiboye *et al.*, 2014). Diabetes mellitus has been recognized as a major risk factor for cardiovascular diseases (CVD), such as atherosclerosis, heart attacks, stroke (Mazumber *et al.*, 2009). About 75% of deaths among men and 57% of death among women with diabetes are attributable to CVD (Muller, 2004). In

Table 4. *Artocarpus altilis* fruit based-diet on serum lipid profile (mmol/l) of alloxan-induced diabetic rats

Groups	HDL	Cholesterol	Triglycerides	VLDL	LDL
A	254.00±4.21[a]	348.00±2.40[a]	64.40±2.01[a]	29.27±1.10[a]	85.77±2.01[a]
B	150.10±2.10[d]	604.00±3.10[d]	120.00±4.10[c]	54.55±1.40[c]	399.35±5.60[d]
C	205.50±1.10[b]	378.00±4.00[c]	84.10±2.10[b]	38.23±2.00[b]	113.23±2.01[c]
D	268.00±2.20[c]	340.00±5.49[b]	82.40±2.00[b]	37.45±1.40[b]	34.55±2.50[b]

Column values with different superscripts are significantly ($p<0.05$) different
Each values is a mean of eight determination ± SEM
Legend: A = Control rats fed with yam flour based-diet, B = Diabetic untreated rats fed with yam flour based-diet, C = Diabetic rats administered with metformin daily and fed with yam flour based-diet , D = Diabetic rats fed with *Artocarpus altilis* fruit-based diet, HDL = High density lipoprotein, LDL = Low density lipoprotein, VLDL = Very low density lipoprotein

the present study, *Artocarpus altilis* fruit based-diet was found to reduce the concentrations of cholesterol, triglycerides, VLDL and LDL with significant increase in HDL concentration (Table 4).

Disturbances in the regulation of the activity of the hormone-sensitive enzyme (lipase) by insulin due to its deficiency or absence has been ascribed to abnormal increase in serum lipid profiles (triglycerides, cholesterol, very low density lipoprotein and low density lipoprotein) in diabetic mellitus patients (Naik, 2011). This might be caused by the reactive oxygen species, prompted by destruction of beta islet cells (as a result of alloxan induction). Lipase is an enzyme which converts triglycerides to free fatty acids and glycerol while insulin is an hormone that inhibits hormone -sensitive lipase in adipose tissue (Murray *et al.*, 2009). Therefore, in the absence of insulin, the plasma level of free fatty acids increases. This is because in the liver, the free fatty acids are catabolized to acetyl CoA, and the excess acetyl CoA is converted to cholesterol, triglyceride and ketone bodies resulting in ketosis (Bako *et al.*,2014). In addition, the high concentration of serum lipoprotein in the diabetic mellitus may also be due to increase in the mobilization of free fatty acids from the peripheral fat depots by glucagons in the absence of insulin (Bako *et al.*, 2014). This may promote the conversion of some fatty acids by liver into triacylglycerol, phospholipids and cholesterol that may be discharged into the blood as lipoproteins (Naik, 2011).

The presence study demonstrated that *Artocarpus altilis* fruit based-diet might promote the presence of insulin, inhibit hormone-sensitive lipase in the adipose tissue and hinder the mobilization of fatty acid from adipose tissue by glucagons. Furthermore, high levels of high density lipoprotein (HDL) have been reported by Khan *et al.* (2003) to be inversely related to the incidence of coronary heart disease. The high concentration of HDL on diabetic rats placed on *Artocarpus altilis* fruit based-diet may promote the removal of cholesterol from peripheral tissue to the liver for catabolism and excretion. These results demonstrated the normolipidaemia activity of the diet, which may be helpful in ameliorating CVD in diabetes mellitus patients (Andallu *et al.*, 2009). This may be attributed to inhibition of oxidative stress and lipid peroxidation by the diet, which promotes regeneration of pancreatic beta cells.

