Review Article

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# Effects of Methanolic Extract of Mistiltoe (*Viscum Album*) on Alloxan Induced Diabetic Albino Rats

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## Abstract

The plant Viscum album is used in folklore medicine to treat various diseases most especially diabetes mellitus. In this study, the antidiabetic potentials of the methanolic extract of Viscum album leave were evaluated using alloxan induced diabetes rats. Diabetes was induced using alloxan and 60mg/kg body weight. The diabetic rats were treated 1500 and 750 mg/kg body weight of viscum album leave extract and glinbenclamide was used as standard drug. During administration of extract, blood glucose level and body weight of the rat were being monitored at three days interval. There was a significant decrease in blood glucose level and body weight at three days interval. There was significant increases in ($p<0.05$) in alloxan induced group after 21 days oral administration of the extract at 1500 and 750 mg/kg. Body weight shows significant reduction in total cholesterol, triglyceride, low lipid density, lipo protein when compared to diabetic control and high density lipo protein showed significant increase (<p0.05) when compared to the diabetic control. Viscum album can be used in management of diabetes mellitus.

**Keywords:** Albino; Alloxan; Diabetic; Effect; Extract; Induced; Methanolic; Mistiltoe; Rats.

## Introduction

Diabetes is a disease condition resulting to the body's ability to produce or respond to the hormone insulin is impaired, resulting in abnormal metabolism of carbohydrates and elevated levels of glucose in the blood. Diabetes is considered a metabolic disease characterized by a disorder in the regulation of carbohydrate metabolism. It results to a high blood glucose level or hyperglycemia. The predictions made by the WHO indicate that the global growth in the world of the prevalence of diabetic patients, mostly type2, will reach 439 million people by 2030 [1]. Huge progress has been made in pharmacological discovery of new treatments. The cost and availability of modern therapies, however, still make it difficult for a great proportion of African populations to fully take benefit of these therapies. The use of antidiabetic plant extracts; thus, will remain a common and unavoidable practice [2]. Accumulating evidence suggests that oxidative cellular injury caused by free radicals contributes to the development of diabetes mellitus [3]. Free radicals are either generated by cellular metabolisms such as glycolysis, mitochonchrial respiration, and xenobiotic detoxification or by exogenous factors such as red-ox reactions. Some are extremely reactive and therefore interact with some "vital" macromolecules including lipids, nucleic acids and proteins [4]. The cells have numerous defense systems (enzymic and nonenzymic antioxidants) to counteract the deleterious effects of ROS and free radicals. Moreover, diabetes also induces changes in the tissue content and the activity of the antioxidant enzymes.

*Viscum album* commonly known as mistletoe and awushie in Igbo language,kauche in hausa,afomo by Yoroba's, libior by Obudu,(Nigeria language)belongs to family loranthaceae, is a semi parasitic plant growing on various host trees and shrubs [5]. The part of the plant majorly used for medicinal purposes is the leaf and twig part. As it (mistletoe) grows on a tree, it uses its roots to anchor on the tree's bark, which allows it to absorb the tree's nutrients. In most cases, mistletoe can harm a tree and cause the branches of the tree to be deformed, but usually it doesn't kill its host. If the host dies, the mistletoe dies. Mistletoe has the potency of producing its own food by photosynthesis, and is able to live on its own, although it is

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mostly found in trees. It's common for a mistletoe plant to grow on top of another mistletoe plant.

*Viscum album* has been claimed to be antidiabetic [6], immunomodulatory [7], bacteriostatic [8] antihypertensive and reduces cholesterol level [9] and therapeutic values for many other ailments. The herb has been analyzed to contain lectins, viscotoxin, polysaccharides and alkaloids as active constituents [10]. The effectiveness of plant to cure ailment in tradomedical practices may not be unconnected with the natural products present in the plant and their abilities to act as radical scavengers.

**Pharmaceutical importance**

As Pharmaceutical Plants in the early 18th Century, Sir John Colbatch (1719-20) stated that mistletoe was a "most wonderful specific remedy for the cure of convulsive distempers." He referred to Viscum album which has since left the realm of witchcraft, herbalism, and quackery to become an accepted pharmaceutical plant. An excellent well-documented account of this transition is given by Kanner (1939) who states that until the middle of the 19th century, the plant enjoyed a prominent place in the medicine and pharmacology of the day. It then fell into disrepute and was not used except as an external treatment for dermatitis (Riehl 1900). Gaultier (1906) discovered a depressor action in its extract which restored its medical prestige in France and subsequently other European countries.

