Effect of crude extract of *Viscum album* (mistletoe) on plasma lipids: an insight into its possible antihyperglycaemic and antihypertensive properties


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Abstract

**Background:** Reports on the hypoglycaemic property of mistletoe leaf is abundant in the literature. In this study, we have investigated this property in relation to the lipid profile in Wistar rats.

**Methods:** Six groups of five rats per group were used for the study. Diabetes was induced with alloxan (60mg/kg, ip) and hypertension by high salt-loading. The treated groups received the crude extract (150mg/kg, oral) for 5 weeks in addition to normal rat feed, water *ad libitum*. Plasma glucose, total cholesterol, triglyceride and LDL-cholesterol levels were determined using standard procedures.

**Results:** The diabetic and the hypertensive rats had about 30.8% and 9.1% increase in blood sugar levels respectively compared to the control. The extract (150 mg/kg, oral) produced about 84.2% and 33.2% (P<0.05) decrease in blood sugar levels in the diabetics and hypertensives respectively. Also the total cholesterol, triglyceride, and LDL-cholesterol levels were increased in both diabetics and hypertensives (P < 0.05). The LDL levels were lowered by the extract by about 21.4% and 24.3% (P<0.05) in the diabetics and hypertensives respectively. Finally the extract also produced about 34.5% and 8.3% depression of blood pressure of the hypertensives and normotensives respectively.

**Conclusion:** We suggest the reduction of plasma lipid fractions by the extract as being responsible for the antihyperglycaemic and antihypertensive activities of the mistletoe extract.

**Key words:** Mistletoe, Extra, Antihyperglycaemic, Lipid fraction, Depression

Introduction

*Viscum album* (mistletoe) is a semi-parasitic plant that bears green leaves and can photosynthesize its food. It is found growing on host plants such as avocado pear, cocoa trees, *abbies alba* and citrus plants. It is well distributed in most parts of the world including Europe, South Korea, France, Canada, Argentina and Africa. Extract preparations (Iscador or Helixor) from *Viscum album* have been reported to be antidiabetic, antihypertensive and anticancerous. The potency of the leaf extract depends on the host plant. Phytochemical analysis of extracts from leaf of *Viscum album* have shown that it contains phenylpropan, flavonoid derivatives, phenolic compounds, n-butanoic fractions, Tanins, Saponins, Lectin, Viscotoxins, arabinogalactans and Choline - a derivative of acetylcholine. The presence of all these compounds probably gives the mistletoe leaves extract its immunoregulatory functions.

The stimulation of insulin secretion from clonal pancreatic beta-cells and decreased serum glucose levels in both diabetic and non-diabetic animals have also been reported. The European mistletoe strengthens the capillary endothelium and reduces blood pressure as well as the heart rate. Its cardiotonic action is thought to be due to it ligands while the hypotensive action is believed to be due to the presence of choline group. With the increase in cases of diabetes and hypertension in the world, series of therapeutic measures have been adopted in the management of these two disorders. Traditionally, various herbs have been used in an
attempt to manage the alarming increase in these disease states yet the quest for further trials is unending. Thus, the leaves of this Nigerian species of mistletoe (extract) are investigated in the current study to provide a scientific basis for the use of this plant in the treatment of diabetes and hypertension.

Materials and Methods

Preparation of the crude extract
The crude extract was prepared as follows; fresh leaves of *Viscum album* from the host plant (citrus) were collected from a local plantation in Akpabuyo, Nigeria during the rainy season. The leaves were first washed free of sand and debris. Wash water was blotted off and the leaves ground to paste. A quantity of the ground sample (100 g) was weighed and soxhlet extracted with 150 ml distilled water at 100°C for 10 hrs. Where larger ground samples were used, extraction was done under reflux with an appropriate volume of water. The extract was slowly evaporated in vacuo. A total yield of 5.0 ± 2.3 g was obtained. Weighed samples of the extract were then used to prepare test solutions of the desired concentrations as has been done previously.

