RESEARCH ARTICLE

ANTILIPIDAEMIC STUDIES OF MISTLETOE (*Loranthus micranthus*) LEAF EXTRACTS ON DIABETIC RATS

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ABSTRACT

This study evaluated the anti-lipidaemic effects of mistletoe (*Loranthus micranthus*) leaves using methanol and aqueous extracts on lipid profiles (triacylglycerols, total cholesterol, high density lipoprotein, and low density lipoprotein) of white albino Wistar rats in the laboratory. Male Wistar rats were used for this study and they were housed to acclimatize in five different cages according to their groups. Each group contained four animals. Diabetes was induced in rats in all but groups 4 and 5 by the intravenous injection of alloxan monohydrate (90mg/kg) dissolved in normal saline through rat tail vein. Group 1 diabetic rats were treated with 600mg/kg body weight concentration of crude methanol extract of *L. micranthus* leaves orally. Group 2 diabetic rats were treated with 600mg/kg body weight concentration of crude water extract of *L. micranthus* leaves orally. Group 3 diabetic rats were treated with 250mg/kg body weight concentration of glibenclamide orally. Group 4 diabetic rats were not treated and served as positive control. Rats in Group 5, which were non-diabetic, received normal saline and served as negative control. The experiments were repeated using different Wistar rats for groups 1, 2, and 4 for the second, third, and fourth weeks. The results of this study showed that both extracts of *L. micranthus* leaves caused a significant (P<0.05) reduction in fasting lipid levels in diabetic and non-diabetic Wistar rats with the effect of the methanol extract being significantly (P<0.05) higher than that of the aqueous extract.

INTRODUCTION

The term 'diabetes' is derived from the Greek word meaning siphon, or the passing through of water and 'mellitus' is Latin for honey sweet, which refers to the excessive flow of urine containing glucose in diabetics (Kobberling, 1971). Diabetes mellitus is a metabolic disorder, which is characterized by hyperglycaemia and lipoprotein abnormalities (Scoppola *et al.*, 2001). It involves changes in lipid metabolism resulting to vast derangements in glucose and lipid homeostasis with disastrous vascular complications (Ugochukwu *et al.*, 2003). The two types of diabetes mellitus are type 1 diabetes mellitus or insulin dependent diabetes mellitus (IDDM) and Type 2 diabetes mellitus or non-insulin dependent diabetes mellitus (NIDDM). The IDDM is an organ-specific autoimmune disorder resulting from specific destruction of the insulin-producing β-cells of the islets of Langerhans of the pancreas (Notkins and Lernmark, 2001; Tisch and McDevitt, 1996). It is a T-lymphocyte-mediated autoimmune disorder in which

specific T cells selectively destroy the insulin producing β cells of the pancreatic islets (Bach and Mathis, 1997). Research works have shown that a great majority of people with type 1 diabetes mellitus inherit traits that put them at risk for this disorder, though not everyone who inherits these traits develops type 1 diabetes mellitus as environmental factors can also trigger the immune system to destroy the insulin-producing cells (Scriver *et al.*, 1995). These environmental factors easily activate autoimmune mechanisms in genetically susceptible individuals, leading to the progressive loss of pancreatic islets β-cells. The environmental triggers have been reported to include minute quantities of the macrolide antibiotic bafilomycin A1 (baf A1), viruses, increased dietary nitrate and nitrite, dairy products and early weaning onto cow milk (Tuomilehto *et al.*, 1997). Bafilomycin causes reduced glucose tolerance and disruption of the pancreatic islets in mice. Type 1 diabetes mellitus has less advanced dyslipidaemia with high arterial disease often developing into myocardial infarction leading to death (Winocour *et al.*, 1986). The NIDDM is a maturity onset, or non-ketotic

diabetes. It begins with a state of insulin resistance / hyperinsulinaemia / normoglycaemia and evolves to one of hypoinsulinaemia/hyperglycaemia (Saltiel and Kahn, 2001). During the development of hyperglycaemia, an increase in apoptosis occurs, culminating in a severe disruption of the islet architecture (Donath *et al.,* 1999). NIDDM has become the most frequently encountered metabolic disorder in the world, especially among developing nations (Zimmet *et al.,* 2001). The main factors contributing to the increasing prevalence of type 2 diabetes are aging (most often occurring after age 40), increasing levels of obesity and lack of physical activity (Goldstein, 2003; Moller, 2001).

