The biochemical effects of lime concentrate ‘Aporo’ and *Mucuna pruriens* seeds extract on Alloxan-induced diabetic rats

Stephen O. Owa, PhD, Adeolu A. Taiwo, BS, Jenifer A. Okosun, BSc, David A. Otohinoyi, BSc, Yvonne O. Akujobi, BSc, Damilola G. Oyewale, BSc, Omodele Ibraheem, PhD, Theresa I. Edewor-Ikupoyi, PhD, and Oyomide S. Adeyemi, PhD, *a*

*a* Biochemistry Unit, Department of Biological Sciences, Landmark University, Omu-Aran, Nigeria

*b* Department of Chemical Sciences, Ladoke Akintola University of Technology, Osun State, Nigeria

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**Abstract**

**Objectives:** This study evaluated the biochemical properties of a local lime concentrate preparation called *Aporo* and an ethanolic extract of seeds of *Mucuna pruriens*.

**Methods:** Six groups of male Wistar rats, each containing five rats, were selected. Diabetes was induced in all rats, except the negative control group, by a single intraperitoneal injection of 150 mg/kg Alloxan. The induced rats, apart from the diabetic control group, were treated by daily oral administration of 5 mg/kg Glibenclamide, 100 mg/kg *Aporo* decoction, an ethanolic extract of *M. pruriens* seed, and a combination of both in equal doses.

**Results:** After 15 days of treatment, the blood glucose level of rats in the positive control group was found to be significantly lower than that of the other rats. However, *Aporo* extract exhibited a significantly higher ability to reduce blood glucose than the standard hypoglycaemic drug Glibenclamide.

**Conclusions:** This study endorses the folk use of *Aporo* in the treatment of diabetes. However, further experimental studies are required to complement the results of the current study.
Keywords: Antidiabetic; Antidyslipidaemic; Antioxidant; Diabetes mellitus; Glucose

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Introduction

Diabetes mellitus has clearly achieved a leading position among the causes of human mortality and morbidity in the world today. This disease, according to statistics, affects approximately 200 million people, which accounts for nearly 2.5% of the human population, and it is also a contributing factor in 30% of all cardiovascular diseases. It is the most common endocrine disorder associated with carbohydrate metabolism, and it occurs as a result of abnormalities or deficiencies in insulin secretion, insulin’s actions or both. There is a growing belief that it is more of a body’s response to a sedentary lifestyle than a putative disease. Diabetes is associated with complications arising from the increase in blood glucose circulating through the body as well as cardiovascular diseases due to the increase in triglyceride and low-density lipoprotein (LDL) levels in the body. In prolonged diabetes, some complications arise such as diabetic ketoacidosis, diabetic retinopathy, neuropathy and nephropathy, which could lead to end-stage renal failure, coma and sometimes death. Thus, it is desirable to provide “natural” and cheap ways to prevent or manage diabetes and to make such remedies readily available to the masses in a traditional form. One such traditional approach is the use of lime concentrate, popularly called Aporo in Nigeria, it is an herbal concentrate. Because the use of Aporo is so widespread, it deserves attention.

Aporo is a decoction traditionally made by locals by boiling whole lime fruits (Citrus aurantifolia), including both the rind and the lime juice, in large pots until most of the water is evaporated and the solution is concentrated to approximately half the original quantity, resulting in a very dark slurry paste. Some other vendors prepare the decoction using Citrus aurantium and Aframomum melegueta. The resulting Aporo is regarded as a wonder-cure for several ailments, including snake bites and scorpion stings, and it also possesses antimicrobial properties. Citrus fruit, from which Aporo is made, has been shown to possess anti-diabetic and hypolipidaemic properties. It has also been shown to have antioxidant properties through its free radical scavenging properties by the presence of phenolic compounds, anthocyanins and vitamin C.

