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Chemical composition of mistletoe extract (*Loranthus micranthus*) and its effect on the protein, lipid metabolism and the antioxidant status of alloxan induced diabetic rats

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The chemical composition of mistletoe and the effect of its aqueous extract on the protein, lipid metabolism and the antioxidant status of diabetic rats was investigated. Twenty male rats, weighing between 90 and 120 g were used. Alloxan was induced into the experimental animals at a dosage of 100 mg/kg body weight. The rats were divided into 5 groups: Group 1 (non-diabetic control) received normal rat feeds (growers mash). Group 2 (diabetic control) received the feeds given to group 1. Group 3 (diabetic) received 20% aqueous extract of mistletoe, group 4 (diabetic) received 40% of the extract while group 5 diabetic rats received 60% of the extract. The duration of the experiment was four weeks. Results indicate that all concentrations of the extract significantly ameliorated the blood glucose, cholesterol, triglyceride, catalase and urea levels of the rats compared with the diabetic group ($P < 0.05$), 40 and 60% of the aqueous extract significantly ameliorated the altered protein status of the rats compared with the diabetic group while none of the concentrations of the extracts administered significantly ameliorated the altered weight of the rats compared with the diabetic and none-diabetic group. The extract possessed antioxidant activity as seen from the total phenolic content, reducing power test and the ameliorating effect on the catalase activity of the diabetic rats. It can be deduced that mistletoe extract has the potentials of ameliorating the hyperglycemia, altered protein, lipid metabolism and the antioxidant status of diabetics.

Key words: Mistletoe extract, alloxan, antioxidant, diabetes, rats

Introduction

Diabetes mellitus is a group of metabolic diseases associated with hyperglycemia, hyperlipedemia, polyuria, polyphagia, ketosis and cardiovascular diseases (King *et al*., 1998). In modern medicine, no satisfactory effective therapy is still available to cure the disease. Though synthetic drugs are used for the management of the disease, there are several drawbacks like resistance to the drugs, severe hypoglycaemia, weight gain as well as high cost of procurement. In the year 2002, about 173 million people suffered from the disease. About two thirds of these people lived in developing countries, making it endemic in these countries. The World Health Organization reported that the number of people with diabetes will increase by 150% in the next 25 years, with an increasing proportion of affected people in the younger age groups (WHO, 2003).This has therefore necessitated the research into plants with anti-diabetic potentials. In addition, although many herbal supplements have been reported for the management of the disease, they are either not easily reachable or lack scientific evidence to support their effectiveness (Vincent and Roger, 2000). Thus, there's need for their scientific investigation to ascertain their effectiveness, toxicity and then provide alternative drugs and therapeutic strategies (Marles and Farnsworth, 1994).Mistletoe is a plant that is well known to the traditional medicine practitioners in Nigeria and Africa

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Table 1. Nutrient composition of the feeds (growers feed) administered to the rats (%).

Nutrient	Percentage composition
Crude protein	16.00
Fat/Oil	5.00
Crude fibre	7.00
Calcium	1.00
Phosphorous (available)	0.45
Lysine	0.75
Methionine	0.36
Salt	0.30

for the management of many metabolic diseases such as hypertension and diabetes Obatomi *et al*.(1994). Mistletoe teas have been used for the prevention and management of strokes in parts of Nigeria and it is also believed to improve the circulatory system and heart function in traditional medicine (Deeni *et al*., 2002). That the plant, possesses some antioxidant activity has been reported (Ademiluyi and Oboh, 2008). However, the mechanism of its anti-diabetic and anti-atherosclerotic actions in diabetes remains unclear. There are indications that this could be as a result of antioxidant activity since some of the phytochemicals present in it could act as antioxidants and bearing in mind that the medicinal values of plants are often attributed to the antioxidant activities of their phytochemical constituents, mostly the phenolics. This thus forms the objective of the study which is designed to investigate the possible role the chemical composition of mistletoe extract will have on the complications that arise from the induction of diabetes in rats using alloxan.

Materials and methods

Preparation of plant materials

The mistletoe (***Loranthus micranthus***) plant used for the experiment was harvested in 2010 and identified by a Taxanomist in the Michael Okpara University of Agriculture, Umudike, Nigeria. The plant was pulverized and dried in an oven at 60^{o}C. It was then ground to flour and stored in an air tight container for further analysis. For the analysis of reducing power, 2 g of the mistletoe flour was dissolved in 20 ml of methanol and left overnight. The mixture was filtered using Whatman No 1 filter paper and the filtrate was used for reducing power assay.