Dyslipidaemia is an abnormal metabolism of lipoproteins. It is used to describe a group of conditions in which there are abnormal levels of lipids and lipoproteins in the blood (Galton, 2003). It is a central feature in untreated diabetes and is associated with increased morbidity and mortality (Ginsberg, 2006). In diabetes mellitus, the composition and amount of the different lipoprotein particles are altered as a result of the dyslipidaemia. The key features of this dyslipidaemia are the elevated levels of triacylglycerols, the reduced levels of HDL cholesterol, and the increased number of small, dense LDL particles (American Diabetes Association, 1993). In both types of diabetes, abnormalities of lipid metabolism are prevalent, but the nature of these abnormalities is different (Cowrie and Harris, 1995). Diabetic dyslipidaemia leads to a diminished activity in the lipoprotein lipase, which leads to a decrease in the VLDL catabolism resulting in increase in the VLDL pool (Ginsberg, 1987). Despite the increased VLDL pool, LDL cholesterol levels may be normal due to increased proportion of VLDL particles being metabolized without conversion to LDL (Howard, 1994). This leads to an increased lipid exchange between triacylglycerol-rich VLDL, HDL, and LDL. In addition, the catabolism of HDL is also increased because of the over-activity of hepatic lipase (Howard, 1994). This results in the generation of smaller, denser lipoprotein particles with abnormal functions and the increased fractional catabolic rate (FCR) of apo A-I leading to a lower HDL cholesterol level.

Complications arising from dyslipidaemia are the leading cause of death in diabetic patients even with good glycaemic control (Wagner *et al.,* 2003). Research works have focused on the management of these metabolites in diabetic patients using lipid lowering drugs (Wagner *et al.,* 2003). Therefore, the effect of methanol and aqueous extracts from *L. micranthus* leaves on lipid profile of diabetic rats was investigated.

Traditional plant remedies have been in use for centuries in the treatment of diabetes, but only a few have been scientifically evaluated (Akhtar and Ali, 1984). In Nigeria, local herbal practitioners claim that extracts from mistletoe leaf is effective in the management of high blood glucose levels that is associated with diabetes mellitus (Osadebe *et al.,* 2004). It has also been reported that the dried leaf samples of the African mistletoe (*Loranthus micranthus*) posses glucose lowering properties in alloxan-induced diabetic mice (Osadebe

et al., 2004). However, the effect of this plant extracts in the management of lipid disorders as a result of diabetes mellitus is not documented.

Mistletoe is a semi-parasitic woody perennial plant commonly found growing on oaks and other deciduous trees. Its semi-parasitic nature is because the plant synthesizes its own chlorophyll but depends on the host for its supply of water and minerals. (Duke 1985). The plant relates to several different species of perennial, evergreen, parasitic shrubs from different genera including the American mistletoe (*Phoradendron leucarpum*), the European mistletoe (*Viscum album*), and the African mistletoe (*Loranthus micranthus*), which is the European equivalent of *Viscum album* and the American equivalent of *Phoradendron. Loranthus micranthus* belongs to the family of African bushy plants called Loranthaceae (Anderson and Phillipson, 1982 and Newall *et al.,* 1996).

