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Phytochemical Constituents and Lipid Lowering Actions of Extracts of *Picralima nitida* Seed and *Tapinanthus bangwensis* leaf in Alloxan-induced Diabetic Rabbits

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ABSTRACT: Diabetes, a metabolic syndrome, is characterized by disordered metabolism including hyperlipidaemia. Orthodox approach of managing diabetes poses risk of inflicting heart diseases amongst others, hence, the search for alternative therapy. In Nigeria, many herbalists have claimed to use coconut water extract of *Picralima nitida* seed and aqueous extract of *Tapinanthus bangwensis* leaf in the management of diabetes, amongst which are their lipid lowering actions. This study aims to provide evidence for the presence of some phytochemicals which have been found to enhance the lipid lowering actions of the plant extracts in the diabetic state. The experimental rabbits (Chinchilla) (1.12 ± 0.09 kg) were grouped into six and extracts administered orally, once daily for five weeks. Groups 1 and 2 (non-diabetic) received only distilled water and coconut water respectively, group 3 (diabetic) received 200 mg/kg body weight aqueous extract of *T. bangwensis* leaf, group 4 (diabetic) received 400 mg/kg body weight of coconut water extract of *P. nitida* seed, groups 5 and 6 (diabetic) received only distilled water and coconut water respectively. The lipid lowering effects of extracts on total lipid and cholesterol concentration in both the plasma and selected tissues (liver, kidney and heart) were assessed. The results revealed that the extracts independently lowered significantly ($p<0.05$) the blood total lipid and cholesterol levels of the diabetic rabbits. Both extracts significantly ($p<0.05$) decreased the total lipid concentrations in the tissues. The extracts revealed the presence of some phytochemicals (Alkaloids, Flavonoids, Saponins amongst others) which possibly contributed to their lipid lowering actions Overall, aqueous extract of *T. bangwensis* leaf and coconut water extract of *P. nitida* seed respectively, can be advocated plants as lipid lowering agents in the management of diabetes.

KEYWORDS: Alloxan, Cholesterol, Diabetes, Lipid, Phytochemicals, *Picralima nitida, Tapinanthus bangwensis*

1.0 Introduction

Diabetes mellitus is a complex metabolic disorder (Ozturk, *et al*., 1996; Petal and Rybczynski, 2003) that involves chronic alterations in fat metabolism amongst others, basically resulting from the secretion of dysfunctional and/or insufficient endogenous insulin by the β-cells of the pancreas. It is one of the world's commonest diseases today; according to recent estimates, the human population worldwide appears to be in the midst of an epidemic of diabetes (NHANES (2009; WHO, 2011; CDC, 2011). The orthodox approach of managing diabetes is faced with a lot of difficulties, this is partly because several of the drugs, aimed in managing diabetes pose a significant risk of inflicting heart disease, cancer (Agabegi and Agabegi, 2008), and no pill or injection to date is able to address the problem of dying pancreatic beta cells, a fundamental dysfunction in diabetes (Bennett *et al*., 2011).

Cholesterol is a form of fat (blood lipids) that comes in two major forms (Low density lipoprotein and High density lipoprotein). In this study, distinctions are not made between the types of cholesterol but on the total cholesterol. High cholesterol levels are as serious as high blood pressure. Heart disease and stroke, two of the leading causes of death for diabetics, have both been linked to high cholesterol levels. In diabetes, high blood lipid levels increase complications greatly (Ozturk *et al*., 1996).

Over the past decade, there has been a resurgence of interest in the investigation of natural materials as a source of potential drug

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substance. Many cultures have folk's medicine tradition that includes the use of plants and plant products and many licensed drugs used today originated from herbs. Traditional medicine has been brought into focus for meeting the goals of primary health care delivery, not only in Africa but also to various extents in all countries of the world (Ali, 2011). In the last few years, researchers have assessed a number of African medicinal plants as potentially emerging alternative/adjunct treatment for diabetes. This has led to an increase in the number of diabetics using these more 'natural' ingredients to help manage their condition. In fact, the World Health Organization estimates that 4 billion people or 80 percent of the world's population use herbal medicine for some aspect of primary health care (WHO, 2011). It had also recommended and encouraged this practice especially in countries where access to conventional treatment is not adequate (WHO, 2002). The popularity of the use of herbal medicine in Nigeria has increased considerably resulting in the listing of herbal medicinal products by the National Agency for Food, Drugs Aministration and Control, NAFDAC (Akinseinde, 2013). Phytochemicals, chemical compounds that occur naturally in plants and suggested to have biological significance (Haneman *et al.*, 2008), are presenting an exciting opportunity for the development of new types of therapeutics. This has accelerated the global effort to harness and harvest those medicinal plants that bear substantial amount of potential phytochemicals showing beneficial effects in combating diabetes and diabetes-related complications.

In Nigeria, many herbalists have claimed to use the coconut water extract of *Picralima nitida* (Common name: Akuamma, Yoruba: Abere) seed and aqueous extract of *Tapinanthus bangwensis* (Common name: African Mistletoe, Yoruba: Afomo) leaf for the treatment for various diseases, including diabetes.