Conclusion

From this study, it can be concluded that the *Artocarpus altilis* fruit based-diet could be very useful in the management of diabetes, probably due to phenolic contents and *in vitro* antioxidant parameters in the fruit.

Acknowledgement

We would like to appreciate all the Biochemistry Programme Technologist of Afe Babalola University, Ado–Ekiti, Ekiti Sate, Nigeria.

References

Adoum, O.A., Micheal, B.O. and Mohammad, I.S. 2012. Phytochemicals and hypoglycaemic effect of methanol stem-bark extract of *Ficus sycomorus* Linn (Moraceae) on alloxan induced diabetic *Wistar albino* rats. African Journal of Biotechnology 11: 4095-4097.

Ajiboye, B.O., Chikezie, G.S., Aganzi, I.Y., Ojo, O.A., Onikanni, S.A., Afolabi, O.B., Anadozie, S. and Sobajo, O. 2016. Effect of *Artocarpus altilis* fruit based-diet on liver and kidney function indices on alloxan-induced diabetic rats. Mediterranean Journal of Nutrition and Metabolism 9: 23–35

Ajiboye B.O., Edobor, G., Ojo, A.O., Onikanni, S.A., Olaranwaju, O.I. and Muhammad, N.O. 2014. Effect of aqueous leaf extract of Senecio biafrae on hyperglycaemic and serum lipid profile of alloxan-induced diabetic rats. International Journal of Diseases and Disorders 2: 59-64

Alemzadeh, R. and Wyatt, D. 2010. Nelson Textbook of Pediatrics.18th ed., p. 231-237. Philadelphia: Jaypee Brothers, Medical Publishers

Ali, N.A., Lüdtke, J., Pilgrim, H. and Lindequist, U. 1996. Inhibition of chemiluminescence response of human mononuclear cells and suppression of mitogen-induced proliferation of spleen lymphocytes of mice by hispolon and hispidin. Pharmazie 51: 667-670.

Andallu, B., Kumar, A.V.V. and Varadacharyulu, N. 2009. Lipid abnormalities in streptozotocin- diabetes: Amelioration by *Morusindica* L Cv. Suguna leaves. International Journal of Diabetes in Developing Countries 29:123-128.

Bako, H.Y., Mohammad, J.S., Waziri,P.M., Bulus, T., Gwarzo, M. Y. and Zubairu, M.M. 2014. Lipid profile of alloxan-induced diabetic wistar rats treated with methanolic extract of *Adansonia digitata* fruit pulp. Science World Journal 9: 19-25.

Dacie, J.V. 1991. Lewis SM. Practical haematology. 7th ed. Edinburgh: Churchill Livingstone.

Ekeanyanwu, R. C., Udeme, A. A., Onuigbo, A. O. and Etienajirhevwe, O. F. 2012. Anti-diabetic effect of ethanol leaf extract of *Cissampelos owariensis* (lungwort) on alloxan induced diabetic rats. African Journal of Biotechnology 11: 6758 -6762

Eyo, J.E., Ozougwu, J.C. and Echi, P.C. 2011. Hypoglycaemic effects of *allium cepa, allium sativum* and *zingiber officinale* aqueous extracts on alloxan-induced diabetic rattus novergicus. Medical Journal of Islamic World Academy of Sciences 19: 121-126.

Fredrickson, D.S., Levy, R.1. and Lees, R.S. 1967. Fat transport in lipoproteins-an integrated approach to mechanisms and disorders. New England Journal of Medicine 276: 34-281.

Gharib, O.A., Sherif, N.H. and Fahmy, H.A. 2013. Possible anti-hemolytic and antioxidant role of ethanolic extract of coriander on irradiated rats. European Journal of Biology and Medical Science Research 1:39-48

Isaac, L.J., Abah, G., Akpan, B. and Ekaette, I.U. 2013. Haematological properties of different breeds and sexes of rabbits. Proceeding of the 18th Annual Conference of Animal Science Association of Nigeria (ASAN): 24-27.