Mistletoe has been reputed to posses medicinal properties with claims of blood pressure lowering and in treating diseases of the spleen, complaints associated with menstruation, infertility, ulcers, and tumours (Barnes *et al.,* 2003). Reports indicate that hypertensive adults treated with mistletoe extracts showed a decreased blood pressure though the extract had a small effect on actually lowering the blood pressure (Weiss, 1985). Certain *in-vivo* and *in-vitro* studies have suggested that some mistletoe constituents, like the alkaloids, are anti-carcinogenic (Khwaja *et al.,* 1986) while other numerous clinical trials have shown that subcutaneous injections of mistletoe extracts relieve carcinogenesis in people with cancer of various organs (Kleijnen and Knipschild, 1994 and Schulz *et al.,* 1997). Others have reported that mistletoe extracts can stimulate insulin secretion from pancreas cells and may improve blood sugar levels in people with diabetes (Gray and Flatt, 1999; Swanson-Flatt *et al.,* 1989). Given both mistletoe uses around the world in the prevention and/or management of such disease/ailments as diabetes, cardiovascular diseases, cancer, cramps, and menopausal hot flushes, this research was primarily designed to determine the effects of mistletoe extracts on the lipoprotein profiles of diabetic Wistar rats.

MATERIALS AND METHODS

Collection and Identification of Plant Materials

Mistletoe leaves were obtained from Nsukka environs. They were identified as *Loranthus micranthus* by Mr. Alfred Ozioko of Bioresources and Development Conservation Programme (BDCP), Aku Road, Nsukka, Enugu State.

Animals

The experimental animals used for this research were male Wistar albino rats of about 8 to 11 weeks old and weight range of 100 to 200g. The rats were obtained from the animal houses of both the Faculty of Biological Sciences and Veterinary Medicine, University of Nigeria, Nsukka (UNN). The animals were kept under standard

conditions for 7 days with water and food *ad libitum* for acclimatization before the experiments commenced.

Animal Stock

Fifty-six male Wistar rats were used for this study. They were acclimatized and housed in separate cages according to their groups. Diabetes was induced in rats by the intravenous injection of alloxan monohydrate (90mg/kg) through the tail vein. Following the induction of diabetes, the rats were divided into five groups of four animals each (n = 4). **Group 1** was diabetic rats treated with 600mg/kg methanol extract (ME) of *Loranthus micranthus* orally. **Group 2** was diabetic rats treated with 600mg/kg of the aqueous extract orally and was called the AE. **Group 3** was diabetic rats treated with 250mg/kg of glibenclamide orally. **Group 4** was diabetic untreated rats called the positive control. **Group 5** was non-diabetic rats given normal saline. The experiments were repeated with different animals for groups 1, 2, and 3 for the second, third, and fourth weeks.

Plant Material

The experiment was carried out in two parts with the same plant material. One part was carried out using methanol extract while the other was csarried out using water (aqueous) extract.

Preparation of the Methanol Extract

The leaves of *Loranthus micranthus* plant were plucked and dried under room temperature for two weeks. The dried leaves were divided into two parts. The first part was pulverised into coarse form. About 500g of the powdered leaves were soaked in 1000ml of 99% methanol. The mixture was left to stand for twenty-four hours with occasional stirring. The mixture was later extracted using a soxhelet extractor to obtain the methanol extract. The extract was concentrated over a water bath at a temperature range of $25^{o}C$ to $30^{o}C$ to obtain 34.32g (yield = 18.28% w/w) of methanol extract.

Preparation of the Aqueous Extract

The second part of the dried leaves were also pulverised into coarse form. About 500g of the powder were soaked in 100ml of distilled water and left to stand for twenty-four hours with occasional soaking. The pulverised leaves were later extracted to get the water (aqueous) extract. Later, the extract was concentrated over a water bath at a temperature of $30^{o}C$ to $35^{o}C$ to obtain 25.73 (yield = 8.87% w/w) of aqueous extract.

Determination of the Concentration of Various Extracts

To determine this, known weights of both extracts were determined separately. The weight of dry crucible was also determined. Later, known weights of both extract were put into the dry crucible, respectively, and their weight determined before heating. The crucible with its content was heated and after the heating, the crucible was weighed with its heated content and the weight recorded.

The concentrations of both extracts were then calculated from the various weights.

Phytochemical Screening of Both Plant Extracts

The preliminary phytochemical analysis on both extracts involved tests for the presence or absence of the following constituents: alkaloids, acidity, carbohydrates, fats and oil, proteins, glycosides, reducing sugar, flavonoids, terpenoids, steroids, resins, tannins, and saponins.