Tapinanthus bangwensis (family-Loranthaceae) is a semi-parasitic evergreen plant found growing on a host of deciduous trees all year round, around the branches of the tree. It depends on its host for minerals and water only, but photosynthesizes its carbohydrate by means of its green leathery, oblong leaves (Osadebe and Uzochukwu, 2006). *Tapinanthus bangwensis* had earlier been reported to be used for the prevention of myocardial infarction, hypercholesterolaemia and cardiovascular diseases by lowering the levels of cholesterol in the blood (Lansky, 1993). Also, in Nigeria and some other parts of Africa, African mistletoe has been used traditionally as antihypertensive and antidiabetic (Obatomi *et al*., 1994; Obatomi *et al*., 1996). However, information is scanty in open scientific literature to support the folkloric use of these plant materials as lipid lowering agent in the diabetic state.

Picralima nitida (family-Apocynaceae) grows as an under storey tree in rain forests and widely distributed in the tropical forests of Africa. When fully grown, it is up to 20m high with white flower in a terminal inflorescence and very large paired fruits (Pods). The fruit contains seeds that are usually embedded in pulpy material known as pulp. The pericarp of the fruit contains latex known as the rind (Nyunaï and Njifutié, 2006). It has been reported that the aqueous extract of *P. nitida* seeds has anti-diabetic activities (Aguwa *et al*., 2001), rich in alkaloids (Tane *et al*., 2002) and possess anti-inflammatory and analgesic actions in rats (Duwiejua *et al*., 2002).

In consonance with the policy of World Health Organization (2002) on medicinal plant materials which include ascertaining their efficacy, this study is aimed at the overall objective of evaluating the lipid lowering actions of the aforementioned herbal extracts (which were often employed traditionally) and the presence of natural product(s) making them lipid lowering agents in the diabetic state, using alloxan-induced diabetic rabbits as model.

Alloxan, a toxic glucose analogue, is a diabetogenic agent that selectively destroys the pancreatic beta-cells through the production of free radicals (Ozturk *et al.*, 1996; Aguwa *et al*., 2001) used in experiment with animals to induce diabetes (Alloxan diabetes). This pancreatic β-cells destruction invariably causes absolute insulin deficiency that leads to hyperglycemia in the diabetic rabbits (Szkudelski, 2001). Chinchilla rabbit breed amongst others are sturdy, well adapted and had features similar to human organs (Cotter *et al*., 1996; Hirakawa *et al*., 2010), hence, the reason for their selection in laboratory investigations.

2.0 Materials and Methods

2.1 Collection and Authentication of Plant Materials

The seeds of *Picralima nitida* (Stapf) T. Durand & H. Durand were purchased from herb seller at 'Baboko market', Ilorin, Nigeria while the leaves of *Tapinanthus bangwensis* (Engler & K. Krause) Danser from citrus tree (host plant) were obtained from a farm settlement in 'Ila-Orangun', Osun State, Nigeria. The plants were authenticated at Forest Research Institute of Nigeria (FRIN) Ibadan, Nigeria. Voucher samples were deposited in the Institute's Herbarium (FHI 109955 and FHI 109972 for *P. nitida* and *T. bangwensis* respectively).

2.2. Animals and Feed

The experimental rabbits (36), (Chinchilla breed) (1.12 ± 0.09 kg) of mixed sexes, purchased from Goshen rabbit farm, Osogbo, Nigeria were employed in this study. Rabbit pellets (a product of Guinea Feeds, Ibadan, Nigeria) and water were made available to the animals *ad libitum* throughout the experimental period. They were acclimatized for a week. The animals were handled according to the stated guidelines of Ethical Committee on the ethical use of animals in research (ETS, 2005).

2.3 Preparation of Plant Extracts

The seed coats of the *P. nitida* seeds were removed with the hand and the *T. bangwensis* leaves were washed free of sand and debris. The seeds and leaves of the *P. nitida* and *T. bangwensis* respectively were air- dried for two weeks at room temperature (28ºC ± 2.00) and pulverized using an electric blender (Holt Star, Model BE 768-2, John Holt product, UK).

Solvent extract of pulverized seeds of *P. nitida* and leaves of *T. bangwensis* were prepared by separately suspending 500g each of the powdered samples in 1000 ml of coconut water and distilled water respectively using the modified method of Gray and Flatt (1999). The mixtures were left to infuse overnight and were thereafter vigorously shaken for 3 hours using a wrist hand shaker for thorough extraction. The extracts were filtered with Whatman No. 1 filter paper and the filtrates concentrated in a rotary evaporator to obtain semi solid extracts. A calculated amount of the residue was weighed and constituted in the solvent used for their extraction to give the doses 200 mg/kg and 400 mg/kg for *Tapinanthus bangwensis* leaf and *Picralima nitida* seed respectively.

2.4 Phytochemical Screening

The extracts were subjected to qualitative and quantitative analysis of their phytochemical constituents (such as alkaloids, flavonoids, saponins, tannins etc) using standard procedures (Harborne, 1998; Trease and Evans, 2002).