Chemicals: Alloxan, Chlorogenic acid, Gallic acid and Glucose oxidase reagent kits used were obtained from Sigma and Aldrich Chemical Company, UK. Every other chemical used for the animal experiments was purchased locally from Associated Laboratories in Aba, Abia State, Nigeria and was of analytical grade.

Animal experiment

Selection of animals and their care

Twenty white albino male rats (Wistar strain) weighing between 90-120 g used for this experiment were purchased from the University of Nigeria, Nsukka in 2010 and were kept in the animal house of the Federal University of Technology (FUTO), Owerri, Imo State, Nigeria. The animals were acclimatized for a period of 7 days to the laboratory conditions prior to the experiment in line with the University's ethics of animals experiments. Rats were housed in well ventilated colony cages with 2 rats per cage at room temperature (27-30^{o}C) with 12 hours of light and dark cycle and they had free access to drinking water and diets (Ad-libithium). The rats were fed with commercial rat feed (growers mash), obtained from Chukwuma Ventures Ltd, Owerri, Nigeria (Table 1).

Induction of diabetes: The rats were fasted for 24 hours before injection of a freshly prepared solution of alloxan intraperitonially at a dosage of 100mg/kg body weight. This single dose of alloxan produced type 1 diabetes having fasting blood sugar level of 204.37± 1.24 mg/dl after five days of injection of alloxan and this diabetic state was maintained throughout the duration of the experiment.

Experimental procedure

The rats were divided into five groups with four animals in each group. Group 1 rats served as the normal (control) and they received 60% aqueous extracts of normal rat feed for a period of four weeks at a dose of 100 mg/kg body weight.Alloxan was injected into the animals of group 2 at a single dose of 100 mg/kg body weight. Those with fasting blood glucose above 200 mg/dl were categorized into groups 2 to 5.

Group 2 (Diabetic control):

The animals of this group received oral administration of 60% aqueous extract of the commercial rat feed (obtained from Chukwuma Ventures Ltd, Owerri) and water for a period of four weeks at a dose of 100 mg/kg body weight.

Group 3 (Diabetic rats with 20% concentration of mistletoe extract):

The animals of this group received oral administration of 20% concentration of aqueous extract of mistletoe flour for a period of four weeks at a dose of 100 mg/kg body weight.

Group 4 (Diabetic rats with 40% concentration of mistletoe extract)

The rats of this group received oral administration of 40% concentration of aqueous extract of mistletoe flour for a period of four weeks at a single dose of 100 mg/kg body weight.

Group 5 (Diabetic rats with 60% concentration of mistletoe extract)

The animals of this group received oral administration of 60% concentration of aqueous extract of mistletoe flour for a period of four weeks. At the end of four weeks, the animals were starved overnight, stunned by blow and sacrificed by decapitation and their blood was collected from their heart using a 10 ml syringe to estimate their fasting blood glucose, serum protein, triacylglycerol, cholesterol and urea. Their liver was collected to investigate their catalase activity using standard procedures while the changes in their body weights were recorded twice in a week throughout the duration of the experiment.

Similarly, the initial and final body weights were measured with an electronic weighing balance. From these, % Fasting Blood Glucose (FBG) reduction and % weight change were calculated using the formula: Percentage change in weight = Initial weight- Final weight x 100.Initial weight: The percentage growth rate was calculated as: Final weight- Initial weight x 100

Experimental duration

The percentage change in fasting blood glucose was calculated as: Initial fasting blood glucose level- Final fasting blood glucose level x 100.Initial fasting blood glucose level

Determination of glucose

The serum glucose was determined using the glucose oxidase method as described by Cooper (1973).

Determination of total proteins: The total proteins in rat the serum was assayed colorimetrically using the method of Bradford (1976). Total proteins react with Bradford reagent to give a blue complex which is measured colorimetrically at a wavelength 595 nm. The standard curve of protein was prepared using serial concentration of bovine serum albumin.

Determination of triglycrides: Triglycerides were analyzed using the method of Biosystems (NCEP, 2001).