Test for Alkaloids (General Tests)

About 2ml of 5% tetraoxosulphate (iv) acid in 50% ethanol was added to 5ml of the methanol and water extracts, respectively. The different mixtures were brought to heat on a boiling water bath for 10 minutes. They were cooled and filtered. To 2ml of each filtrate was added few drops of: Mayer's Reagent (Potassium mercuric iodide solution) Dragendorff's Reagent (Bismuth potassium iodide solution), Wagner's Reagent (Iodine in potassium iodide solution) Picric acid solution (1%).

The remaining filtrates were placed separately in 100ml separator funnels and made alkaline with dilute ammonia solution. The aqueous alkaline solution, of each extract, was separated and extracted with two 5ml portions of dilute sulphuric acid. Both extracts were tested with a few drops of Mayer's, Wagner's, and Dragendorff's reagents. Alkaloids showed up as milky precipitate with one drop of Mayer's reagent and a reddish brown precipitate with one drop of Wagner's reagent.

Test for Acidity

About 0.1g of both extracts were placed in clean dry test tube and sufficient water poured into the mixtures. The mixtures were warmed in a hot water bath and allowed to cool. A wet blue litmus paper was dipped into each of the mixture and the colour change observed. A colour change to red indicated acidity.

Test for Carbohydrate (Molisch's Test)

One gram of each extract (methanol and water extracts) was boiled with 2ml of distilled water and then filtered. Concentrated sulphuric acid was gently poured down the sides of each test tube to form a lower layer. A purple interface of ring indicated the presence of carbohydrates.

Test for Fats and Oils

One gram of each extract was pressed between a clean filter paper. The filter paper was observed for translucency which indicated the presence of oils in the extracts.

Test for Proteins (Million's Test)

Two drops of Million's Reagent were added, respectively, to both extracts in a test tube. The formation of white precipitate indicated the presence of proteins.

Test for Glycosides (Fehling's Test)

About 5ml of a mixture of equal parts of Fehling Solutions I and II were added to about 5ml of each extract

and then heated on a water bath for 5 minutes. A brick red precipitate showed the presence of reducing sugar.

Test for Reducing Sugars (Fehling's Test)

About 1g of both extracts were shaken vigorously with 5ml of distilled water and later filtered. The filtrate was used for the Fehling's test. Fehling's Test: To 1ml portion of the filtrate were added equal volumes of Fehling's Solutions I and II and boiled on a water bath for few minutes. A brick red precipitate indicated the presence of reducing sugars.

Test for Saponins (Fehling's Method)

About 20ml of water was poured into 0.25g of both extracts in a 100ml beaker and boiled gently on a hot water bath for 2 minutes. Both mixtures were respectively filtered out and allowed to cool. The filtrates were used for the Fehling's test. A reddish precipitate indicated the presence of Saponins.

Test for Tannins (Ferric Chloride Method)

One gram of both extracts was boiled respectively with 50ml of distilled water. Each was filtered and the filtrate used for the test. To about 3 ml of the respective filtrate, few drops of ferric chloride solution were added. A greenish black precipitate indicated the presence of tannins.

Test for Flavonoids (Ammonium Test Method)

About 10ml of ethylacetate were added to 0.2g of both extracts. Both mixtures were treated on a water bath for 3 minutes. Each mixture was cooled, filtered and the filtrate used for the ammonium test. Ammonium Test: A quantity, 4ml of each of the filtrates was shaken with 1ml of ammonia solution. The layers were allowed to separate and the yellow colour in the ammoniacal layer indicated the presence of flavonoids.

Test for Resins (Precipitation Test)

About 0.2g of both extracts was washed with about 15ml of 95% ethanol and the mixture poured into 20ml distilled water in a beaker. The formation of a precipitate indicated the presence of resins.