Mucuna pruriens (Linn), a bean-like leguminous plant, is another potential cultural remedy for diabetes. Apart from its common use as a treatment for infertility and as an aphrodisiac, M. pruriens seeds are also known for their high L-DOPA contents, which make them highly useful in traditional treatment of Parkinson’s disease. Studies carried out by Majekodunmi et al. have shown that this seed possesses anti-diabetic properties.

This research was therefore aimed at evaluating the antidiabetic, antidyslipidaemic and antioxidant effects of Aporo and ethanolic extracts of seeds of M. pruriens when administered singly and in combination with equal dosages.

Materials and Methods

M. pruriens and Aporo

Seeds of M. pruriens were obtained from the International Institute of Tropical Agriculture (IITA), Ibadan. The seeds were washed, air-dried at approximately 25°C and subsequently reduced into a coarse powder using dry commercial mill. Aporo was purchased from a manufacturer at Adodo Palace, Egbe, Kogi state, Nigeria. It remained refrigerated at 2°C–8°C until required for use.

Preparation of seed extract

The seeds were washed thoroughly with distilled water, air-dried, powdered using a commercial mill and soaked in 90% ethanol for 72 h. Milled seed (1 kg) was soaked in 200 ml of ethanol and was stirred intermittently. It was then sieved using a fine muslin cloth, after which the mixture was filtered through Whatman filter paper. The extract was concentrated using a rotary evaporator (Stuart, Germany) and then dried in a water bath (Clifton) to remove the remaining solvent. A golden brown, oily semi-viscous fluid was obtained. The same milled seeds were extracted twice, and an oily extract of 36.17 g was obtained with a yield of 3.617%. It was stored in an air tight container and refrigerated at 2°C–8°C until required for use.

Drugs and chemicals

Alloxan monohydrate was a product of Oxford Laboratories Reagent, London. Finetest glucose strips were obtained from Infopix Co., Ltd, Kyunggi-Do, Korea. A glucose kit was obtained from Randox Diagnostics (Crumlim, UK). HDL, total glycerides and total cholesterol kits were products of Agape Diagnostics, Kerralu, India. Glibenclamide tablets were products of Swiss Pharma Nigeria Ltd and were obtained from the Landmark University Health Centre. All other reagents were of analytical grade.

Anti-diabetic studies

Experimental animals

Apparently healthy male Wistar rats weighing approximately 187.5 g were obtained from Covenant Farms, Olodo, Ibadan, Oyo state. These animals were housed in standard polypolyene cages and maintained under controlled room temperature (25 ± 5°C) with a 12-h light/dark cycle. The cages were cleaned on a daily basis. The rats were acclimatized for two weeks before the commencement of the experiment. The animals had access to a rat pellet diet and clean water ad libitum.
**Experimental induction of diabetes**

Leaving aside five rats to serve as a negative control group, diabetes was induced in overnight-fasted rats (12 h) by intra-peritoneal injection of 150 mg/kg Alloxan dissolved in 0.9% ice-cold saline immediately before use. The same volume of 0.9% NaCl injectable solution was administered to the negative control rats. Seventy-two (72) hours after Alloxan administration, blood samples were withdrawn from the rat tails, and glucose levels were determined using a glucometer to confirm the development of diabetes. The rats exhibiting blood glucose levels above 250 mg/dl were considered diabetic and were used for further experimentation.

**Experimental design**

The animals were sub-divided into six groups of five rats each. The rats were grouped as follows:

<table>
<thead>
<tr>
<th>Group</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Negative Control rats received 1 ml of normal saline daily for 15 days.</td>
</tr>
<tr>
<td>II</td>
<td>Positive Control rats underwent induction of diabetes and received 1 ml of normal saline.</td>
</tr>
<tr>
<td>III</td>
<td>Diabetic Standard Control rats were administered a standard reference drug, Glibenclamide, dissolved in 1 ml normal saline at a dose of 5 mg/kg body weight.</td>
</tr>
<tr>
<td>IV</td>
<td>Diabetic test rats were administered the <em>Aporo</em> decoction dissolved in 1 ml normal saline at a dose of 100 mg/kg body weight.</td>
</tr>
<tr>
<td>V</td>
<td>Diabetic test rats were administered the <em>Aporo</em> decoction and <em>M. pruriens</em> ethanolic seed extract mixed in equal dosages of 100 mg/kg body weight and dissolved in 1 ml normal saline.</td>
</tr>
<tr>
<td>VI</td>
<td>Diabetic test rats were administered <em>M. pruriens</em> ethanolic seed extract at a dose of 100 mg/kg body weight dissolved in 1 ml normal saline.</td>
</tr>
</tbody>
</table>

