Hypoglycemic effect of Phragmanthera incana (klotzsch) on alloxan-induced diabetic albino rats

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Anti-diabetic effect of Phragmanthera incana, a mistletoe species growing on Theobroma cacao (Cocoa) and Cola nitida (Kolanut) were investigated based on its ethnomedicinal claims for treatment and management of diabetes in Nigeria. Diabetes was induced by intraperitoneal injection of alloxan (100mg/kg) in albino rats. After 4 days monitoring, rats with RBS of ≥150mg/dl were considered diabetic and included in the study. Eight groups of diabetic rats (n=5), and a ninth group of non-diabetic rats were used for the study. Three groups of diabetic rats were assigned to each extract and administered doses of 200, 400 or 800mg/kg. One group was administered with glibenclamide and the eighth group was left untreated. RBS were monitoring within 24 hours and days 3, 7, 10 and 14 post-administration of extracts. The extracts reduced RBS within 1 hour post-administration of the extract, and through the course of the experiment. Twenty four hours post-administration of extracts, rats administered 800mg/kg exhibited the lowest RBS, but in the overall, 400mg/kg dose achieved the best control of blood glucose levels. The extracts achieved a more significant control of blood glucose levels than glibenclamide. This study justifies the use of this plant in ethnomedicine for management of diabetes mellitus.

Key words: Phragmanthera incana, theobroma cacao, cola nitida, alloxan, diabetes.

INTRODUCTION

Mistletoe is a common name for various parasitic plants belonging mostly to Loranthaceae family (Watson, 2001; Judd et al., 2002). Ancient Europeans believed that the mistletoe plant held magical powers to bestow life and fertility, bring peace, and protect against disease. Northern Europeans associated the plant with Norse goddess of love, Freya, and developed the custom of kissing underneath mistletoe branches. Christians incorporated this custom into their Christmas celebrations, and kissing under a mistletoe branch eventually became a part of secular Christmas tradition (Drury, 1987). Mistletoe is especially interesting botanically because it is a hemiparasite (partial parasite). Mistletoe is also capable of growing on its own; like other plants as it can produce its own food by photosynthesis. However, it is more commonly found growing as a parasitic plant (Runyon et al., 2009).

Mistletoe is used mainly in Europe as a treatment for cancer (Kovacs, 2004; Augustin et al., 2005). While American mistletoe is toxic, European mistletoe is considered to have medicinal properties till today (Krenzelok et al., 1997). Currently, it is best used as an adjuvant therapy with cancer chemotherapy or radiotherapy (Mengs et al., 2002; Bock et al., 2004). Mistletoe has been used in medicine to prove much of its older frame as “all healer”. The white-berried mistletoe (Viscum album) has been documented as a traditional treatment for diabetes and high blood pressure.

Mistletoe extracts represent the most unorthodox oncology therapy in Germany (Bock et al., 2004; Orhan et al., 2005). V. album has been also reported to reduce heart rate, stimulate the immune system, relax spasms and exert sedative, diuretic and anti-cancer effects (Bown, 1995).

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A tea prepared from leaves of mistletoe is used traditionally to treat diabetes in the West Indies. This treatment has been shown also to relieve the diabetic symptoms of severely hyperglycaemic streptozotocin-diabetic mice, including polydipsia, hyperphagia and body weight loss (Swanston-Flatt et al., 1989). In Nigeria, the Hausa and Fulani tribes of Northern Nigeria use mistletoe in the treatment of cancers and inflammations. (Abubakar et al., 2007). The African mistletoe, Loranthus bengwensis L. (Loranthaceae), has been widely used in Nigerian folk medicine to treat Diabetes mellitus (Obatomi et al., 1994).