2.5 Induction of Diabetes

The rabbits were fasted overnight before they were given a single intraperitoneal injection of aqueous alloxan monohydrate (Sigma-Aldrich, USA) at a dose of 300 mg/kg b. w. to make them diabetic. After 6 hours, they were given 5% D - Glucose solution to drink to counter the hypoglycaemic shock phase (Lenzen, 2008). The fasting blood glucose level of blood samples drawn from the tail vein puncture was determined after 72 hours using One-touch ultraeasy glucometer (Lifescan Johnson and Johnson Company, Milipitas). Rabbits showing blood glucose level greater than 180 mg/dL were selected for the study (Lacy and Kostianosvky, 1963) to indicate diabetic rabbits.

2.6 Animal Grouping and Extract Administration

The experimental rabbits (mixed sexes) were grouped into six (6). Extracts were administered orally at the dose of 200 mg/kg and 400 mg/kg for *Tapinanthus bangwensis* and *Picralima nitida* respectively, once daily for five (5) weeks. The dosages were arrived at with series of preliminary studies to establish the concentration at which the extracts exhibited the safest and highest hypoglycaemic activities, 200 mg/kg and 400 mg/kg for *Tapinanthus bangwensis* and *Picralima nitida* respectively. Preliminary studies also indicated both extracts possessed hypoglycaemic activity; lowering the plasma glucose level of the diabetic rabbits to the normal range of $\geq 75 \leq 115$ mg/dL within five (5) weeks of administration.

The animals were grouped and treated as follows:

A: Non diabetic rabbits placed on distilled water only (NDDW) (Control)

B: Non diabetic rabbits placed on coconut water only (NDCW) (Control)

C: Diabetic rabbits placed on distilled water extract of *T. bangwensis* leaf (DDWT)

D: Diabetic rabbits placed on coconut water extract of *P. nitida* seed (DCWP)

E: Diabetic rabbits placed on distilled water only (DDW) – (Positive control)

F: Diabetic rabbits placed on coconut water only (DCW) – (Positive control)

2.7 Collection and determination of weight of organs

At the end of the fifth week of experimentation, the rabbits were anaesthetized with ether. The animals were sacrificed by cutting the jugular vein. The animals were dissected and each of their kidneys, hearts and livers were removed. The organs were blotted in clean tissue paper, weighed, and thereafter homogenized in 0.25M sucrose solution as described by Akanji and Yakubu (2000). The homogenates were thereafter transferred into specimen bottles and kept frozen for 24 h prior analyses.

2.8 Determination of Blood Glucose and Lipid Profile

Blood glucose concentrations were read using One-touch ultraeasy glucometer (Lifescan Johnson and Johnson Company, Milipitas, CA) following the procedures as outlined by the manufacturer. The total lipid in the plasma / tissue was determined according to the method described by Frings and Dunn (1970). The plasma cholesterol concentration of the experimental rabbits was determined using the Multicare machine (Biochemical System International, Srl, Arezzo-Italy). The total cholesterol of the tissue homogenate was determined by the method of Zlatkis *et al.*, (1953).

2.9 Statistical Analysis

All data were subjected to analysis of variance using the model for randomized complete block design (Steel and Torrie, 1980). Significant differences between treatment means were determined at 5% confidence level using Duncan's Multiple Range Test (SPSS 16).

3.0 Results

Table 1 shows the phytochemical composition of the aqueous extract of *T. bangwensis* leaf and coconut water extract of *P. nitida* seed, revealing the presence of alkaloids, flavonoids, saponins, tannins, amongst others. Out of all the phytochemicals revealed in the extracts, alkaloids constituted the highest percentage (Table 1). There was significant ($p<0.05$) amount of flavonoids in the aqueous extract of *T. bangwensis* leaf compared with coconut water extract of *P. nitida* seed, while oxalate was not detected in both extracts.

The total lipid were significantly ($p<0.05$) increased in diabetic rabbits (DDW, DCW) when compared with the normal (NDDW, DCW) rabbits. However, rabbits administered the test plant extracts (DDWT and DCWP) had significant ($p<0.05$) reduction in their plasma total lipid compared to the untreated groups (Figure 1).

The total lipid concentration in the selected tissues of the extract treated diabetic rabbits was significantly ($p<0.05$) reduced compared to those of the untreated diabetic rabbits which exhibited significantly high level of total lipid in their tissues (Figure 2). The lipid concentration in the tissues of the extracts treated groups (DDWT and DCWP) was not significantly different from those of the non diabetic groups.

There was significant ($p<0.05$) reduction in plasma cholesterol concentration of diabetic rabbits admininstered with aqueous extract of *T. bangwensis* leaf and coconut water extract of *P. nitida* seed (DDWV and DCWP) when compared to non diabetic rabbits (Figure 3).

The cholesterol concentration of the selected tissues of diabetic rabbits placed on distilled water extract of *T. bangwensis* leaf and coconut water extract of *P. nitida* seed were significantly ($p<0.05$) reduced compared to the diabetic non treated groups which showed significantly ($p<0.05$) high cholesterol levels in all their tissues in contrast to those of the non diabetic rabbits groups (Figure 4).