All administrations were performed orally using sterile cannulas, and the daily treatments lasted for 15 days. The selection of dosages was premised on a previous report by Majekodunmi et al. that showed that doses in rats of up to 100 mg/kg for 12 weeks caused no significant adverse effects. The rats were weighed before commencement of the experiment, weekly during the experiment and 24 h before they were sacrificed. Blood was collected from the tail by tail snip using a pair of surgical scissors on the 6th and 13th days of the experiment to determine blood glucose concentrations; blood was collected in fluoride oxalate sample bottles after the rats were sacrificed to determine the final blood glucose concentration. Handling of animals was consistent with international best practices as approved by the Landmark University Ethics Committee.

**Collection of blood samples**

The rats were sacrificed under mild anaesthesia (diethyl ether) 24 h after the last treatment. An aliquot (2 mL) of blood was collected into fluoride oxalate sample bottles for the analysis of blood glucose concentration. Another 5 mL of the blood was collected in plain sample bottles and centrifuged at 4000 x g (Anke TDL-5000B, Shanghai, China) for 5 min to yield the serum, which was carefully aspirated with a Pasteur pipette into sample bottles for the other biochemical assays.

**Biochemical assays**

Blood glucose levels after alloxan induction on the 6th and 13th days of the experiment were estimated by the enzymatic glucose oxidase method using a commercial auto-coding glucometer (Finetest). Rat plasma glucose estimation, serum cholesterol, triglycerides and HDL-cholesterol were analysed using Randox assay kits (Crumlin, UK). LDL—cholesterol was estimated according to the method described by Friedewald et al. Total serum protein was determined using the biuret reaction as described by Gornal et al. with slight modification: potassium iodide was added to the biuret reagent to prevent the precipitation of Cu²⁺ ions. The antioxidant activity of the *Aporo* decoction and *M. pruriens* extracts were assayed by estimating reduced glutathione (GSH) and lipid peroxidation levels in rat serum. GSH was estimated by the method of Ellman, while lipid peroxides were estimated by the method of Varshney and Kale. Where applicable, the absorbance was measured using a Jenway UV/Vis Spectrophotometer (Staffordshire, UK).

**Statistical analysis**

The results were expressed as the mean ± standard deviation. The data were statistically analysed using one-way analysis of variance (ANOVA) as the primary test, followed by Tukey’s test for multiple comparisons using SPSS software version 19 (IBM, 2010). Mean values at p < 0.05 was considered statistically significant.

**Results**

**Effect of treatments on blood glucose concentration**

At day 6 of treatment, the positive (diabetic) control group, as well as all other treated groups apart from the *Aporo*-treated group, showed a significant (p < 0.001) increase in blood glucose when compared with the negative control group (Figure 1). The *Aporo*-treated group, however, showed a significant (p < 0.05) decrease in blood glucose relative to the positive (diabetic) control group. At day 13 of treatment, there was a significant (p < 0.01) increase in the blood glucose of the positive control group when compared with the negative control group (Figure 1). Additionally, a decrease in the blood glucose level was recorded in all treated groups. After termination of treatment, the blood glucose concentration was significantly decreased in the groups treated with *Aporo*, Glibenclamide and a combination of *M. pruriens* and *Aporo*. 
Effect of treatments on serum lipid profile