Test for Steroids and Terpenoids

About 9ml of ethanol was poured into 1g of the extract. It was refluxed for a few minutes and then filtered. The filtrate was concentrated to 2.5ml on a boiling water bath and 5ml of hot water was added. The mixture was allowed to stand for 1 hour and the waxy matter filtered off. The filtrate was extracted with 2.5ml chloroform using a separating funnel. Later 1ml of concentrated sulphuric acid was poured into about 0.5ml of the chloroform extract in a test tube. The appearance of a reddish-brown interface showed the presence of steroids. Another 0.5ml of the chloroform extract was evaporated to dryness on a water bath and heated with 3ml of concentrated sulphuric acid for 10 minutes on a water bath. A grey colour indicated the presence of terpenoids.

Acute Toxicity Test (LD$_{50}$)

The median lethal doses (LD$_{50}$) of the methanol extract and aqueous extract were determined in mice using the oral route of administration (Lorke, 1983). Both extracts did not cause death in mice at dose concentrations above 2900mg/kg. Consequently, the methanol extract and aqueous extract of mistletoe (*Loranthus micranthus*) are considered non toxic (Lorke, 1983) at such concentration.

Preparation of Normal Saline

A quantity, 0.9g of sodium chloride was weighed and dissolved in a little quantity of distilled water. The volume was finally made up to 100ml.

Determination of Total Cholesterol Concentration

This was done using the method described by Randox laboratories U.K using Randox kits.

Procedure: Test tubes were set and labelled RB (Reagent Blank), STD (Standard) and SAM (Sample) accordingly. About 10µl of distilled water, standard, and serum sample were pipetted into the RB, STD, and SAM test tubes respectively. Lastly, 1000µl of the reagent was added to all the three sets of test tubes (Reagent Blank, Standard, and Sample). The solutions were mixed properly, incubated at 25°C for 10 minutes and the absorbance of the sample (A$_{sample}$) was measured against the reagent blank within 60 minutes at 500nm wavelength. The concentrations of cholesterol in the serum samples were then determined.

Determination of High Density Lipoprotein (HDL)-Cholesterol

This was also carried out using the method described by Randox laboratories U.K using Randox kits. The procedure involved two steps:

Precipitation Step: Using a micropipette, a 500µl quantity of each sample was pipetted into their corresponding test tubes. About 1000µl of the precipitant was also pipetted into all the test tubes using a micropipette. They were mixed properly and allowed to stand for 10 minutes at room temperature. The mixtures were centrifuged for 10 minutes at 4,000rpm. After centrifugation, the clear supernatant was separated off and used for the next step.

Cholesterol CHOD-PAP Assay: A quantity, 100µl of distilled water was pipetted into test tubes labelled RB (Reagent Blank) only. This was followed by the addition of 100µl of the standard into the second test tube labelled STD (Standard). Later, 100µl of the supernatant was also pipetted into test tubes labelled SNT (Supernatant). Finally, 1000µl of the reagent was pipetted into all the different test tubes (Reagent Blank, Standard, and Supernatant). They were mixed thoroughly and incubated for 10 minutes at 25°C. After the incubation, the absorbance of the sample (A$_{sample}$) and standard (A$_{standard}$) were measured against the reagent blank at 500nm wavelength. The HDL concentration in the supernatant was then calculated.

Determination of Low Density Lipoprotein (LDL)-Cholesterol

To assay the serum LDL-cholesterol level, the method described by Randox laboratories U.K using Randox kits was employed. A 100μl of the serum was pipetted into a centrifuge tube followed immediately by the addition of 1000μl of the precipitation reagent. They were mixed thoroughly and allowed to stand for 10 minutes at 25°C. The mixture was then centrifuged for 15 minutes at 3500rpm. The supernatant was collected and its cholesterol concentration determined within an hour of centrifugation. Distilled water of about 50μl was pipetted into test tubes labelled RB (Reagent Blank). This was followed by 50μl each of the standard solution and the supernatant which was pipetted into test tubes labelled STD (Standard) and SNT (Supernatant), respectively. Finally, 1000μl of the reagent solution was pipetted into all the different test tubes (Reagent Blank, Standard, and Supernatant). They were mixed and incubated for 10 minutes at 25°C. After incubation, the absorbance of the sample (A_{sample}) and standard ($A_{standard}$) against the reagent blank was measured at 500nm wavelength and the LDL concentration calculated.