Mazumder, P.M., Farswan, M. and Parcha, V. 2009. Effect of an isolated active compound (Cg^{-1}) of *Cassia glauca* leaf on blood glucose, lipid profile and atherogenic index in diabetic rats. Indian Journal of Pharmacology 41: 182-186.

Merck, M. 2012. Haematologic reference ranges. Mareck Veterinary Manual. Retrieved from *http://www.merckmanuals.com/.*

Miliauskas, G., Venskutonis, P.R. and Van-Beek, T.A. 2004. Screening of radical scavenging activity of some medicinal plants and aromatic plant extract. Food Chemistry 85: 231-237.

Moller, D. 2004. New drug targets for type 2 diabetes and the metabolic syndrome: A review. Nature 414: 821-827.

Murray, R. K., Bender, D.A., Botham, K. A., Kennelly, P. J., Rodwell, V. W. and Weil, P. A. 2009. In Harper's Illustrated Biochemistry. 28th ed. New York: McGraw Hill/Lange Medical Book.

Naik, P. 2011. Biochemistry Textbook. 3rd ed. India: Jaypee Brother Medical Publisher.

Nelson, D.C. and Cox, M.M. 2010. Lehninger Principles of Biochemistry. 4th ed. New York: W.H. Freeman and Co..

Puntel, R.L., Nogueira, C.W. and Rocha, J.B.T. 2005. Krebs cycle intermediates modulate thiobarbituric reactive species (TBARS) production in rat brain in vitro. Neurochemical Research 30: 225-235.

Rajagopal, A., Rao, A.U., Amigo, J., Tian, M., Upadhyay, S.K., Hall, C., Uhm, S., Mathew, M.K., Fleming, M.D., Paw, B.H., Krause, M. and Hamza, I. 2008. Haem homeostasis is regulated by the conserved and concerted functions of HRG-1 proteins. Nature 453: 1127-1131.

Singleton, V.L., Orthofer, R. and Lamuela-Raventós, R.M., 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Fo253 Czech. Journal of Food Science 26(4): 242-253

Szabo, C., Ischiropoulos, H. and Radi, R. 2007. Peroxynitrite: biochemistry, pathophysiology and development of therapeutics. Nature Reviews Drug Discovery 6: 662-680

Tietz, N.W. 1990. Serum triglyceride determination. In Clinical Guide to Laboratory Tests. 2nd ed., p. 554-556. Philadelphia, USA: W.B. Saunders Co,

Togun, V.A., Oseni, B.S.A., Ogundipe, J.A., Arewa, T.R., Hammed, A.A., Ajonijebu, D.C. and Mustapha, F. 2007. Effects of chronic lead administration on the haematological parameters of rabbits – a preliminary study, p. 341. Proceedings of the 41st Conferences of the Agricultural Society of Nigeria. Nigeria.

Wali, U., Saidu, Y., Ladan, M.J., Bilbis, L.S. and Ibrahim, N.D. 2013. Antioxidant Status and Lipid Profile of Diabetic Rats Treated With Antioxidant Rich Locally Prepared Nutraceutical. International Journal of Colorectal Disease 1: 03-38

Waterman, P.G. and Mole, S. 1994. Analysis of Phenolic Plant Metabolites. Oxford, UK: Blackwell Scientific

Publications.
Wiley, D.M., Kim, J.D., Hao, J., Hong, C.C., Bautch, V.L. and Jin, S.W. 2011. Distinct signaling pathways regulate sprouting angiogenesis from the dorsal aorta and the axial vein. Nature Cell Biology 6: 686-692.
World Health Organization (WHO). 1994. WHO Study Group Report on Prevention of Diabetes Mellitus, p. 1-92. Geneva: WHO.
Yakubu, M.T., Akanji, M.A. and Oladiji, A.T. 2008. Alterations in serum lipid profile of male rats by oral administration of aqueous extract of *Fadogia argrestis* stem. Research Journal of Medicinal Plants 2: 66-73.