Antidiabetic property of some Nigerian medicinal plants

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The antidiabetic properties of extracts of three Nigerian medicinal plants - Ocimum basilicum L. (Lamiaceae, formerly Labiatae), Phyllanthus niruri L. (Euphorbiaceae) and Viscum album L. (Viscaceae or Loranthaceae) parasitic on Psidium guajava L. (Myrtaceae) on normoglycemic and alloxan-induced diabetic rats were studied. The extracts (258.3 g, 6.89% w/w of P. niruri, 189.17 g, 6.76% w/w of V. album and 131.50 g, 6.41% w/w of O. basilicum), obtained by 48 h cold maceration in methanol: methylene chloride (1:1), were evaluated for hypoglycemic and oral glucose tolerance effects in normoglycemic rats and antihyperglycemic effect in alloxan (100 mg/kg i.p) diabetic (blood glucose level ≥ 200 mg/dl) rats. The results showed that acute oral administration of the extracts to normoglycemic rats caused a mild to moderate non-dose-related reduction in blood glucose levels. The extracts significantly (P < 0.05) suppressed the acute postprandial rise in blood glucose to varying extents. Extract of O. basilicum lowered the elevated glucose level to 13.67% at 180 min while P. niruri and V. album reduced the glucose level to 16.87 and 17.33%, respectively. In alloxan diabetic rats, the extracts caused a significant (P < 0.05) non-dose-related reduction in blood glucose with 52.31 (O. basilicum), 44.29 (P. niruri) and 16.71% (V. album) maximum reduction at 6 h.

Key words: Alloxan, diabetes mellitus, Nigerian medicinal plants.

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder characterized by hyperglycemia due to defective insulin action, insulin secretion or both. In various parts of the world, several medicinal plants are employed in the management of DM (Verspohl, 2002; Akah et al., 2002; De Sousa et al., 2004; Colea, 2006). In Nigeria, medicinal plants popularly used for the treatment of diabetes include Ocimum basilicum L. (Lamiaceae, formerly Labiatae), Phyllanthus niruri L. (Euphorbiaceae) and Viscum album L. (Viscaceae or Loranthaceae) parasitic...
on *Psidium guajava* L. (Myrtaceae). *O. basilicum*, also known as 'Sweet basil', is an annual common garden herb (Simon, 1995) believed to have originated from central Africa and Southeast Asia.

Currently, it is cultivated extensively in Nigeria where it is commonly called curry in the southeastern parts and serves as a culinary herb and source of essential oil for use in foods, flavors, and fragrances. The leaves are used to reduce plasma cholesterol in Morocco (Amrani et al., 2006) and as treatment for diabetes in Nigeria. The nematicidal (Chatterjee et al., 1982), fungistatic (Reuveni et al., 1984), antimicrobial (Ntezurubanza et al., 1984), antiulcer (Singh, 1999a), anti-inflammatory (Singh, 1999b; Benedec et al., 2007), antiplatelet aggregation (Tohti et al., 2006; Amrani et al., 2009), hypolipidemic, antioxidant (Amrani et al., 2006), antihyperglycemic and hypolipidemic (Zeggwah et al., 2007) properties have been documented. Documented biologically-active constituents of the essential oil include methyl chavicol, eugenol, linalool, camphor, and methyl cinnamate (Nishida et al., 1984). The isolation of triterpene acids - betulinic, oleanolic, ursolic, 3-epimaslinic, alphatolic and euscaphic acids from the genetically transformed hairy roots of the plant (Murugaiyah and Chan, 2007) and the lignans phyllanthin, hypophyllanthin, phyltetralin (Murugaiyah and Chan, 2006), cubebin dimethyl ether, urinatetralin (Elkahmi et al., 2006), and niranthin (Murugaiyah and Chan, 2007).