Table 1: Phytochemicals in coconut water extract of *P. nitida Seed* and aqueous extract of *T. bangwensis* leaf

Parameters (mg/100g)	**Coconut water extract of *P. nitida* seed**	**Aqueous water extract of *T. bangwensis* leaf**
Alkaloids	36.50 ± 3.89[a]	33.50 ± 3.00[b]
Saponins	11.00 ± 3.90[a]	9.00 ± 2.40[b]
Phenols	5.50 ± 2.30[a]	ND
Terpenoids	9.00 ± 1.90[a]	11.3 ± 0.98[b]
Tannins	15.00 ± 2.00[a]	10.2 ± 2.00[b]
Oxalates	ND	ND
Steroids	4.90 ± 1.24[a]	2.50 ± 0.15[b]
Cyanogenic glycosides	ND	ND
Flavonoids	11.10 ± 2.22[a]	28.80 ± 0.30[b]
Ascorbic acid	4.90 ± 0.98[a]	6.00± 0.23[b]

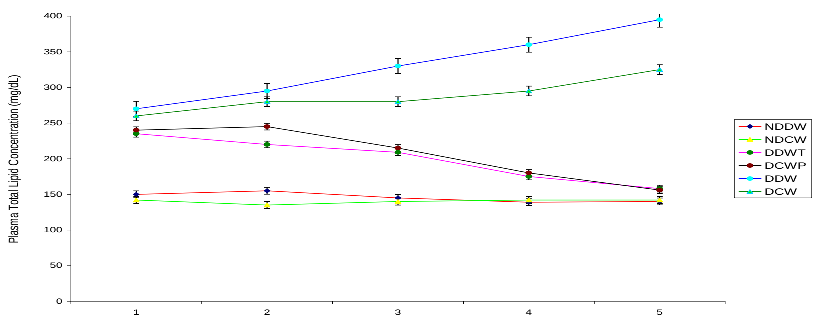

Figure 1: Plasma total lipid concentration of alloxan-induced diabetic rabbits administered extracts of *T. bangwensis* leaf and *P. nitida* seed for five weeks

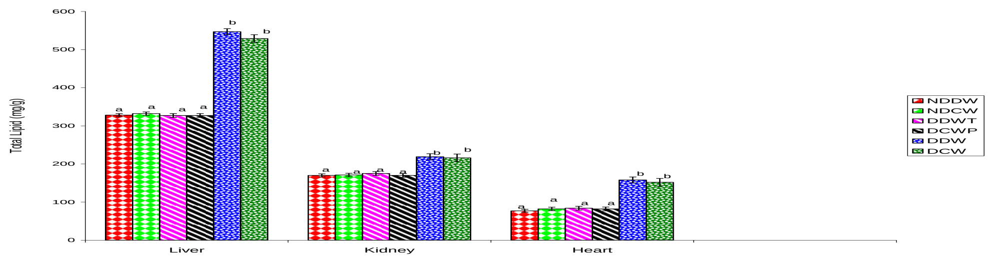

Figure 2: Lipid concentrations in selected tissues of alloxan-induced diabetic rabbits administered extracts of *T. bangwensis* leaf and *P. nitida* seed

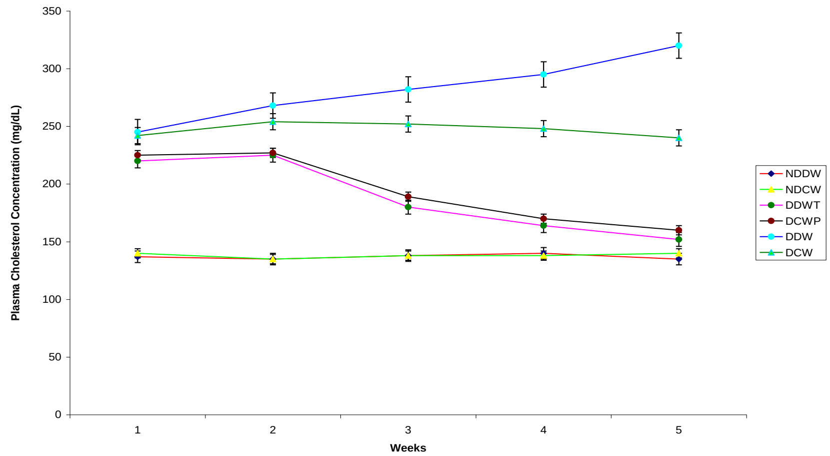

Figure 3 Plasma total cholesterol concentration of alloxan-induced diabetic rabbits administered extracts of *T. bangwensis* leaf and *P. nitida* seed for five weeks