Determination of Triacylglycerol Concentration

Serum triacylglycerols determination was done using the method described by Randox laboratories U.K using Randox kits.

Procedure: This involved setting test tubes according to Reagent Blank (RB), Standard (STD), and Sample (SSAM). Ten micro-litres each of the sample and standard were pipetted into test tubes labelled SAM and STD, respectively. Later, 1000μl of the reagent was pipetted into all the test tubes: Reagent Blank, Standard, and Sample. The test tube contents were mixed thoroughly and incubated for 10 minutes at 25°C. The absorbance of the Sample (A_{sample}) and Standard ($A_{standard}$) were measured against the reagent blank at 500nm wavelength. Finally, the concentration of triacylglycerol in the serum was determined.

Statistical Analysis

The results of the experiment were presented as mean±SEM and were subjected to One Way Analysis of Variance (ANOVA). The difference between the means were tested using post Hoc L.SD at $P<0.05$ significance level.

RESULT AND DISCUSSION

Diabetes mellitus is the fastest growing metabolic disease in the world and as the knowledge of its multifactorial and heterogeneous nature increases so does the need for more challenging and appropriate therapies. This therapy is essential in order to reduce the disruption in the metabolism of lipoprotein causing dyslipidaemia, which further results to other complex cardiovascular and nephritic disorders. Therefore, the effect of the extracts of *Loranthus micranthus* leaves on lipid profile in serum of diabetic rats was investigated.

In this study, the results of the phytochemical analysis of the *Loranthus micranthus* leaf methanol extract and aqueous extract indicated the presence of important compounds such as alkaloids, glycosides, flavonoids. The alkaloid fraction is particularly present in the methanol extract but absent in the aqueous extract. It also contains glycosides in greater proportion than the aqueous extract. The saponin content is high in the aqueous extract but absent in the methanol extract. From the result stated earlier, it could be inferred that the antilipidaemic property of the methanol extract of *Loranthus micranthus* could be a function of its alkaloid and glycoside fractions. Though the aqueous extract exhibited some antilipidaemic property, it is not as effective as that from the methanol extract. The general inference therefore, is that the methanol and aqueous extracts of *Loranthus micranthus* posse hypoglycaemic and antilipidaemic properties which could be as a result of the presence of alkaloids and glycosides found in the extracts.

Table 1. Phytochemical properties of extracts

Constituents	Aqueous extract	Methanol extract
Alkaloids	-	++
Carbohydrates	+++	+++
Fats and Oils	-	-
Proteins	++	-
Glycosides	++	+++
Reducing Sugar	++	++
Flavonoids	+++	++
Terpenoids	+	++
Steroids	++	++
Resins	+++	+++
Tannins	++	++
Saponins	+	-

+: Present in trace concentration; ++: Present in moderately high concentration; +++: Present in very high concentration; -: Absent.

Table 2. Acute toxicity test (LD_{50})

PHASE A		
EXTRACTS (METHANOL AND AQUEOUS)	**DOSE (mg/kg)**	**MORTALITY/GROUP**
Methanol and Aqueous	10	0/4
Methanol and Aqueous	100	0/4
Methanol and Aqueous	1000	0/4
PHASE B		
EXTRACTS (METHANOL AND AQUEOUS)	**DOSE (mg/kg)**	**MORTALITY/GROUP**
Methanol and Aqueous	1600	0/3
Methanol and Aqueous	2900	0/3
Methanol and Aqueous	5000	0/3