Acute toxicity test
Seventy male white Wistar mice (18–20 g) were randomly assigned to seven groups of ten mice per group. The animals were allowed to acclimatize to their environment before the commencement of the experiments. The control group received normal saline while each of the test groups was injected intraperitoneally with one of the following doses of the extract: 25, 50, 100, 200, 400 and 800 mg/kg body weight. The maximum volume injected was 0.5ml. The injected mice were returned to their cages, water and feed were given *ad libitum*. The mortality in each cage was assessed 24 hours after administration of the extract. The percentage mortalities were calculated using the log probit method and plotted against the log _50_. The results were subjected to statistical analysis of the regression.

Determination of glucose level and lipid profile in the rats
Thirty male albino Wistar rats of 0.22 kg average body weight were obtained from the animal house of the Department of Physiology, University of Calabar, Nigeria. The rats were randomly assigned into three batches of ten animals each: batch 1 = normotensives, batch 2 = diabetics and batch 3 = hypertensives. Each batch was further divided into two groups of five animals per group. A group from each batch was treated with the crude extract (150 mg/kg, oral) daily for 5 weeks. All animals were fed daily with the normal rat feed (Pfizer Nig. Ltd) and water *ad libitum* for 6 weeks.

To induce diabetes, the animals were injected with alloxan (60 mg/kg i.p) daily for 3 days. Hypertension was induced by placing the animals on high salt diet consumption (8% NaCl and 1% saline for drinking) for a period of 6 weeks. In the diabetic batch (treated and untreated groups) blood samples were collected by cardiac puncture under light ether anesthesia. They were stored in fluoride oxalate bottles at -4°C for glucose determination using the Biosystem kit. Copper reduction method was employed.

For batches 1 and 2, blood samples were collected via the carotid artery at the end of in vivo blood pressure (BP) measurements. The BP in these groups was measured by standard method. The animals were anesthetized with sodium pentobarbitone (50 mg/kg ip.), the trachea was intubated and the carotid artery, cannulated for blood pressure (BP) measurements. The BP was recorded on the polygraph machine (SN 000237, M/N 30) using the BP transducer (M/N MB 5049/41-6A). The mean arterial pressure (MAP) was calculated by using the formula: MAP = DP + 1/3 (SP-DP), where SP and DP represented the systolic and diastolic pressures respectively. Simultaneously, the heart rate was monitored via a tachometer on a different channel of the same recording machine. Animals with MAP below 140mmHg were not considered hypertensive, and were therefore discarded. At the end of every experiment blood samples (5 ml) were taken via the carotid artery for analysis, before sacrificing the animal. For cholesterol level determination, blood
samples were subjected to ultra centrifugation at 2000 revolutions per minute (RMP) for 5 minutes to separate plasma from serum. Enzymatic cholesterol colorimetric kit was used for analysis and the absorption measured at 550 nm. The method of Siedel et al was used to determine total cholesterol (TC) while triglyceride (TG) was also determined by the method of Sullivan et al.

Low density lipoproteins (LDL) was calculated from the total plasma cholesterol and triglyceride (TG) obtained based on Friedewald et al. Friedewald equation states that LDL = TC - HDL + TG/2.23, where HDL is the high density lipoprotein.

Data analysis
Data are presented as mean ± SEM. One way analysis of variance (ANOVA) was used where the comparison involved more than two groups. Student’s unpaired t-test was used to compare difference between two groups. Difference between group means were considered to be significant at P<0.05.

Results

Acute toxicity test
The acute toxicity studies (Figure 1) showed that the crude extract from the leaves of Viscum album had LD50 value of 0.4g/Kg mice i.p. The high dose recipients were found to be immobile and cold to touch with piloerection and mean rectal temperature of about 26 ± 2.1°C.

Blood sugar levels in hypertensive and diabetic rats
Blood sugar (Figure 2) rose from about 5.49 ± 0.05 mmol/L (normoglycaemic level) to about 7.94 ± 0.3 mmol/L in the diabetic untreated rats, representing about 30.8% increase. In hypertensive untreated animals (Figure 2) the increase was from 5.49 ± 0.05 mmol/L to 5.99 ± 0.2 mmol/L thus showing about 9.1% increase and this was significant (P < 0.05). Following the administration of the extract (150 mg/kg oral daily for 5 weeks), the blood glucose level in diabetic and hypertensive rats were significantly lowered (Figure 2). Blood glucose level in extract-treated diabetics decreased from 7.94 ± 0.05 mmol/L to 4.31 ± 0.38 mmol/L representing about 84.2% decrease (P < 0.05). In the extract-treated hypertensives, the decrease in blood glucose was from 5.99 ± 0.2 mmol/L to 4.0 ± 0.1 mmol/L (33.2% decrease, P<0.05).