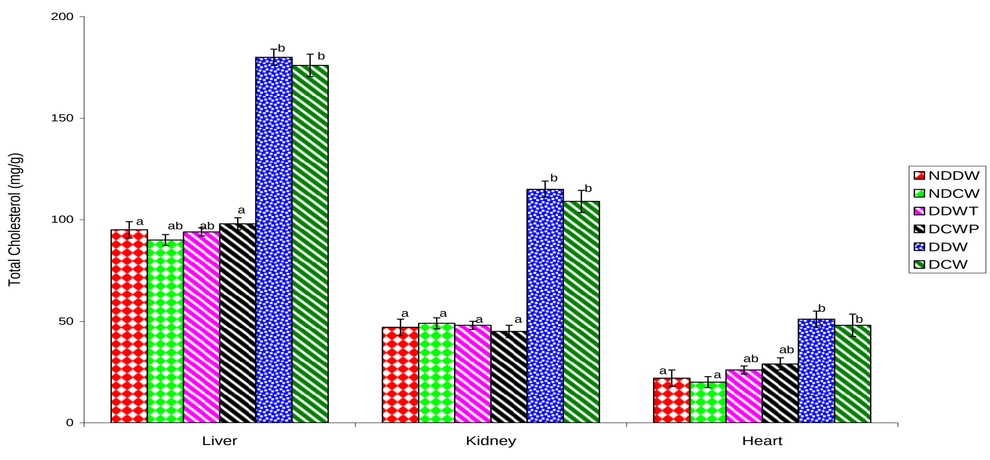

Figure 4: Total cholesterol concentrations in selected tissues of alloxan-induced diabetic rabbits administered extracts of *T. bangwensis* leaf and *P. nitida* seed

Bars with different notations are significantly different ($p<0.05$).

4.0 Discussion

The diabetic rabbits were administered with coconut water extract of *Picralima nitida* seed (DCWP) and distilled water extract of *Tapinanthus bangwensis* leaf (DDWT) respectively and the extracts (Table 1) revealed the presence of various phytochemicals including alkaloids, flavonoids, saponins, terpenoids etc. which are varieties of plant-derived compounds reported to be responsible for protection against diseases (Haneman and Zidenberg-Cherr, 2008). Also, several workers implicated alkaloids, flavonoids, saponins, cyanogenic glycosides and terpenoids etc in many plants such as *Vernonia amygdalina*, *Occium gratissimum*, *Emelia sonchifolia* (red tassel flower) and *Leonotis leonurus,* used in ethnomedical practice for the management of diabetes (Loew and Kaszkin, 2002; Igbaakin, 2005; Monago and Ogbonnaya, 2009; Atangwho *et al*., 2010; Oyedemi *et al*., 2011). Flavonoids as component of human diets have beneficial effects as antioxidants, capable of neutralizing free radicals which are implicated in heart disease, strokes and cancer (Lenzen *et al*., 1996) all of which are complications of high concentration of plasma/tissue lipid, hence, the lipid lowering action of the test extracts. Earlier study reported the protective action of flavonoids against oxidative stress induced cellular damage(s) and also the ability of flavonoids to regenerate pancreatic β – cells (Tiedge *et al*., 1997). The presence of flavonoids in the extracts may enhance their antihyperlipidaemic activity (Haliwell, 1994). Moreso, the presence of ascorbic acid which is also a good antioxidant is capable of preventing lipid peroxidation in both plasma and tissues (Halim *et al*., 1997).

Alkaloids are reported by Jeffery and Harborne (Jeffery and Harborne, 2000) to help in improving cardiac conditions by reducing blood pressure, increasing circulation of blood and inhibiting the accumulation of atherosclerosis all of which are complications of diabetes. Consequently, the occurrence of alkaloids which accounts for the most significant concentration of all the phytochemicals determined may be partly responsible for the usefulness of the extracts as antidiabetic agents (Tiwari and Rao, 2002).

Saponins had also been reported to be useful in the treatment of hypercholesterolaemia. Saponins cause a depletion of body cholesterol by binding with cholesterol, thus preventing its reabsorption and consequently, increasing its excretion from the body (Lansky, 1993; Lipkin, 1995) and consequently enhancing the lipid lowering action of the test extracts.

Following the administration of alloxan, there is usually a characteristic increase in blood glucose level (Lenzen, (2008). In our previous study, administration of aqueous extract of *T. bangwensis* leaf and coconut water extract of *P. nitida* seed respectively decreased blood glucose level in diabetic rabbits (DDWV, DCWP) (Adegoke and Oloyede, 2013). The blood glucose levels in untreated diabetic rabbits (DDW, DCW) when compared with normal rabbits were significantly ($p<0.05$) raised (Adegoke and Oloyede, 2013).

The phytochemicals, flavonoids and alkaloids as revealed to be present in the test extracts may be responsible for the hypoglycaemic action as also observed using *Vernonia amydalina* and *Occium gratissium* extracts in diabetic rats (Igbaakin, 2005); soybean extract in diabetic rabbits (Bhathena and Velasquez, 2002). The significant ($p<0.05$) reduction in the glucose level in the diabetic rabbit group placed on coconut water extract of *P. nitida* seed (Adegoke and Oloyede, 2013), may in part be as a result of health benefits of coconut water which had earlier been documented to improve insulin secretion, improve utilization of blood glucose, being completely non- toxic and capable of relieving stress on pancreas (CRC, 2007).