Determination of serum cholesterol: The total cholesterol in the serum was determined by the method of **Stein (1986).**

Determination of Urea: The method of Fawcett and Scott (1960) was used for the assay.

Catalase activity of the rats: The catalase activities in the liver of the animals was determined using the method of Sinha (1972).

Total phenolic assay of mistletoe flour: The total phenolic content of the mistletoe flour was measured using the Folin-ciocalteau method (Singleton *et al*., 1999).

Determination of reducing power: The reductive capacity of mistletoe flour was determined by assessing the ability of the extract to reduce $FeCl_3$ solution as described by Ademiluyi and Oboh (2008) and modified by Eleazu *et al*. (2011).

Statistical Analysis

Data was analyzed statistically using the Statistical Package for Social Science (SPSS) 15.0 windows version. Results are presented as mean ± standard deviation. One way analysis of variance (ANOVA) was used for comparison of the means. Differences between means were considered to be significant at $P < 0.05$ using the Duncan Multiple Range Test.

Results and Discussion

Analysis of the nutritive constituents of the commercial feed that were administered to the rats indicated that it contained 16% Protein, 5% Fat, 7% Crude fibre, 1% Calcium, 0.45% Phosphorous, 0.75% Lysine, 0.36% Methionine and 0.3% salt (Table 1).Alloxan is known to destroy the β-cells of the islet of the Langerhans of the pancreas that function in the regulation of insulin secretion and thus leads to an increase in the blood concentration of glucose and type 1 diabetes mellitus (Eleazu *et al*., 2010). Hence, the increase in the blood glucose of the diabetic animals. However, findings from this study showed that administration of all concentrations of the extract significantly ameliorated the blood glucose level of the rats compared with the diabetic control ($P < 0.05$) (Table 2). In addition, there were no observed significant differences ($P > 0.05$) in the ameliorative effects of 40% and 60% aqueous extracts of mistletoe flour indicating that the ameliorative effect of mistletoe on hyperglycemia is not dose dependent.Diabetes mellitus has been associated with altered protein metabolism as much of the structural proteins are used in gluconeogenesis. This is because insulin is an important physiological factor that plays a key role in the maintenance of protein balance, since it not only stimulates the uptake of amino acids and protein synthesis, but also inhibits protein degradation (Pathak and Dhawan, 1988).

Table 2. Effect of mistletoe extract on biochemical parameters in rats.

	Percentage Concentration of Mistletoe Extract				
	Control	**Diabetic**	**20%**	**40%**	**60%**
Glucose (mg/dl)	$101.30^{d} \pm 0.35$	$204.37^{a} \pm 1.24$	$144.73^{b} \pm 0.13$	$123.05^{c} \pm 0.59$	$118.06^{c} \pm 2.82$
Protein (mg/ml)	$59.0^{c} \pm 0.07$	$55.2^{a} \pm 0.02$	$56.0^{a} \pm 0.05$	$58.6^{c} \pm 0.02$	$56.9^{b} \pm 0.02$
Cholesterol (mg/dl)	$103.50^{a} \pm 0.45$	$184.06^{e} \pm 1.86$	$111.72^{d} \pm 0.64$	$107.02^{b} \pm 0.81$	$108.11^{c} \pm 0.54$
Triglyceride (mg/dl)	115.00 ± 0.07^{a}	165.12 ± 0.01^{e}	140.08 ± 0.00^{d}	134 ± 0.00^{c}	130.05 ± 0.01^{b}
Catalase(µmol/mg protein)	$8.67^{ab} \pm 4.47$	$7.60^{c} \pm 3.8$	$8.10^{b} \pm 6.58$	$8.65^{ab} \pm 2.22$	$8.60^{a} \pm 9.13$
Urea (mg/dL)	$6.47^{a} \pm 0.07$	$15.42^{e} \pm 0.03$	$13.84^{d} \pm 0.03$	$13.50^{c} \pm 0.06$	$13.14^{b} \pm 0.03$

$^{a-d}$Means in the same row with different superscripts are significantly different (P<0.05) (N = 4 animals per group).

Table 3. Effect of mistletoe extract on the body weight of diabetic and non-diabetic rats (g)

	Percentage concentration of mistletoe extract				
Parameter	**Control**	**Diabetic group**	**20%**	**40%**	**60%**
Body weight	105.78 ± 12.61^{a}	89.23 ± 13.07^{a}	102.79 ± 13.31^{a}	96.66 ± 13.15^{a}	93.19 ± 13.96^{a}

N = 4 animals per group.