The positive (diabetic) control group showed a significant (p < 0.001) decrease in serum HDL levels when compared with the negative control group. Meanwhile, treatment with *M. pruriens* as well as *Aporo* alone significantly increased the HDL levels relative to the positive (diabetic) control. The positive (diabetic) control group showed an increase (p < 0.01) in serum LDL when compared with the negative control (Figure 2). All treated groups exhibited decreases in serum LDL levels. Treatment with *M. pruriens*, *Aporo* or Glibenclamide did not affect the level of rat serum total cholesterol relative to the positive control. However, *Aporo* treatment

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**Figure 1:** Effects of the various administrations on fasting blood glucose (mg/dl, days 6 and 13) and final plasma glucose (Day 15) levels of normal and alloxan-induced diabetic rats. (Values are the mean ± SD for 4–5 animals in each group. Values are statistically significant at p < 0.05.)

**Figure 2:** Effects of the various administrations on serum lipid (mg/dl) profile of normal and alloxan-induced diabetic rats. (Values are the mean ± SD for 4–5 animals in each group. Values are statistically significant at p < 0.05.)
decreased \((p < 0.05)\) the level of rat serum triglycerides relative to the positive control group.

**Effect of treatment on serum total protein levels**

The concentration of total protein in rats in the positive (diabetic) control group was significantly lower when compared to the negative control group (Figure 3). However, treatment with Glibenclamide, *Aporo* and the combination of *M. pruriens* and *Aporo* significantly elevated the protein level when compared with the positive control group (Figure 3).

**Effect of treatment on reduced glutathione levels**

Rats treated with *Aporo* alone had higher \((p < 0.05)\) GSH concentrations when compared to other groups (Figure 4). The positive (diabetic) control group and the group treated with a combination of *M. pruriens* and *Aporo* showed lower GSH levels when compared to the negative control.

**Figure 3:** Effects of the various administrations on serum total protein of normal and alloxan-induced diabetic rats. (Values are the mean ± SD for 4–5 animals in each group. Values are statistically significant at \(p < 0.05\).)

**Figure 4:** Effects of the various administrations on serum reduced glutathione (\(\mu\)mol/ml) levels of normal and alloxan-induced diabetic rats. (Values are the mean ± SD for 4–5 animals in each group. Values are statistically significant at \(p < 0.05\).)
Effect of treatment on lipid peroxidation levels

The positive (diabetic) control group showed significantly higher levels of lipid peroxidation when compared with the negative control group (Figure 5). Meanwhile, the treated groups had significantly reduced lipid peroxidation relative to the positive (diabetic) control group with the Aporo treatment, which displayed the highest reduction in lipid peroxidation ($p < 0.001$) when compared to the positive control group.

Discussion

A number of herbs have been demonstrated to be effective in lowering blood glucose levels in alloxan-induced diabetic animals. The two herbs tested in this study (Aporo and M. pruriens tested individually and by co-administration) have provided insight into the potentials of herbal medications. Although M. pruriens is reputed to possess anti-diabetic properties, results from this study showed that the Aporo decoction has an improved ability to decrease blood glucose at a dose of 100 mg/kg. The results revealed that the Aporo decoction is better than Glibenclamide, a standard hypoglycaemic agent whose primary mechanism of hypoglycaemic action is increasing endogenous insulin secretion.

The phytochemical analysis of the Aporo decoction carried out by Adeleye et al. showed the presence of bioactive compounds including tannin, flavonoid, alkaloids, phylloquinone, anthocyanin, reducing sugar, saponin and anthraquinone. Aporo is rich in anthocyanins. Anthocyanins are powerful plant pigments that have been characterized as some of the most powerful antioxidant sources in lowering blood sugar by increasing endogenous insulin secretion and conferring additional beneficial health effects. Thus, the ability of Aporo to lower blood sugar concentrations may be linked to the presence of this compound. Contributing to the high antioxidant properties is the richness of the antioxidant vitamin C.