An antidiabetic agent could exert a beneficial effect in the diabetic situation by enhancing insulin secretion and/or by improving/mimicking insulin action (Gray and Flatt, 1997). The test plants extracts indicate the presence of natural product(s) which possibly directly or indirectly stimulate insulin secretion amongst others (Adegoke and Oloyede, 2013).

Furthermore, the normal effects of insulin on lipid metabolism is to decrease lipolytic activity in the adipose tissues, promote uptake of fatty acids and chylomicrons from the plasma by peripheral and adipose tissues and enhancement of lipogenesis and lipid storage in tissues (William, 1999) resulting in low plasma lipid. When insulin is deficient as in diabetes, clearance of lipids from the plasma will be reduced resulting in hyperlipidaemia. The ability of the extracts to reduce the level of plasma lipid (Figure 2) may probably be due to the presence of bioactive components (flavonoids and alkaloids) of the extracts which possess insulin-like actions which might have acted by promoting the uptake and storage of fatty acids by the adipose tissues (Mathe, 1995; Zhang, *et al.,* 2008).

Consequently, plasma hyperlipidaemia as observed in the diabetic untreated rabbits, can result in the deposition of lipids in other tissues such as kidney and heart leading to tissue damage (Stroev, 1986). The reduction of lipid concentration (Figure 3) in the selected tissues of diabetic rabbits placed on the test plant extracts (DDWT and DCWP) might be associated with the effect of the extracts resulting in lipid lowering action.

Likewise, the reduction in the serum total cholesterol levels (Figure 4) following the administration of the extract may be attributed to reduction in the concentration of acetyl CoA resulting from decreased β-oxidation of fatty acids since acetyl CoA is a key substrate in the biosynthesis of cholesterol (Zhang, *et al.,* 2008). Although acetyl coA level was not measured, in diabetics, since glucose is not available for energy production, the body reverts to the use of lipids which results in increased β-oxidation of fatty acids. This leads to enormous production of acetyl-coA which is the precursor for cholesterol synthesis (Rang *et al*., 1995).

Similar effect was observed in a similar study with a different species of mistletoe leaf (*Loranthus micranthus*) in which its aqueous extracts did not induce adverse alterations in biochemical parameters such as total cholesterol (Bernard, 1990). This may explain why the cholesterol levels in the plasma of rabbits administered the aqueous extract of *T. bangwensis* is close to the control (Figure 4). The hypocholesterolaemia observed in this study may also be attributable to the presence of saponin which possess cholesterol lowering effect (Lansky, 1993; Lipkin, 1995). Saponins cause depletion of serum cholesterol thereby preventing its reabsorption and increasing its excretion (Malinon, 1997). Another possible explanation for the observed effect of test extracts on serum cholesterol levels may be based on the fact that herbs are generally rich in vitamins (Edem and Usoh, 2009). Vitamin such as ascorbic acid is a good antioxidant capable of preventing hypercholesterolaemia in both plasma and tissues (Tiwari and Rao, 2002). The high level of cholesterol in the plasma leads to its deposition in other tissues such as the kidney and the heart as revealed in this study (Figure 4) which may consequently result into complications such as fatty liver, cardiovascular disorders and heart attack (Earle, 1990). The ability of the extracts to reduce the levels of cholesterol and lipid in the tissues of diabetic rabbits may be due to the presence of bioactive components of the extracts which possess insulin-like actions that can decrease lipolytic activity, promote uptake of fatty acids by adipose tissue, enhance lipogenesis and storage and possess cholesterol lowering action. Flavonoids and alkaloids had been reported to exert potent hypolipidaemic effects in diabetes (Zhang, 2008).

Conclusion

Overall, the plant extracts (aqueous extract of *T. bangwensis leaf* and coconut water extract of *P. nitida* seed) (which were often employed traditionally in diabetes) independently demonstrated to possess lipid lowering activity as revealed by their antihyperlipidaemic and antihypercholesterolaemic, actions etc. thus alleviating the biochemical disorders (hyperlipidaemia and hypercholesterolaemia) associated with diabetes. Hence, both extracts may be good candidates as lipid lowering agents in the management of diabetes.

References

Adegoke, B. M. and Oloyede, O. B. (2013). Antihyperglycaemic and antihyperproteinaemic activity of extracts of *Picralima nitida* seed and T*apinanthus bangwensis* leaf on alloxan-induced diabetic rabbits. International Journal of Innovation & Applied Studies 3(4): 1125-1131.

Agabegi, E. D. and Agabegi, S. S. (2008). Step-up to medicine (Step-Up Series)". Hagerstwon, MD: Lippincott Williams & Wilkins. pp. 185.

Aguwa, C. N. Ukwe, C. V. Inya-Agha, S. I. and Okonta, J. M. (2001). Antidiabetic effect of P*icralima nitida* aqueous seed extract in experimental rabbit model. *J. Nat. Remedies* 1(2): 135–139.

Akanji, M. A. and Yakubu, M. T. (2000). α- tocopherol protects against metabisulphite – induced tissue damage in rats. Nigerian Journal of Biochemistry and Molecular Biology; 16: 147-148.

Akinseinde, K. (2013). NAFDAC to Work with herbal practitioners to improve herbal medicine. Tribune Newspaper: Health News.