Lipid profile in the diabetic and hypertensive rats
Figure 3 shows the lipid profile of animals in the three study batches (normotensive, diabetic and hypertensive). There were significant increases in the total cholesterol (TC), triglycerides (TG) and low density lipoproteins (LDL) in the untreated diabetics and hypertensives when compared with the normotensive untreated group (P < 0.05). The levels of the high density lipoprotein-cholesterol (HDL-c) in both the untreated hypertensives and diabetics was not significantly different from that of the normotensive untreated group (P>0.05).
Effect of crude extract of *Viscum album* (mistletoe) of E. E. Nkanu, A. E. Eno, O. E. Ofem, J. O. Imoru and F. B. Unoh

**Figure 2. Histogram showing the effect of aqueous extract (150 mg/kg) on blood glucose level of the controls, hypertensive and diabetic rats. Each point represent mean ± SEM**

Treatment of the diabetic and hypertensive groups with the extract (150mg/kg oral) produced significant lowering effect of plasma lipids (P<0.05) (Figure 3). The total cholesterol (TC) level was lowered from 1.43 ± 0.03 mmol/L to 1.22 ± 0.03 mmol/L (14.6% decrease) in the diabetics, in the hypertensives the TC decreased from 1.77 ± 0.04 mmol/L to 1.40 ± 0.05 mmol/L (20.7% decrease). These decrease were significant (P<0.05 in both cases). The LDL-c level decreased significantly from 0.98 ± 0.07 mmol/L to 0.77 ± 0.04 mmol/L (21.4% decrease) in the treated diabetics and 1.11 ± 0.04 mmol/L to 0.84 ± 0.05mmol/L (24.3% decrease) in the hypertensives. The HDL-c levels in the hypertensives and diabetics that received the extract treatment were not significantly different from their respective (controls) untreated groups, (P>0.05) (Figure 3). Also in the normotensive groups, there were no significant difference between the untreated and treated normotensives regarding the blood levels of TC, TG, LDL-c and HDL-c.

**Blood pressure experiment**

The results of the blood pressure experiments are summarized in Table 1. The control mean arterial pressure (MAP) of normotensive rats was 90.2 ± 4.3mmHg and the control heart rate (HR) was 139.8 ± 1.6 beats/min. Salt-loading for 6 weeks raised the MAP to 145.3 ± 6.2mmHg and the HR to about 165.6 ± 4.8 beat/min.

**Figure 3. Histogram showing effect of the aqueous extract (150 mg/kg) on the total cholesterol (TC), High density Lipoprotein- cholesterol (HDL-c), low density lipoprotein cholesterol (LDL-c) and Triglyceride (TG) levels of normotensive, diabetic and hypertensive rats. Each point represents the mean ± SEM**

However, extract treatment produced a reduction of MAP to a level of 95.2 ± 3.8mmHg, representing approximately 34.48% reduction. Furthermore, the HR was reduced to approximately 99.5 ± 6.4 beats/min. This is approximately 39.92% decrease in hypertensive heart rate produced by the extract. However, the effects of the extract on normotensive rats was slight and not statistically significant (P>0.05). In this group, the extract reduced the MAP by approximately 8.31% and the HR by approximately 10.44% when compared with untreated counterparts (Table 1).

**Table 1. Effect of crude extract (150 mg/kg oral) of *Viscum album* (mistletoe) on blood pressure and heart rate of male rats**

<table>
<thead>
<tr>
<th>Experimental Groups</th>
<th>MAP (mmHg)</th>
<th>HR (beats/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normotensives (untreated)</td>
<td>90.2 ± 4.3</td>
<td>139.8 ± 1.6</td>
</tr>
<tr>
<td>Normotensive (treated)</td>
<td>82.7 ± 5.5</td>
<td>125.2 ± 7.4</td>
</tr>
<tr>
<td>Hypertensives (untreated)</td>
<td>145.3 ± 6.2</td>
<td>165.6 ± 4.8</td>
</tr>
<tr>
<td>Hypertensive (treated)</td>
<td>95.2 ± 3.8*</td>
<td>99.5 ± 6.4*</td>
</tr>
</tbody>
</table>

Data: mean values ± SEM. n=5.