This present study was based on African mistletoe, Phragmanthera incana, a woody parasitic shrub, which stems up to 2m long; of secondary jungle and bush savanna areas. Young parts and perianth are more or less densely covered with brown hairs with red berries. The plant is very variable in the shape and size of the flowers and leaves (Burkill, 1985). Phragmanthin, a single peptide with low molecular weight (10.7kD) and haemaglutinin activity was isolated and purified to homogeneity from fresh leaves of P. incana (Fasanu and Oyedapo, 2008). Metabolic studies of phragmanthin on Sprague – Dawley rats revealed that it caused significant reduction in the levels of plasma inorganic phosphate, haemoglobin, activities of L- alanine aminotransferase and alkaline phosphatase, muscle glycojen and blood sugar. Though, a slight elevation of the levels of plasma L – aspartate aminotransferase activity and creatinine was exhibited (Fasanu and Oyedapo, 2008). However, the effect of this mistletoe on diabetes is yet to be investigated. This study was therefore aimed at evaluating the probable changes in fasting and or random blood glucose (Random Blood Sugar – RBS) levels of diabetic albino rats administered with the methanol extract of P. incana harvested from cocoa or kolanut trees.

MATERIALS AND METHODS

Plant Sample Collection

Phragmanthera incana (mistletoe) were harvested from two plant hosts [Theobroma cacao (Cocoa) and Cola nitida (Kolanut)] in a forest at Alesan Obolode, Owo metropolis, Nigeria. Authentication of the mistletoe species was carried out at the Forestry Research Institute of Nigeria with Forest Herbarium Index 108925. A voucher specimen was submitted at the Department of Botany, University of Ibadan Herbarium.

Plant Sample Extraction

The samples were washed, air dried and ground to powder and kept dry in an air-tight container. Cold extraction method for 72 hours with methanol was used and were concentrated using rotary evaporator. The concentrated extracts were further dried on water bath at a very low temperature of about 40\(^\circ\)C to remove all solvents.

Experimental Animals

Albino rats were obtained from and housed at Experimental Animal Unit of the Faculty of Veterinary Medicine of the University of Ibadan. The rats were allowed access to water and pelleted rat feed ad libitum. The rats were humanely handled according to the Animal Use Ethical Guide stated by the Faculty of Veterinary Medicine Committee on animal right and uses.

Antidiabetes Studies

Diabetes was induced in albino rats weighing between 120-200g with alloxan (100mg/kg). Following an overnight fast, fasting blood sugar levels (FBs) of the rats were measured using Accu-check glucometer and diabetes was induced by intraperitoneal injection of alloxan. The rats were monitored for 4 days and rats with blood glucose level of ≥150mg/dl were considered diabetic and included in the study. Forty diabetic rats were randomly and equally divided into eight groups. Three groups were designated for treatment with the graded doses of P. incana extract harvested from Cocoa tree, 3 groups were treated with P. incana extract harvested from Kolanut tree, one group was treated with glibenclamide, a standard anti-diabetic drug, and the 8th group of diabetic rats was left untreated. A 9th group of non-diabetic and untreated rats were included as control for the study. Treatment with methanol extracts of P. incana at the doses of 200mg/kg, 400mg/kg or 800mg/kg commenced four days post induction of diabetes with alloxan. Thereafter, random blood sugar levels (RBS) of all the rats were measured post-administration of the extracts over a period of twenty-four hours, beginning at 1, 3, 6, 12 and 24 hours, and on days 1,3, 7, 10 and 14 post-administration of the extract.

Statistical Analysis

Data were presented as mean and the standard error of mean of the results from this study. The results were analyzed by paired sample t-test descriptive statistical mode of analysis using the Statistical Package for Social Sciences (SPSS 15) to compare the differences in the mean values of the different rat groups.

RESULTS

All the doses of the extracts of P. incana reduced blood glucose levels within the first hour post-administration of
the extracts (Figure 1 & 2). There was a significant reduction in the blood glucose levels of rats administered with the different doses of *P. incana* extract harvested from Cocoa compared to the untreated diabetic rats. Although, the different doses of the extract did not cause significant reduction compared within the doses, the reductions caused were statistically significant compared to glibenclamide, particularly within 12 hours post-administration of the extract (Figure 1).