Ali, A. A. (2011). Trends and challenges of traditional medicine in Africa. African Journal of Traditional Complementary and Alternative Medicine 8(5S): 115-123.

Atangwho, I. J., Ebong, P. E., Egbung, G. E., Akpaso, M. I. and Asuquo, E. E. (2010). Histological effect of combined extracts of *Vernonia amygdalina* and *Azadirachta indica*. Research Journal of Agriculture and Biological Science 6(4): 514-521.

Bennett, W. L., Maruthur, N. M., Singh, S., Segal, J. B., Wilson, L. M., Chatterjee, R., Marinopoulos, S. S., Puhan, M. A. (2011). Comparative effectiveness and safety of medications for type 2 diabetes: An update including new drugs and 2-drug combinations. Annals of Internal Medicine 154 (9): 602-613.

Bernard, C. (1990). Insulin resistance diabetese mellitus. Diabetes News XI(4): 6.

Bhathena, S. J. and Velasquez, M. T. (2000). Beneficial role of dietary phytoestrogens in obesity and diabetes. American Journal of Clinical Nutrition 76(6):1191-1201.

Centers for Disease Control and Prevention, (CDC) (2011). National diabetes fact sheet: national estimates and general information on diabetes and prediabetes in the United States, Atlanta, GA: U.S. Department of Health and Human Services.

Cotter, C. S., Avidano, M. A., Stringer, S. P. and Schultz, G. S. (1996). Inhibition of proteases in Pseudomonas otitis media in chinchillas. *Otolaryngol Head Neck Surg* 115**:** 342-351.

Duwiejua, M., Woode, E. and Obiri, D. D. (2002). Pseudo-akuammigine, an alkaloid from *Picralima nitida* seeds has anti-inflammatory and analgesic actions in rats, Journal of Ethnopharmacology 81: 73-79.

Earle, K. A. (1990). Diabetic nephropathy and cardiovascular disease: Familiar factors. Diabetes News XI (4): 5.

Edem, D. O. and Usoh, I. F. (2009). Biochemical changes in wistar rats on oral doses of mistletoe (*Loranthus micranthus*). American Journal of Pharmacology and Toxicology 4: 94-97.

European Treaty Series (2005). European convention for the protection of vertebrate animals used for experimental and other scientific purposes. European Treaty Series, Strasbourg, ETS-123.

Frings, C. S. and Dunn, R. T. (1970). A Colorimetric method for determination of total serum lipids based on the sulfo-phosphovanihin reaction. American Journal of Clinical Pathology 53: 89.

Gray, A. M. and Flatt, P. R. (1997). Nature's own pharmacy: The diabetes perspective. Proceedings of the Nutrition Society 56:507–517.

Gray, A. M. and Flatt, P. R. (1999). Insulin -secreting activity of the traditional antidiabetic plant *Viscum album* (Mistletoe). Journal of Endocrinology 160: 409–414.

Halim, A. B., El-Ahmady, O., Hassab-Allah, S. Abdel-Galil, F., Hafez, Y. and Darwish, A. (1997). Biochemical effect of antioxidants on lipids and liver function in experimentally-induced liver damage. Annals of Clinical Biochemistry 34: 656-663.

Haneman, K. and Zidenberg-Cherr, S. (2008). Facts about phytochemicals, In: Nutrition and Health Info-Sheet for Health Professionals. UC Cooperative Extension Center for Health and Nutrition Research p. 1-4.

Harborne, J. B. (1998). Phytochemical Methods. *A Guide To Modern Techniques of Plant Analysis.* 2nd Edition. Chapman and Hall, London. p. 50 – 94.

Haliwell, B. (1994). Free Radicals, antioxidants and human disease: Curiosity, cause or constipation? Lancet 344: 721-724.

Hirakawa**,** Y., Sasaki**,** H., Kawamoto, E., Ishikawa**,** H., Matsumoto, T., Aoyama, N. Kawasumi, K. and Amao, H. (2010). Prevalence and analysis of *seudomonas aeruginosa* in chinchillas. *BMC Veterinary Research* 6: 52.

Igbaakin A. P. (2005). Biochemical assessment of the efficacy of solvent extracts of *Vernonia amygdalina* and *Occium gratissium* in the treatment of alloxan – induced diabetic rats, A Ph. D. Thesis submitted to the Dept. of Biochemistry, University of Ilorin, Nigeria, unpublished.

Jeffery, B. and Harborne, D. (2000). Arsenal for survival: Secondary plant products. Taxonomy 49: 435-449.

Lacy, P. E. and Kostianosvky, M. (1963). Method for the isolation of intact islets of langarhans from the rat pancreas. *Diabetes* 16: 35-39.

Lansky, P. S (1993). Plants that Lower Cholesterol. Acta Horticulture 332(2): pp 4 – 7.

Lenzen, S. Drinkgren, J. and Tiedge, M. (1996). Beneficial effects of cells against β- oxidation in diabetes. Free Radical Biology and Medicine 20: 463–466.

Lenzen, V. (2008). The mechanisms of alloxan- and streptozotocin-induced diabetes. Diabetologia 51(2): 216–226.