In the acute toxicity test, administration of 10, 100, 1000mg/kg respectively of the methanol extract and aqueous extract caused no death in the first phase of the test. In the second phase of the test, no death was also recorded even up to 5000mg/kg doses of the methanol extract and aqueous extract on test mice, respectively. Therefore, the methanol extract and aqueous extract of *Loranthus micranthus* were considered not toxic even at 4900mg/kg and 5000mg/kg doses. The result of the lethal toxicity test (LD$_{50}$) indicated that both the methanol and aqueous leaf extracts of *Loranthus micranthus* may not contain toxic compounds which are lethal to test animals. At a dose 5000mg/kg body weight, both extracts did not kill the test animals.
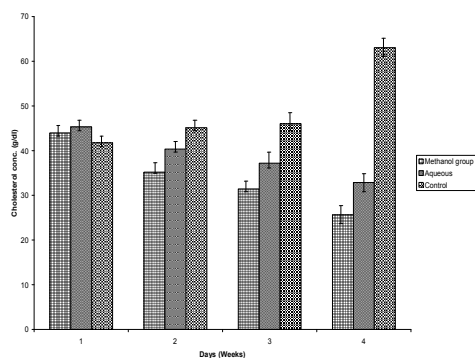

Fig. 1. Effect of extracts on cholesterol level of diabetic rats

Total cholesterol levels of diabetic rats treated with the methanol extract were significantly (P<0.05) lower than those of the control rats. The cholesterol levels of diabetic rats treated with both extracts were not significantly (P>0.05) reduced in the first week of administration. However, the results show that with continuous administration, the cholesterol level in treated diabetic rats was significantly lower (P<0.05) in the fourth week of treatment.
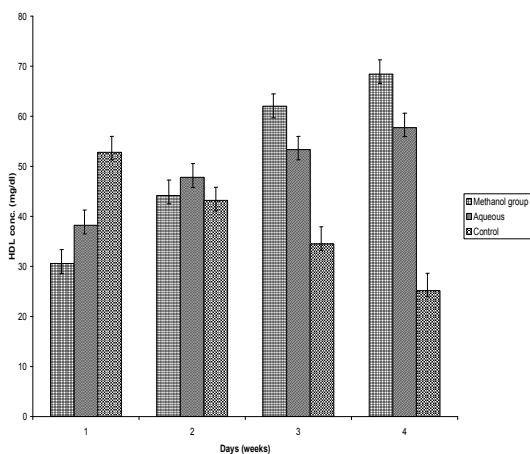

Fig. 2. Effect of extracts on HDL level of diabetic rats

HDL levels of diabetic rats treated with the methanol extract did not significantly (P>0.05) increase more than those of the control rats. Within the first week of administration of extracts, the HDL levels of diabetic rats treated with both extracts were not significantly (P>0.05)

increased. The result also indicate that with continuous administration, the HDL level in treated diabetic rats was not significant (P>0.05) in the fourth week of treatment.
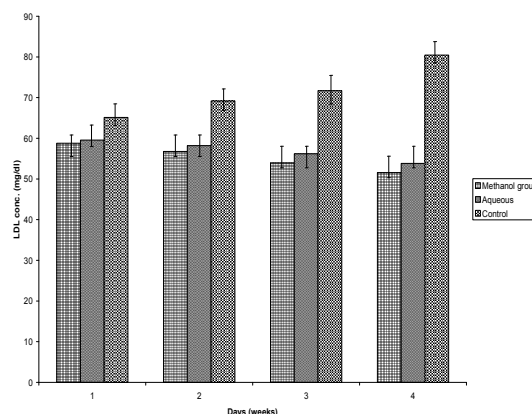

Fig. 3. Effect of extracts on LDL level of diabetic rats

The low density lipoprotein LDL levels of diabetic rats treated with the methanol and aqueous extracts of *Loranthus micranthus* leaves were not-significantly (P>0.05) affected when compared to those of the control rats. With prolonged administration of both extracts, the LDL levels also remained non-significant.
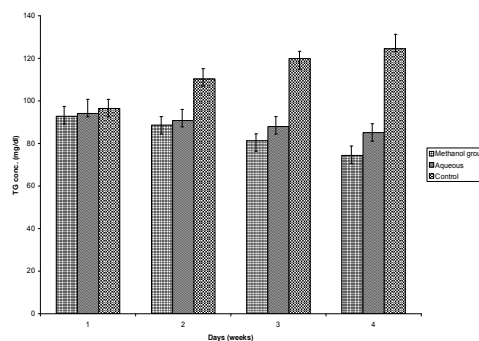

Fig. 4. Effect of extracts on triacylglycerol level of diabetic rats

Serum triacylglycerol TG level was significantly (P<0.05) higher in diabetic rats compared to the control rats. Triacylglycerol levels of diabetic rats treated with both extracts decreased significantly (P<0.05) in the treated animals. The results show that with continuous administration of the extracts, the triacylglycerol level in treated diabetic rats decreased significantly (P<0.05) in the fourth week of treatment.