*P. incana* extract harvested from Kolanut tree significantly reduced the blood glucose levels of rats compared to the untreated diabetic rats. Rats administered with the extract had significantly lower blood glucose levels compared to rats administered with glibenclamide. There was an insignificant difference between the blood glucose of rats administered with the extract and the non-diabetic rats at the end of the study. At the end of 24 hours, the highest dose of the extract (800mg/kg) had the most profound
Table 1. Random blood sugar (RBS) levels of rats administered with *Phragmanthera incana* extract harvested from *Theobroma cacao* (Cocoa).

<table>
<thead>
<tr>
<th></th>
<th>0 hr</th>
<th>Day 1</th>
<th>Day 3</th>
<th>Day 7</th>
<th>Day 10</th>
<th>Day 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>200mg/kg</td>
<td>466.2±57.92</td>
<td>248.8±57.23</td>
<td>309.8±48.38</td>
<td>158.8±18.81</td>
<td>185.2±17.52</td>
<td>130.8±62.08</td>
</tr>
<tr>
<td>400mg/kg</td>
<td>344.2±44.56</td>
<td>294.4±27.52</td>
<td>183.0±36.8</td>
<td>129.8±64.56</td>
<td>125.0±36.00</td>
<td>98.2±24.64</td>
</tr>
<tr>
<td>800mg/kg</td>
<td>379.8±52.56</td>
<td>214.8±40.96</td>
<td>199.6±28.24</td>
<td>191.4±29.52</td>
<td>197.2±61.36</td>
<td>175.2±17.84</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>429.7±13.11</td>
<td>214.0±3.33</td>
<td>239.7±6.44</td>
<td>261.7±20.22</td>
<td>206.3±37.56</td>
<td>159.0±58.00</td>
</tr>
<tr>
<td>Diabetic Ctrl</td>
<td>308.8±46.16</td>
<td>363.6±44.88</td>
<td>399.2±72.96</td>
<td>397.2±64.56</td>
<td>402.6±62.08</td>
<td>417.2±54.56</td>
</tr>
<tr>
<td>Non-Diabetic</td>
<td>81.2±20.96</td>
<td>115.0±23.2</td>
<td>110.0±12.4</td>
<td>92.4±4.88</td>
<td>97.8±5.92</td>
<td>81.4±10.32</td>
</tr>
</tbody>
</table>

Table 2. Random blood sugar (RBS) levels of rats administered with *Phragmanthera incana* extract harvested from *Cola nitida* (Kolanut).

<table>
<thead>
<tr>
<th></th>
<th>0 hr</th>
<th>Day 1</th>
<th>Day 3</th>
<th>Day 7</th>
<th>Day 10</th>
<th>Day 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>200mg/kg</td>
<td>174.4±16.48</td>
<td>138.6±22.32</td>
<td>121.0±6.4</td>
<td>106.6±8.32</td>
<td>97.4±16.96</td>
<td>99.6±11.2</td>
</tr>
<tr>
<td>400mg/kg</td>
<td>218.5±80.32</td>
<td>141.6±32.32</td>
<td>120.8±8.56</td>
<td>113.0±25.2</td>
<td>90.0±21.2</td>
<td>78.8±14.56</td>
</tr>
<tr>
<td>800mg/kg</td>
<td>299.8±59.36</td>
<td>286.0±10.4</td>
<td>264.4±6.24</td>
<td>248.8±17.68</td>
<td>151.8±23.76</td>
<td>127.2±10.16</td>
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<tr>
<td>Glibenclamide</td>
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</tr>
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effect on the blood glucose, but 400mg/ml of each of the extract had a better overall hypoglycemic effect as observed in the course of monitoring on days 3, 7, 10 and 14 (Table 1 & 2).