*V. album* is a species of Mistletoe and common bushy plant which grows as an epiphyte on the branches of deciduous trees. It is widely distributed in tropical and subtropical Africa, in Asia and Europe (EMEA, 1999). Mistletoes have been used in the treatment and management of many diseases for many years, both in traditional and complementary medicine in some parts of Africa. It has been reported to possess the ability to lower blood pressure, slow the heart beat, stimulate the immune system, relax spasms and exert sedative, diuretic and anti-cancer effects (Bown, 1995). It is chiefly used to lower blood pressure and heart rate, ease anxiety and promote sleep. In low doses it can also relieve panic attacks and headaches and improve the ability to concentrate (Chevallier, 1996). *V. album* is also effective in the management of chronic metabolic disorders such as diabetes (Obatomi et al., 1994) and has been shown to relieve diabetic symptoms of severely hyperglycaemic streptozotocin-diabetic mice, including polydipsia, hyperphagia and body weight loss (Swanston-Flatt et al., 1989). A number of biological effects, such as anticancer, antimycobacterial, antiviral, apoptosis-inducing and immunomodulatory activities have been reported for mistletoes (Onay-Ucar et al., 2006) as well as hypoglycemic, glucose lowering or antihyperglycemic (Ohiri et al., 2003; Orhan et al., 2005; Nwaegerue et al., 2007; En et al., 2008) effects. Extracts of the plant has also been shown to contain insulin-releasing constituents (Gray and Flatt, 1999) and possess insulinotropic and mild glucagonostatic (Eno et al., 2008) properties. Leaves and twigs of mistletoe have been reported to contain a-amyrin, tyramine, quercitin, syringin and flavoyedorin A and B (Duke, 1985).

Due to their widespread documented use in the treatment of diabetes, *O. basilicum, P. niruri* and *V. album* were selected for study to compare the potency of their antidiabetic activity and identify the most promising for further development.

**MATERIALS AND METHODS**

**Animals**

Adult albino rats (100 to 200 g) of either sex bred in the laboratory
animal facility of the Department of Pharmacology and Toxicology, University of Nigeria, Nsukka were used for the study. The animals were kept in steel cages within the facility maintained at normal environment temperature and natural dark/light cycle and allowed free access to water and standard pelleted feed. Animals were allowed a 2 week acclimatization period before commencement of the experiments. All animal experiments were conducted in compliance with the National Institute of Health Guide for Care and Use of Laboratory Animals (Pub No. 85 – 23, revised 1985).

Drugs
Glibenclamide tablets (Daonil™, 5 mg; Aventis Pharma) was used.

Equipment
Glucometer and test strips (Accu-Chek Active; Roche Diagnostics GmbH, Germany)

Collection, preparation and extraction of plant materials
Fresh leaves of O. basilicum were purchased from the open market in Nsukka while whole plants of P. niruri were collected from bushes in a farm in Orba, Nsukka, Enugu State, Nigeria. Fresh leaves of V. album parasitic on Psidium guajava (Myrtaceae) were collected along Bauchi road, Jos, Plateau State, Nigeria. The plant materials were collected within the months of March and April. The plant materials were individually identified and authenticated at the International Centre for Ethnomedicine and Drug Development (InterCEDD), Nsukka, Enugu State, Nigeria. The leaves were sorted to remove bad parts, dried under the sun (V. album and P. niruri) or shade (O. basilicum) and milled to a coarse powder. The powdered plant materials (2.05 kg of O. basilicum; 3.75 kg of P. niruri and 2.8 kg of V. album) were separately extracted with a mixture of methylene chloride: methanol (1:1) by cold maceration for 48 h. The filtered extracts were concentrated in a rotary evaporator under reduced pressure to afford 131.50 g of O. basilicum, 258.3 g of P. niruri and 189.17 g of V. album extracts.

Pharmacological activities tests

Experimental design
The antidiabetic potentials of extracts of the plants selected were evaluated using normoglycemic and diabetic rats. The hypoglycemic effect and oral glucose tolerance were studied in normoglycemic rats while the antihyperglycemic effect was assessed in alloxan diabetic rats. Extracts were administered orally by gavage at 100, 200 and 400 mg/kg.

Hypoglycemic effect of extracts on normoglycemic rats
Animals fasted overnight were randomly divided into eleven groups (n = 6). Groups I to III received extract of O. basilicum (100, 200 or 400 mg/kg), respectively. Groups IV to VI were given extract of P. niruri (100, 200 or 400 mg/kg) while groups VII to IX received extract of V. album (100, 200 or 400 mg/kg), respectively. Groups X and XI served as the control and received glibenclamide (0.2 mg/kg) or 17% v/v Tween 80 (2.5 ml/kg). Extracts were administered orally. The blood glucose level of each animal was measured by tail snipping using a glucometer prior to (fasting blood glucose, FBG) and at 0.5, 1, 2, and 4 h after extract administration.