## Materials and Method

Mill grander, Timer, Beaker, Weighing balance, Glucometer, Razor blade, Syringe and needle, spectrophotometer, analytical weighing balance, centrifuge Methanol, Alloxanglucose, randox, triglyceride, total cholesterol, high density lipo protein reagents.

**Collection of Plant Materials**

Mistletoe (*Viscum album)* leaves used for this study wasobtained from Neem tree (host plant) in MubiNorth Local government Area of Adamawa State. Department of Agricultural Engineering, Federal Polytechnic Mubi confirmed the botanical identification ofthe plant leaf. The voucher samples are kept in the Polytechnicherbarium for reference.

**Preparation of The Methanolic Plantextract**

The plant materials were shade-dried and ground to powder. Extraction was performed at room temperature. Five hundred grams (500g) of the dried and ground powdered plant materials were soaked in 1Litre of 70% methanol for 72 hours and stirring intermittently. The extracts were filtered and filtrate were concentrated at 400C and kept in a desiccator for use. Thus, the percentage (%) yield of the extract was calculated using the formula:

$$\text{Yield (\%)} = \frac{\text{Weight of extract recovered}}{\text{Weight of dry powder}} \times 100$$

**Animals**

Male Wistar rats (100-140g) were obtained from the Federal University of Technology, Yola, Adamawa State; from biochemistry department. They werehoused in cages and kept in a room where a12-hour light/dark cycle was maintained. They were allowedfree access to water and feed diet (product of Pfizer NigeriaLtd) throughout the period of the experiment

**Induction of Diabetes in Rats**

After two weeks of acclimatization, the rats were subjected to a 16 hour fasting. Diabetes was induces with a single intraperitoneal injection of alloxan at a dose of 60mg/kg body weight. The alloxan was freshly dissolved in normal saline [11]. The injection volume was prepared to contain 1.0 ml/ weight, [12]. After 3 days, blood glucose levels were measure using glucometer (Accu-Cheek) and the animals with a glucose concentration of more than 230 mg/dl were classified as diabetic and thus, selected for the research [13]. The extracts and glibenclamide were administered on a daily basis to the experimental rats for 21 days by gastric intubation. Blood sugar levels were determined every three days (3days) using glucometer. Their body weight was also determined weekly. At the end of 21 days, the rats were anaesthetized using chloroform and sacrificed 24 hours after the last treatment. Blood samples were collected in specimen bottles, allowed to clot, centrifuged and serum were collected for the biochemical parameter analyses.

**Treatment**

Twenty male Wistar albino rats were used in this study. The rats were randomized and divided into five groups of four animals each.

Group I: Normal, received normal saline solution (0.9% NaCl w/v, 5 ml/kg).

Group II: Diabetic, received alloxan (60 mg/kg body weight) once

Group III: Diabetic, rats were treatedwith methanolic extract of Viscum album (1500mg/kg body weight)

Group IV: Diabetic rats were treated with methanolic extract of Viscum album (750 mg/kg body weight).

Group V: Rats induced with diabetes and treated with glibenclamide 5mg/kg body weight

## Measurement of Blood Glucose Levels

Fasting blood glucose levels was determined using the method described by [14]. Exactly 1 ml of glucose working reagent containing glucose oxidase, peroxidase and 4- amino antipyrine as chromogen was pippetted into all the required test tubes. Then 20µL of the test samples was pippetted into the sample test tubes. Carefully, 20µL of standard reagent was pippetted into the standard test tube. They were all incubated at room temperature for 30 minutes and absorbance was read spectrophotometrically at 510nm.

Serum Lipid Profile Determination

**Total Cholesterol Estimation:** This was carried out using the method described by [15].

**Procedure:** Exactly 1 ml of the cholestrol reagent was added to all the required test tubes. 10µL of the sample was added to the test sample test tube, 10µl of standard reagent was added to the standard test tube and none to blank. It was incubated at room temperature for 20min.The absorbance of the test sample and the standard was read at 505nm and the concentration of the sample was calculated using the formula;

$$\frac{\text{Absorbance of test}}{\text{Absorbance of Standard}} \times \text{Concentration of Standard}$$

**Determination of Serum Triacylglycerols**

The serum triacylglycerols level was determined by method of [16]. Triglycerides are converted into quinoneimine dye. Quinoneimine dye is formed at a rate proportional to triglyceride concentration in the serum. It is detected and quantified spectrophotometrically. The value was expressed in the unit of mg/dl.