*Significantly different from untreated group, P<0.05.
Discussion

The results obtained from the current study suggested that the crude extract from the Nigerian species of *Viscum album* (mistletoe) is moderately toxic. This suggestion is based on the very high LD$_{50}$ value (0.4g/kg mice, i.p.) of the leaf extract. It is documented in the literature that diabetes (type I and II) and hypertension are amongst the mistletoe-treatable diseases. However, the mechanism(s) by which this extract reduces hyperglycaemia in diabetic patients and hypertension in hypertensives are still speculative. In the diabetics, it is suggested that an agent in the extract could evoke a stepwise stimulation of insulin production from clonal pancreatic beta-cells, thereby promoting insulin availability in blood plasma, with a resultant depression of circulating glucose levels$^{19}$.

In the present study, we have obtained evidence that could suggest that, the reduction of plasma lipid levels by the extract as an additional mechanism. In fact, it has already been known that high plasma cholesterol and low levels of the high density lipoproteins HDL (i.e. the “good” cholesterol) are very critical in the assessment of risk factors for coronary heart disease (CHD) or an important diagnostic parameters for the disease$^{20,21}$. Our investigations showed that the elevated levels of total cholesterol, glucose, triglycerides and LDL-c in diabetic animals were markedly reduced by the crude mistletoe leaf extract. It had earlier been suggested that hyperglycaemia was closely related to hypercholesterolemia, hypertriglyceridaemia and elevated LDL. These are all documented as risk factors for CHD$^{22-24}$. Therefore, the reduction of plasma glucose level by the crude mistletoe extract could not be unrelated from its marked depression of plasma lipid fraction levels in the diabetics, suggesting a possible dual pathway mechanism employed by the mistletoe crude extract administration.

Reports had suggested that salt-loading in rats led to insulin resistance which led to the impairment of one of the many metabolic steps situated downstream of phosphatidylinositol-3 kinase activity and insulin signaling$^{25,26}$. It is therefore not surprising that in our salt-induced hypertensive animals, the blood glucose levels were markedly higher than those of normotensive controls, as their hypertensive condition were induced by prolonged salt-loading. Salt-loading in rats is said to produce impaired glucose uptake in peripheral tissues$^{25,26}$. Therefore, it is unlikely that hypertension caused by methods other than salt-loading could affect blood glucose level.

Apart from elevated blood glucose in the hypertensive group, the results also showed elevated levels of TC, TG and LDL-c. These observations point to the existence of an indirect relationship between the plasma lipid fraction levels and hypertension, as was the case in diabetes. Lipids had been repeatedly shown to predict cardiovascular events$^{17-19,21}$ and there was a call for physicians to pay particular attention to a patient’s blood lipid levels when treating high blood pressure$^{27}$. Mistletoe leaf extract had no effect on the heart rate but produced a possible depressor effect on the peripheral blood vessels$^{28}$. It was therefore very apparent that the vasodilator effect of the extract coupled with depressed lipid fraction levels culminated in the reduction of arterial blood pressure.

Interestingly, the extract did not alter the lipid profile in normoglycaemic and normotensive animals. Also, in the diseased conditions (diabetes and hypertension), the plasma levels of the “good” cholesterol (HDL-c) were not significantly altered. A similar result had earlier been reported for the effect of extract of *Sievia rebaudiana* leaves on blood glucose levels$^{30}$. The marked depression of the LDL-c (“bad” cholesterol) levels in diseased (diabetes or hypertension) by the mistletoe extract could provide an insight into the possible mechanism of action employed by the crude extract.

In conclusion, the results from this study agree with earlier reports that mistletoe leaf extract could stimulate insulin surge from the pancreatic beta cells in diabetes. However, our study implicates its reduction of plasma lipid fractions particularly the “bad” cholesterol (LDL-c) as a possible additional mechanism in both diabetes and hypertension.
Acknowledgment

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References


23 Erkelens DW. Insulin resistance syndrome and type 2 diabetes mellitus. Am J Cardiol 2001; 88: 38F-42F.