Oral glucose tolerance in normoglycemic rats
Animals fasted for 16 h but with free access to water were randomly divided into groups (n = 6) as described already. One hour after extract administration, the rats were fed with glucose (4 g/kg) solution. The blood glucose level of animals in each group was measured before (0) and at 30, 60, 90, 120, 150 and 180 min after the glucose load.

Anti-hyperglycemic activity test in alloxan diabetic rats

The antihyperglycaemic effect of the extracts was studied in alloxan-induced diabetic rats. Animals were fasted for 18 h but allowed free access to water. At the end of the fasting period, the basal fasting blood glucose (FBG) levels of the rats were determined. Subsequently, diabetes was induced by single intraperitoneal injection of alloxan monohydrate (100 mg/kg) and normal feeding maintained thereafter. Twenty four hours later, blood was drawn from each rat by tail snipping and the blood glucose level measured to establish diabetes. Animals with blood glucose level ≥ 200 mg/dl were considered diabetic and used for the study. The diabetic animals were randomly divided into eleven groups (n = 6) and treated as described earlier. Blood glucose was measured before (0 h) and at 0.5, 1, 2 and 4 h after extract administration.

Statistical analysis
Data obtained was analyzed using One Way ANOVA (SPSS version 16) and expressed as Mean ± SEM. Data was further subjected to least significant difference (LSD) post hoc test for multiple comparisons and differences between means accepted significant at P < 0.05.

RESULTS

Extractive yield of the plant materials
The extraction process afforded 131.5 g (6.41% w/w) of O. basilicum extract, 258.3 g (6.89% w/w) of P. niruri extract, and 189.17 g (6.76% w/w) of V. album extract.

Effect of extracts on blood glucose level of normoglycemic rats
Acute oral administration of the extracts to normoglycemic rats caused a mild to moderate reduction in blood glucose level over time. The extracts provoked a non-dose-related but comparable magnitude of reduction in glucose level. Extracts of O. basilicum and P. niruri caused maximal reduction of 14.51 and 17.08% at 200 and 400 mg/kg, respectively. V. album extract caused 15.49% maximal reduction at 100 mg/kg (Table 1).
Table 1. Effect of extracts on blood glucose level of normoglycemic rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>0 h</th>
<th>0.5 h</th>
<th>1 h</th>
<th>2 h</th>
<th>4 h</th>
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<tr>
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<td>71.33 ± 2.73</td>
<td>72.17 ± 2.57</td>
<td>71.17 ± 2.95</td>
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<td>200</td>
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<td>75.83 ± 2.63</td>
<td>69.33 ± 2.55</td>
<td>64.83 ± 2.76*</td>
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<td>400</td>
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<td>70.33 ± 2.35</td>
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<td>P. niruri</td>
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<td>86.50 ± 5.54</td>
<td>88.33 ± 2.79</td>
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<td>82.83 ± 4.92</td>
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<td>200</td>
<td>77.17 ± 4.83</td>
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<td>81.33 ± 5.19</td>
<td>82.83 ± 4.92</td>
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<td>76.67 ± 2.85</td>
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<td>400</td>
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<td>81.50 ± 5.93</td>
<td>78.33 ± 9.46</td>
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<td>70.17 ± 2.63</td>
<td>72.67 ± 1.86</td>
<td>68.67 ± 2.03</td>
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</table>

*P < 0.05 compared to zero hour (0 h) value (One Way ANOVA; LSD post hoc test); Values of blood glucose level shown are Mean ± SEM; Values in parenthesis represent percent reduction in blood glucose calculated relative to 0 min values; NR = No reduction.