Procedure: Just 1 ml of the sample reagent was added to all the required test tubes. 10µL of the sample was added to the test sample test tube, 10µl of standard reagent was added to the standard test tube and none to blank. It was incubated at room temperature for 15min. The absorbance of the test sample and the standard was read at 500nm and the concentration of the sample was calculated using the formula;

$$\frac{\text{Absorbance of test}}{\text{Absorbance of Standard}} \times \text{Concentration of Standard}$$

**High Density Lipoprotein Cholesterol (HDL-c) Estimation**

This was determined using assay kit as described by [17].The value was expressed in the unit of mg/dl.

Procedure: The proteins were precipitated using phosphotungstic acid, in the presence of magnesium and all other cholesterol in the solution. 1 ml the sample reagent was added to the required test tubes, 10µl of the sample was added to the test sample test tube, and 10µl of standard reagent was added to the standard test tube and none to blank. It was incubated at room temperature for 15min. The absorbance of the test sample and the standard were read at 500nm and the concentration of the sample was calculated using the formula;

$$\frac{\text{Absorbance of test}}{\text{Absorbance of Standard}} \times \text{Concentration of Standard}$$

## Statistical Analysis

The data were expressed as mean and standard error of mean. Statistical evaluation of data was performed using oneway analysis of variance ANOVA followed by Duncan's multiple range test (DMRT) (Duncan 1957) and consider significant at p>0.05 using SPSS software.

## Result, Data Presentation Analysis and Discussion

Percentage Yield

The Percentage yield following the methanolic extraction of leaves extract of Mistletoe (*Viscum album*) is shown in (**Table 1**).

***Table 1:*** *Percentage yield of methanolic extract of the leaves of Viscum anlbum.*

| Leave | % yield |
|---|---|
| *Viscum album* | 11.4 |

***In Vivo* Study**

**Body Weight**

Figure 1: shows the effect of oral administration of methanolic extract of *Viscum albumleave* and Glibenclamide on body weight of diabetic and non-diabetic rats for 21 days. The diabetic control group showed a sharp decrease in body weight when compared with the normal control group. The extract group showed a relative improvement in body weight when compared to the diabetic control group.

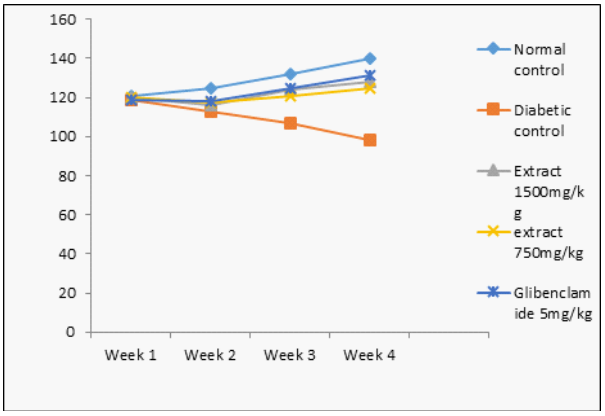


***Figure 1:*** *Effect of Methanolic Leaf Extract of Viscum album on Body Weight Alloxan induced Albino rats.*

**Assessment of Fasting Blood Glucose Level**

Table 2: shows the effect of daily oral treatment of methanolic extracts of *Viscum album* leave and Glibenclamide (standard drug) on serum blood glucose after 21 days of treatment (after induction with Alloxan). The blood glucose levels of diabetic control rats increased significantly ($P < 0.05$) when compared with the normal control. In the treated diabetic group, a significant decrease in blood glucose levels compared with diabetic control group was observed. There is also a significant decrease ($P < 0.05$)in the glibenclamide treated group.

***Table 2:*** *Effects of methanolic leave extract of Viscum album and Glibenclamide on fasting blood glucose level in experimental rats after 21 days of treatment.*

| GROUPS | TREATMENT | BLOOD GLUCOSE LEVEL (mg/dl) |
|---|---|---|
| A | NC + 0.9% NaCl | 76.92 ±1.37$^{a}$ |
| B | DC | 184.45±3.26$^{c}$ |
| C | D + 1500mg/kg b.w | 133.88±1.96$^{b}$ |
| D | D + 750mg/kg b.w | 136.14±1.69$^{b}$ |
| E | D + Glibenclamide5mg/kg b.w | 84.84±9.94$^{a}$ |

*Values are means ±SEM of four replicates for each group (P< 0.05).*
*NC = normal control*
*DC = diabetic control*
*D =Diabetic*

**Assessment of Lipid Profile**

The effect of oral administration of methanolic extract of *Viscum album* leave and glibenclamide on serum HDL-cholesterol, LDL-cholesterol, and Triacylglycerols (TG) on diabetic and normal rats for 21 days is presented in (**Table 3**): The result showed a significant (P<0.05) increases in the level of triacylglycerols (TG), and LDL-cholesterol in diabetic control group when compared with normal control group. There was a significant (P<0.05) decrease in the level of HDL-cholesterol in diabetic control group compared with normal control rats.