DISCUSSION

The decreases in the serum lipid profile level in treated diabetic rats with the extracts could at least in part, result from the inactivation of the enzymes involved in the glycation end product reactions in the treated diabetic rats. Lipid profile which is altered in the serum of diabetic patients appears to be a significant factor in the development of premature atherosclerosis and includes an increase in triacylglycerol and total cholesterol levels (Orchard, 1990; Betteridge, 1994). High serum cholesterol

concentrations were observed in all the diabetic rats in the research carried out. This reduction in the cholesterol concentration follows a similar pattern by both extracts. The results of this study indicate that with continuous administration of both extracts, the cholesterol concentrations in diabetic rats treated with the methanol and aqueous extracts reduced significantly when compared with the non-treated diabetic rats.

Low concentration of high density lipoprotein metabolism is a characteristic feature in un-treated diabetes mellitus (Howard, 1994). However, HDL increases in diabetic patient with good glycaemic control (Eckel *et al.,* 1981). This finding is consistent with lower VLDL clearance and LPL activity since HDL, especially HDL$_2$, increases during the lipolytic process (Eisenberg, 1984). The result of this research work indicates a significant increase in the fasting HDL concentration in diabetic rats. This increase also occurred gradually in the non-fasting state. However, the HDL concentration in the non-fasting states in the diabetic rats did not increase significantly following the administration of the methanol and aqueous extract.

There are no consistent changes observed in LDL concentrations in diabetes mellitus (Barrett-Connor *et al.,* 1982). However, there is a very strong evidence that lowering the LDL cholesterol by drugs reduces the morbidity and mortality from cardiovascular disease in diabetes mellitus (Cullen, 2003). The result of this research indicates that the methanol and aqueous extracts of *Loranthus micranthus* did not significantly affect the LDL metabolism in diabetic mellitus.

Triacylglycerol metabolism is affected in diabetes mellitus (Scoppola *et al.,* 2001). The results of this research finding show that both the methanol and aqueous extracts of *Loranthus micranthus* significantly reduced the triacylglycerol concentrations in diabetic rats. The triacylglycerol concentrations in the diabetic rats reduced with continuous administration of both extracts, however with the methanol extract having a greater significant reduction in the triacylglycerol level of the diabetic rats. Above all, the results of this research indicate that the crude methanol extract of *Loranthus micranthus* leaf is suspected to affect the metabolism of cholesterol in treated diabetic rats. It can also be inferred that the methanol and aqueous extract of *Loranthus micranthus* does not have a significant effect in the metabolism of HDL and LDL in diabetic mellitus.

CONCLUSION

Aberrations in lipid metabolism are common in diabetes mellitus and are predisposing factors to dyslipidaemia (Fuller *et al.,* 1983). The methanol and aqueous extracts of mistletoe *Loranthus micranthus* leaf extracts have hypoglycaemic and antilipidaemic properties by affecting the serum lipid profile of diabetic rats. The antilipidaemic property of the methanol extract is significantly greater than the aqueous extract. These alterations in serum lipid profile in diabetic rats could be beneficial in preventing diabetic complications as well as improving lipid metabolism in diabetics.

Suggestions for further research

Further work needs to be carried out in these two fractions to obtain their pure fractions in order to understand their mechanism of action. Other parts of the *Loranthus micranthus* plant, especially the stem and root should be investigated for antilipidaemic activities. The plant should be investigated for its bioactive compound(s), which should be purified and used for bioassay-directed experiments. The glycoside and alkaloid present in the crude methanol extract of *Loranthus micranthus* leaf should be further investigated for antilipidaemic properties.

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