Effect of extracts on oral glucose tolerance in normoglycemic rats

Oral administration of heavy glucose load to normoglycemic animals significantly (P < 0.05) increased the blood glucose level by over 150% within 30 min and progressively reduced to 47.84% at 180 min. Single oral pre-treatment of the animals with the extracts significantly (P < 0.05) suppressed the acute postprandial rise in blood glucose to varying extents. The effect of the extracts was non-dose-related. Extract of V. album reduced the glucose level from 120.97% (400 mg/kg) at 30 min to 13.33% (200 mg/kg) at 180 min (Figure 1). At similar time intervals, extract of P. niruri lowered the blood glucose from 126.22% (100 mg/kg) to 16.87% (100 mg/kg) (Figure 2). Also, extract of O. basilicum lowered glucose level from 112.33% (400 mg/kg) at 30 min to 13.67% (200 mg/kg) at 180 min (Figure 3). A comparison of the magnitude of inhibitory effect based on the level of increase in blood glucose level was of the order O. basilicum > V. album > P. niruri (Table 2).

Effect of extracts on blood glucose level of alloxan diabetic rats

Single oral administration of the extracts lowered elevated blood glucose level of alloxan diabetic rats to varying extents. Extracts of O. basilicum and P. niruri caused significant (P < 0.05) non-dose-related reduction in blood glucose. The effect of V. album extract was also non-dose-related. Extract of O. basilicum caused the most consistent and greatest reduction in blood glucose with maximum inhibitory effect (52.31%) evoked by the 100 mg/kg dose occurring at 6 h. Extract of P. niruri (100 mg/kg) caused a maximum inhibitory effect (44.29%) at 6 h while V. album extract (200 mg/kg) evoked 16.71% at same time (Table 3).

DISCUSSION

In this study, single oral administration of the extracts to normoglycemic rats reduced fasting
blood glucose and suppressed post-prandial rise in blood glucose following glucose meal. They also lowered blood glucose in alloxan diabetic rats following single oral treatment. These findings are consistent with earlier reports on the antihyperglycemic or antidiabetic effects of these plants (Ramakrishnan et al., 1982; Khanna et al., 2002; Ohiri et al., 2003; Orhon et al., 2005; Nwaegerue et al., 2007; Zeggwah et al., 2007; Eno et al., 2008). The ability of the extracts to reduce fasting blood glucose in normal rats suggests they may possess inherent hypoglycemic effect. Suppression of post-prandial hyperglycemia is an important index of glycemic control in diabetes. Chronic hyperglycaemia in DM is a risk factor constantly fuelled by postprandial elevation of blood glucose. As such, control of postprandial hyperglycemia in diabetes is of immense importance due to its close relation to the risk of micro- and macro-vascular complications and death (Balkau, 2000; Ceriello, 2005).
Interestingly, in addition to lowering blood glucose through hypoglycemic effect, extracts of these plants may also be capable of suppressing postprandial hyperglycemia which usually occurs after meals. With the exception of *V. album*, the extracts also effectively lowered blood glucose in alloxan diabetic rats on single oral administration. These effects are indicative of effective glycemic control by extracts of these plants.

In the antihyperglycemia test in alloxan diabetic rats, a comparison of the potency of blood glucose lowering effect showed that extracts of *O. basilicum* and *P. niruri* caused more pronounced and consistent effect than *V. album*. The poor glucose lowering effect caused by extract of *V. album* in diabetic rats in this study was rather surprising given the magnitude of effect from the study by Ohiri et al. (2003). However, findings similar to that from this study have also been documented elsewhere where extract of mistletoe (6-25% by weight of diet, 1 g/400 ml infusion in place of drinking water) significantly reduced the hyperphagia and polydipsia associated with streptozotocin-diabetes but failed to alter plasma glucose (Swanson-Flatt et al., 1989). The
reasons for this may not be unrelated to the effect of the host trees on the constituents and hence biological activities of mistletoes. Differences in the host tree have been shown to determine the apoptosis-inducing properties (Bussing and Schietzel, 1999) and antioxidant capacity (Onay-Ucar et al., 2006) of extracts of Mistletoes. Antioxidant capacity has also been reported to depend on the time of harvest (Onay-Ucar et al., 2006). Elsewhere, variation in host plant species has also been shown to determine the antidiabetic properties of the African mistletoe (Loranthus bengwensis) in streptozotocin-diabetic rats (Obatomi et al., 1994). It is thus likely, that the disparity in the potency of blood glucose lowering effect of V. album in this study...
Table 2. Effect of extracts on oral tolerance in normoglycemic rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>0 min (mg/kg)</th>
<th>30 min (mg/kg)</th>
<th>60 min (mg/kg)</th>
<th>Blood glucose level (mg/dl) 0 min</th>
<th>30 min</th>
<th>60 min</th>
<th>120 min</th>
<th>150 min</th>
<th>180 min</th>
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<td>167.83 ± 21.45</td>
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*P<0.05 compared to 0 min and 30 min values respectively (One Way ANOVA; LSD post hoc test); Values of blood glucose level shown are Mean ± SEM; Values in parenthesis represent percent increase in blood glucose calculated relative to 0 min values.