In lipid metabolism, Alloxan is known to induce hyperlipidaemia in diabetic animals by increasing free fatty acid mobilization from the peripheral fat deposits. The results obtained show no significant differences in the level of total serum cholesterol. This indicates that although the treatments influence the blood glucose, they have little effect on the lipid status of diabetic animals. Thus the treatments may not inhibit mobilization of free fatty acids; the lack of inhibition may be due to the doses utilized or the duration of the treatments in the study. However, Aporo showed the ability to reduce atherogenic index by significantly elevating serum HDL levels relative to the diabetic control group. This suggests the potential of Aporo to improve diabetes-related complications such as atherosclerosis. Aporo also caused a significant reduction in serum triglyceride levels.

Much evidence has been recorded in the past few years supporting the fact that diabetes is worsened by stress. Glutathione (GSH) is a tripeptide that contains a free thiol group and is a major antioxidant in the human body. GSH provides reducing equivalents for glutathione peroxidase (GPx)-catalysed reduction of hydrogen peroxide and lipohydroperoxides to water. The reduced level of GSH in the diabetic group may suggest increased use due to ensuing oxidative stress; however, the significantly higher levels of GSH in the diabetic rats treated with the Aporo decoction is evidence that the GSH was preserved. This is potentially due to decreased oxidative stress, which could be attributed to the antioxidant properties of the Aporo decoction. Further evidence of increased oxidative stress in the diabetic state is based on increased lipid peroxidation or reduced antioxidant reserves such as antioxidant enzymes. Under normal conditions, the amount of lipid peroxides in the body tissue serum is low; however, much research on the effect of diabetes on the antioxidant state of the body has shown that elevated levels of lipid peroxides occur in the plasma of diabetic rats. This effect is evidenced in the elevated lipid peroxide levels.
observed in the diabetic control rats. However, treatment of diabetic rats with either Glibenclamide, *M. pruriens*, Aporo or the combination of *M. pruriens* and *Aporo* reduced lipid peroxidation relative to the positive (diabetic) control group. The group treated with *Aporo* decoction showed much lower levels of lipid peroxidation relative to the negative control, indicating the potential of *Aporo* to reduce oxidative stress in addition to decreasing blood glucose concentrations in the diabetic state.

Protein metabolism in the insulin-deficient state is catabolic, so the body is continually breaking down body proteins to compensate for required energy that is not being provided by the metabolism of glucose. Thus, in the diabetic state, wasting occurs, causing decreased total protein levels. The results obtained from this study showed that there was a significant decrease in the levels of total protein of the diabetic rats; however, all treatments were able to restore the total protein levels, as seen by the significant elevation when compared with the diabetic and negative control groups.

Co-administration of the *M. pruriens* extract with *Aporo* had no added advantage in improving the lipid profile and the antioxidant status of the diabetic animals. *Aporo* has been implicated for antioxidant properties, most likely due to the high level of vitamin C and anthocyanin contents. The high antioxidant property of the *Aporo* decoction may be the rationale of its wide usage in the management of various diseases in Nigeria. This ability is especially important in the management of diabetes and in the diminution of its complications which are believed to be largely due to the activities of free radicals. Additionally, according to Lachin and Reza, plants having the ability to scavenge free radicals in the body are supposed to have the ability to restore the function of pancreatic tissues by increasing the rate of insulin secretion.

**Conclusion**

*Aporo* possesses antidiabetic properties, which may be linked to its antioxidant activity. Because oxidative stress is associated with the diabetic condition, the use of a strong antioxidant decoction such as *Aporo* could be relevant in managing hyperglycaemia and diabetic complications. Our data suggest that *Aporo* is better used alone than in combination with *M. pruriens*, as they are more effective individually than when combined. We recommend further investigations to explore the use of *Aporo* as adjuvant therapy in the management of diabetic condition.

**Conflict of interest**

The authors have no conflict of interest to declare.

**Authors’ contributions**

SOO conceived and designed the study. AAT, JAO, DAO YOA and DGO collected and organized the data. SOO, OI, TIE and OSA guided the data collection, analysis and interpretation. SOO, AAT, OI, TIE and OSA drafted the initial manuscript, and OSA wrote the final manuscript. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

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