This explains the decrease in the protein levels of the diabetic animals. In addition, only 40% and 60% concentrations of the administered extract significantly ameliorated the altered protein contents of the animals (Table 2) ($P < 0.05$) compared with the diabetic group. Hypercholesterolemia and hyper-lipidaemia are recognized complications of diabetes mellitus (Sharma *et al*., 1996) resulting from alterations in lipid metabolism characterized by elevated levels of cholesterol and triglycerides and this could account for the elevated levels of cholesterol and triacylglycerol in the diabetic animals. The abnormally high concentration of serum lipids in diabetes is mainly due to an increase in the mobilization of free fatty acids from the peripheral depots, since insulin inhibits hormone sensitive lipase. In addition, increase in the cholesterol levels in the hepatic tissue might be due to an increase in the transport of chylomicron cholesterol to the liver (Chauhan *et al.,*1987). As there is a close relationship between the total cholesterol level and the occurrence of atherosclerosis, the significant reduction in the total cholesterol and triglyceride levels of the diabetic animals after 20%, 40% or 60% administration of the extract (Table 2) ($P < 0.05$) confirms that the plant can be beneficial in preventing atherosclerotic conditions, thereby reducing the possibility of coronary heart disease. This justifies the folklore use of this plant in the management of hypertension.

Renal disease is one of the most common and severe complications of diabetes. The liver is the predominant source of urea production in the body. Urea is a breakdown product of protein diet. Significant elevations in the serum urea levels of the diabetic animals indicate their impaired renal function. Conversely urea synthetic capacity is impaired in patients with liver disease (Haussinger *et al.*, 1990). All concentrations of the extract administered significantly ameliorated ($P < 0.05$) the altered renal function of the diabetic rats (Table 2).

Catalase is an enzymatic antioxidant that is widely distributed in all animal tissues and the highest activity is recorded in the red cells and the liver. Catalase detoxifies hydrogen peroxide (Eleazu *et al*., 2010) and protects the tissue from highly reactive hydroxyl radicals. Thus the reduction in the levels of these enzymes as a result of the alloxan that was injected into the animals may result in a number of deleterious effects due to the accumulation of superoxide radicals and hydrogen peroxide.

Some studies have reported no alterations in the activity of red cell catalase in diabetics (Dohi *et al*., 1998). However, results obtained for this antioxidant enzyme are in agreement with earlier reports by Udoh *et al*. (2007) who reported a decrease in the red cell catalase activity of diabetics. Diabetes is strongly co-related with oxidative stress induction. The recovery of the antioxidant enzyme in the extract, treated group (Table 2), justifies the antioxidant potentials of the plant, making it useful in arresting oxidative stress and this is the most significant finding in this study. In addition, there are indications that mistletoe mimics insulin action by binding to the α-subunits of receptors that are transduced to the β-receptors, promoting rapid autophosphorylation of a specific tyrosine residue of each β-subunit and inducing a conformational change which results in increased antioxidant status of the animals. This further leads to the amelioration of the altered protein and lipid metabolism of the diabetic rats.

The loss in weight in the diabetic animals as evidenced in Table 3 would be expected. This is attributed to the alloxan which was used to induce type 1 diabetes in the diabetic animals. The destruction of the pancreas results in the utilization of non-carbohydrate moieties such as protein for the synthesis of glucose.

Table 4. Percentage change in glucose, weight and growth rate

Parameter	20% Mistletoe extract	40% Mistletoe extract	60% Mistletoe extract
Weight	12.23±3.87	8.47±2.69	2.95±1.44
Growth rate	44.97±6.82	32.57±2.73	10.68±3.54
Fasting blood glucose (decrease)	- 42.88±0.52	- 21.47±0.23	-16.54±3.37

Table 5. Total phenolic content of mistletoe flour

Sample	Phenolic acid equivalent (mg CAE/g fw)
Mistletoe flour	0.24 ± 0.06 mg CAE

CAE =Chlorogenic Acid Equivalent, fw = fresh weight.