Table 3. Effect of extracts on blood glucose level of alloxan diabetic rats

<table>
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<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Pre-Diabetic FG</th>
<th>Diabetic Pre-treatment</th>
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<td>316.83 ± 44.49</td>
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<td>P. niruri</td>
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<td>339.00 ± 67.09</td>
<td>363.00 ± 72.02</td>
<td></td>
</tr>
<tr>
<td>V. album</td>
<td>100</td>
<td>80.33 ± 4.79</td>
<td>316.33 ± 47.03</td>
<td>341.50 ± 61.28</td>
<td>348.50 ± 65.87</td>
<td>338.50 ± 68.04</td>
<td>316.50 ± 68.88</td>
<td>326.83 ± 69.08</td>
</tr>
<tr>
<td>200</td>
<td>74.00 ± 3.30</td>
<td>354.17 ± 33.19</td>
<td>357.33 ± 32.31</td>
<td>325.17 ± 26.17</td>
<td>306.17 ± 30.27</td>
<td>295.83 ± 36.22</td>
<td>295.00 ± 41.17</td>
<td></td>
</tr>
<tr>
<td>400</td>
<td>78.67 ± 4.70</td>
<td>363.17 ± 29.53</td>
<td>353.00 ± 51.01</td>
<td>352.67 ± 36.93</td>
<td>297.17 ± 47.08</td>
<td>225.33 ± 60.69</td>
<td>339.50 ± 65.20</td>
<td></td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>0.2</td>
<td>87.33 ± 2.49</td>
<td>407.00 ± 28.04</td>
<td>370.00 ± 42.23</td>
<td>355.50 ± 40.27</td>
<td>315.33 ± 41.49</td>
<td>287.33 ± 53.78</td>
<td>293.17 ± 53.76</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>71.67 ± 2.88</td>
<td>350.83 ± 46.34</td>
<td>316.67 ± 55.82</td>
<td>305.83 ± 61.19</td>
<td>277.00 ± 67.60</td>
<td>265.67 ± 67.19</td>
<td></td>
</tr>
</tbody>
</table>

*P<0.05 compared to Diabetic Pre-treatment value (One Way ANOVA; LSD post hoc test); FG = Fasting blood glucose; Values of blood glucose level shown are Mean ± SEM; Values in parenthesis represent percent reduction in blood glucose calculated relative to Diabetic Pre-treatment values.
compared to the report by Ohiri et al. (2003) may derive from differences in the host tree and site of plant collection.

Studies on these plants have not documented any known noxious effect. Elsewhere, toxicological studies on extracts of *O. basilicum* and *P. niruri* have consistently revealed LD$_{50}$ values > 5 g/kg (data not shown). Earlier studies on *V. album* also gave i.p. LD$_{50}$ value of 4.18 ± 0.96 g/kg in mice (Ohiri et al., 2003). Thus, the risk of acute intoxication from these plants may be highly remote.

**Conclusion**

Findings from this study have shown that extracts of *O. basilicum*, *P. niruri*, and *V. album* exhibited hypoglycemic and antihyperglycemic effects in normoglycemic and diabetic rats, respectively and suppressed post-prandial hyperglycemia in normoglycemic rats. These findings provide a rationale for the use of these plants in treatment of diabetes and impetus for further development as antidiabetic remedies. Based on the potency of antihyperglycemic effect in diabetic rats, *O. basilicum* and *P. niruri* are recommended for further studies.

**Conflict of interests**

The authors have not declared any conflict of interest

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