***Table 3:*** *Effects of methanolic extract of Viscum album leaveand Glibenclamide on serum lipid profile in Alloxan induced diabetic rats.*

| GROUPS | TREATMENT | TC (mg/dl) | TG(mg/dl) | HDL-C (mg/dl) | LDL-C (mg/dl) |
|---|---|---|---|---|---|
| A | NC + 0.9% NaCl | 159.76±1.57 | 20.73±2.16 | 505.20±2.13 | 349.5±4.61 |
| B | DC | 517.61±6.04 | 128.65±1.56 | 29.18±5.15 | 73.74±0.64 |
| C | D + 1500mg/kg b.w | 161.88±2.32 | 120.26±0.32 | 96.06±1.22 | 41.77±3.91 |
| D | D + 750mg/kg b.w | 204.12±1.47 | 98.93±1.85 | 71.20±8.70 | 113.14±2.38 |
| E | D + Glibenclamide 5mg/kg b.w | 196.30±3.09 | 127.90±1.25 | 131.53±5.04 | 39.19±1.38 |

*Values are means ±SEM of four replicates for each group. Values with different superscript (a, b, c, d, e) on the same column are statistically different (P< 0.05).*

*TC = Total Cholesterol*
*TG = Triglycerides*
*HDL-C = High Density Cholesterol Lipoprotein*
*LDL-C = Low Density Cholesterol Lipoprotein*

The result obtained from this research work shows that there was asignificant increase(P<0.05) in fasting blood glucose (FBS) levels as observed in the diabetic control group compared to the normal control. The oral administration of the plant extract showed a significant reduction (P<0.05)in glucose level compared to the group treated with the Glibenclamide (standard drug) (Table2). The group treated with 1500mg/kg of the extract showed high reduction of glucose level close to normal compared to the group treated with 750mg/kg body weight of the extract. The hypoglycemic activity of the extract may be due to its protective action against Alloxan-mediated damage to the pancreatic ß-cells and also possibly through regeneration of damaged ß-cells or increased insulin release or secretion which is in accordance with (Subramoniam et al., 2012). Some of these phytochemicals (saponins, flavonoids, tannin and alkaloids) are believed to be responsible for the blood glucose lowering effects of these plant materials. It may also be that the extract regulate the absorption of glucose in the gastrointestinal tracts thereby reducing the postprandial glucose or regulate the metabolism of glucose in the liver by reducing hepatic glucose production.

Groups administered with oral dosage of extract (1500 and 750mg/kg body weight) and Glibenclimide 1.4mg/kg body weight (drug control) showed significant decrease (p<0.05) in serum lipid level when compared with the diabetic control group. The positive control showed increased TC, TG and LDL-C, and increased HDL-C. The lipid lowering effect of the extract may be due to the presence of phytochemicals such as flavonoids, saponins (marounek et al., 2007). The arthrogenic index of all treated groups were significantly decreased (p<0.05) compared with the positive control group. The most effective group was those of drug control and the least effect was observed in the group treated with 750mg/kg body weight of extract. The elevation of Atherogenic index in the positive control group may be due to the increased TC, TG and LDL-C and decrease in HDL-C level. This agreed with a previous research that elevation of serum TC, TG and LDL-C along with decreased HDL-C level are known to cause hyperlipidemia which is responsible for initiation and progression of atherosclerosis impasse.

HDL-C function in the transport of cholesterol away from the peripheral tissue to the liver, thus preventing the genesis of atherosclerosis. The observed significant increase in the level of HDL-C further point to the cardiac protection activity of the extract.

## Conclusion

The results of this study indicated that the extract of *Viscum album* leavepossess hypoglycemic and hypolipidemic. Therefore it may be useful in controlling diabetes and its related complications. Despite the use of advanced oral hypoglycemic agents for the management of diabetes, use of herbal remedies is gaining higher importance because these oral hypoglycemic agents have drawbacks and limitations.

## Recommendations

This project work aimed at determining the glucose lowering effect of *Viscum album* in alloxan induced diabetic rats recommend further research on the plant on the following:

1. To check the effects of the leave extract on the activities of some selected diabetic related carbohydrate metabolizing enzymes.

2. To assess the effect of the leave and composite extract on endogenous antioxidant such as glutathione.

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