**DISCUSSIONS**

Methanol extracts of *Phragmanthera incana* harvested from host plants *Theobroma cacao* (Cocoa) and *Cola nitida* (kolanut) both exhibited significant hypoglycemic effects in the alloxan-induced diabetic rats. The extracts had hypoglycemic effects within 1 hour post-administration of the extract, which was sustained throughout the course of the experiment. The hypoglycemic effect of the extracts was comparable to the standard anti-diabetic drug, glibenclamide. The extracts at the dose of 800mg/kg exhibited the most profound hypoglycemic effect 24 hours post-administration of the extracts, but the extracts at 400mg/kg exhibited the best control of the blood glucose levels through the course of the study. *P. incana* harvested from kolanut exhibited more hypoglycemic effect compared to that harvested from cocoa. This correlated well with the phytochemical screening of the mistletoes growing on the two plant hosts which showed differences in their phytochemical profiles (Ogunmefun et al., 2013). The two extracts showed consistent and sustained lowering of the blood glucose levels throughout the 14-day period of observation, despite the single dose of the extract administered on day 0.

In this study, the rats administered with the extracts of *P. incana* exhibited a better controlled blood glucose levels compared to rats administered with glibenclamide. Diabetes mellitus was induced in all the rats by intraperitoneal injection of alloxan, a toxic glucose analogue proven to induce an insulin-dependent diabetes when administered to rodents and many other animal species by selectively destroying insulin-producing β-cells of the Islet of Langerhans in the pancreas (Lenzen, 2008). It has been successfully used to experimentally induce diabetes and reversal of damage can be determined by monitoring of blood glucose levels. Random and or fasting blood glucose levels are essential parameters for determination of diabetes status of a patient and monitoring of response to interventions employed for management of diabetes mellitus. Response to anti-diabetic drugs is determined by the ability of the drug to lower and maintain normal blood glucose levels. Blood levels need to be properly controlled in diabetic subject to prevent development of its associated complications such as eye, kidney and or heart disease, as well as nerve damage, amongst other complications (Adler et al., 2003; Boulton et al., 2005; Boyle, 2007; Keenan et al., 2007).

This study has demonstrated the anti-diabetic effect of *Phragmanthera incana* species harvested from different plant hosts, confirming the ethno traditional use of the plant for management of diabetes mellitus. Another African mistletoe *Loranthus bengwensis* L. (Family: Loranthaceae) that have been proven to possess anti-diabetic effect. This mistletoe significantly decreased
blood glucose levels of streptozocin-induced diabetic rats monitored for 28 days. The researchers also reported a variation in the anti-diabetic property of the mistletoes from different plant hosts (Obatomi et al., 1994). Recently, Osadolor and Ojewe (2008) investigated the hypoglycemic effect of the aqueous extract of *L. bengwensis* in normal (non-diabetic) rats and reported that it significantly lowered blood glucose levels.

Other medicinal plants to possess anti-diabetic property include *Vernonia amygdalina* (Nwaoguikpe, 2010) which lowered blood glucose levels in alloxan-induced diabetic rats. Atangwho et al. (2013) further 999evaluated the anti-diabetic effect of fractions of the extract in relation to their antioxidant effect. Oyagbemi et al. (2010) reported the anti-diabetic property of *Cnidoscolus aconitifolius* administered to alloxan-induced diabetic rats. The anti-diabetic effect of *Garcinia kola* was also reported by Udenze et al. (2012) in alloxan-induced diabetic rats and Adaramoye (2012) reported the anti-diabetic effect of kolaviron, a biflavonoid isolated from *G. kola* in streptozocin-induced diabetic rats.

In conclusion, *P. incana* growing on host plants *Theobroma cacao* (Cocoa) and *Cola nitida* (kolanut) are confirmed to have anti-diabetic property and further studies are recommended to explore the bioactive principle responsible for the hypoglycemic effect of *P. incana* with probable establishment of the mechanism(s) of glucose uptake involved.

REFERENCES