Lipkin, R. (1995). Saponins and your health: One of nature's protective phytochemicals. Science News. 148:392-393.

Loew, D. and Kaszkin, M. (2002). Approaching the problem of bioequivalence of herbal medicinal products. Phototherapy Research 16:705-711.

Malinon, R. (1997). Blood cholesterol lowering properties of dietary saponins. American Journal of Clinical Nutrition 6:42 – 45.

Mathe, D. (1995). Dyslipidaemia and diabetes: Animal models. Diabete et Metabolisme 21(2): 106–111.

Monago, C. C. and Ogbonnaya, A. E. (2009). Effects of aqueous extract of *Emelia sonchifolia* on some liver enzymes in dithizone induced diabetes in rabbits. Nigerian Journal of Biochemistry and Molecular Biology 24(1): 8-15.

NHANES (2009). National Health and Nutrition

Examination Survey 2002-2009.
Nyunaï, N. and Njifutié, N. (2006). *Picralima nitida* (Stapf) T. Durand & H. Durand" Record from Protabase.
Obatomi, D. K. Bikomo, E. O. and Temple, V. J. (1994). Antidiabetic properties of african mistletoe in streptozocin - induced diabetic rats. Journal of Ethnopharmacology 43: 13-17.

Obatomi, D. K., Aina, V. O. and Temple, V. J. (1996). Effect of african mistletoe on blood pressure in spontaneously hypertensive rats. Pharmaceutical Biology 34: 124-127.
Okoro, V. M. O., Ezeokeke, C. T., Ogundu, U. E. and Chukwudum, C. (2010). Phenotypic correlation of bodyweight and linear body measurement in Chinchilla rabbits (*Orycotolagus cuniculus*). Journal of Agricultural Biotechnology and Sustainable Development 2(2): 27-29.
Osadebe, P. O. and Uzochukwu, I. C. (2006). Chromatographic and antimotility studies on extracts of *Loranthus Micranthus* Linn. Journal of Pharmaceutical and Allied Sciences 3: 263-268.
Oyedemi, S. O., Yakubu, M. T. and Afolayan, A. J. (2011). Antidiabetic activities of aqueous leaves extract of *Leonotis leonurus* in streptozotocin induced diabetic rats. Journal of Medicinal Plants Research 5(1):119-125.
Ozturk, Y. Attan, V.M. and Yildizoglu-Ari, (1996). Effects of Experimental diabetes and insulin on smooth muscle functions. American Society of Pharmacology and Experimental Therapeutics 48: 69-112.
Petal, M. and Rybczynski, P. (2003). Treatment of non-insulin dependent diabetes mellitus. Expert Opin. Invest. Drugs 12: 623-633.
Rang, A. P., Dale, M. M. and Ritter, J. M. (1995). Pharmacology, 3rd edition Churchill Livingstone publishers, New York. pp. 409-413.
Steel, R. G. D. and Torrie, J. H. (1980). Principles and Procedures of Statistics: A Biometric Approach. 2nd ed., McGraw Hill Books Co., New York, USA,
Schmelzer, G. H. & Gurib-Fakim, A. (Editors). PROTA (Plant Resources of Tropical Africa), Wageningen, Netherlands.
Stroev, E. A. (1986). Biochemistry, Russian Edition, Mir Publisher, Moscow. pp. 472.
Szkudelski, T. (2001). The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. Physiology Research 50: 536-546.
Tane, P., Tene, M. and Sterner, O. (2002). Picranitine, A new indole alkaloid from *Picralima nitida* (Apocynaceae). Bulletine of Chemical Society of Ethiopia. 16:165-188.
Tiedge, M., Lortz, S., Drinkgren,J. and Lenzen, S. (1997). Possible protection of pancreatic glucose toxicity. Diabetes 46: 1733–1742.
Tiwari, A. K. and Rao, J. M. (2002). Diabetes mellitus and multiple therapeutic approaches of phytochemicals: present status and future prospects. Current Science 83(1): 30-37.
Trease, G. E. and Evans, W. C. (2002). Pharmacognosy, 15th edition. W.B. Saunders Company Limited, Toronto. pp. 333 – 393.
William, F. G. (1999). Review of Medical Pharmacology, 20th Edition. Mc Graw Hill York, pp 870.
World health Organization (2011). World Health Statistics 2011: Facts and Figures about Diabetes, WHO Statistical Information System.
World Heatlth Organization (2002). General guidelines for methodologies on research and evaluation of traditional medicine. World Health Organization, Geneva. 1: 184.
Zhang, Y., Li, X., Zou, D., Liu, W., Zhu, N., Huo, L., Wang, M. Hong, J., Wu, P., Ren, G., Ning, G. (2008). Treatment of type 2 diabetes and dyslipidaemia with the natural plant alkaloid berberine. Journal of Clinical Endocrinology and Metabolism 93(7): 2559-2565.
Zlakis, A., Zak, B. and Boyle, A. J. (1953). A new method for the direct determination of serum cholesterol. Journal of Laboratory and Clinical Medicine 41: 486-492.