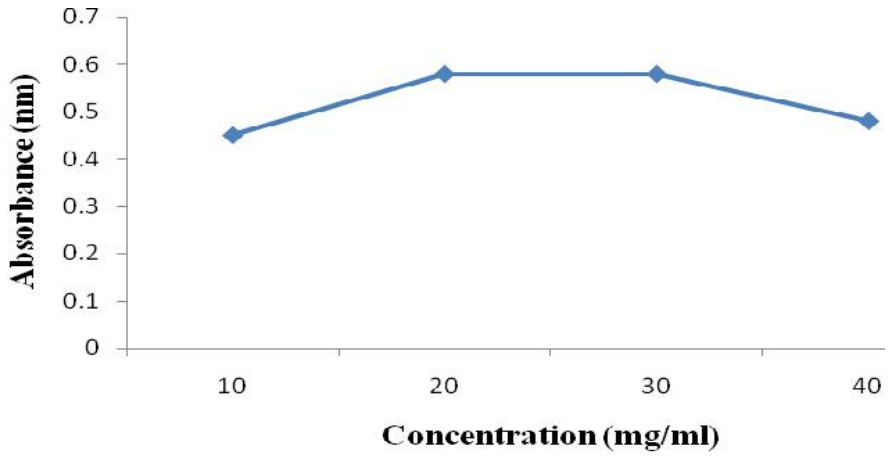

Figure 1. Reductive capacity of mistletoe

This was evidenced in the decrease in the protein content of the diabetic rats. The loss of structural proteins like muscle protein in increased gluconeogenesis together with increased lipolysis and increased synthesis of ketone bodies results in severe weight loss. None of the concentrations of the extracts administered significantly ameliorated the weight loss observed in the diabetic animals compared with the diabetic control ($P > 0.05$).There were 12.23±3.87%, 8.47±2.69% and 2.95±1.44% increase in the weights of the diabetic rats respectively, after administration of 20%, 40% and 60% extracts of mistletoe to the diabetic rats. Similarly, we recorded 44.97±6.82%, 32.57±2.73% and 10.68±3.54% increase in growth rates after administration of 20%, 40% and 60% extracts of mistletoe to the diabetic rats while we recorded 42.88±0.52%, 21.47±0.23% and 16.54±3.37% decrease in fasting blood glucose levels after administration of 20%, 40% and 60% extracts of mistletoe respectively to the diabetic animals (Table 4). The study shows that 20% of the aqueous extract of mistletoe flour was most effective in ameliorating the hyperglycemic status of the diabetic rats with a corresponding increase in body weights and growth rates. In recent years phenolic compounds have attracted the interest of researchers because they show promise of being powerful antioxidants that can protect the human body from free radicals, the formation of which is associated with normal metabolism of aerobic cells (Ademiluyi and Oboh, 2008). Results obtained in table 5 indicate that mistletoe contains significant quantities of phenol, suggesting its antioxidant potentials. Kirkosyan *et al*. (2003) reported that phenolic compounds in plants possess antioxidant activity and may help protect cells against the oxidative damage caused by free radicals.Reducing power is a measure of the ability of the methanolic extracts to reduce Fe^{3+} to Fe^{2+}. Reducing power has been one of the antioxidant capability indicators of medicinal herbs (Duh and Yen, 1997). This is because antioxidants are strong
reducing agents and this is principally because of the redox properties of their hydroxyl groups and structural relationships of their chemical structure (Oboh and Rocha, 2007). Results obtained from the reducing power test as shown in Figure 1 indicate the antioxidant potentials of the extract. Maximum reductive capacity of the extract was observed to occur at 30 mg/ml. Correlation between glucose and catalase as shown in Table 6 was significant (- 0.968) and this indicates that

Table 6. Correlation between glucose and catalase.

	Catalase
Glucose	-0.968**

**Correlation is significant at 0.01 level.

elevated blood glucose levels could lead to a significant decrease in antioxidant status of the diabetic animals and vice versa.

Conclusion

The result of the study reveals that mistletoe has considerable antioxidant potentials as seen from the total phenolic content, reducing power test and its effect on the red cell catalase activity of the diabetic animals, justifying its therapeutic use in herbal medicine. In addition, the plant has proved to have anti-diabetic and anti-lipidemic potentials and could be useful in managing the complications arising from diabetes mellitus such as hypercholesterolemia, hyper-triglyceridemia, impaired renal function, protein metabolism and antioxidant status.

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