Peperomia pellucida in diets modulates hyperglycaemia, oxidative stress and dyslipidemia in diabetic rats

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1. Introduction

Diabetes mellitus is a major public health problem in the developed as well as developing countries. It is ranked as the seventh cause of death in the world and third when it’s fatal complications are taken into consideration[1]. It is a disease characterized by chronic hyperglycaemia and glucosuria produced by an absolute or relative insufficiency of insulin. The ailment may result into the development of further metabolic and anatomic disturbances among which is lipemia, hypercholesterolaemia, weight loss, ketosis, arteriosclerosis, and gangrene, pathologic changes in the eye, neuropathy, renal disease and coma[2,3]. It is known that diabetes is a condition within the body where the beta cells of the islets of Langerhans in the pancreas do not produce enough insulin and/or the insulin receptors are not working properly. This results to an inadequate supply of insulin and therefore elevated blood glucose level. The symptoms of diabetes include increased blood glucose; increased appetite and thirst, unexplained weight loss, weakness, decreased blood pressure and blurred vision[4]. Diabetes is also associated with significant oxidative stress which has been reported to be a major contributory factor to several diabetic complications[5]. Reactive oxygen species (ROS) are important part of the defense mechanisms against infection, but its excessive generation has been implicated in the pathogenesis of vascular disease[6-8]. Diabetic patients have an increased incidence of vascular disease and it has been shown that free radical activity is...
elevated during diabetes[9,10]. Increased oxidative stress has also been proposed to be one of the major causes of the hyperglycemia-induced trigger of diabetic complications. Hyperglycemia stimulates ROS formation from a variety of sources. These sources include oxidative phosphorylation, glucose autooxidation, NAD(P)H oxidase, lipooxygenase, cytochrome P450 monoxygenases, and nitric oxide synthase. Normal levels of antioxidant defense mechanism is not sufficient for the eradication of free radical induced injury, therefore administrations of antioxidants from a natural origin have a promising role to play. Several antioxidants of plant material are experimentally confirmed and widely used as more effective agents against oxidative stress[11,12].

Peperomia pellucida (P. pellucida) (L.) HBK (Piperaceae) is popularly known in Nigeria as shiny bush or riri and is used locally for hypertension, diabetes and generally as tonic for healthy well being. It is an herbaceous plant with succulent alternate and ovaite leaves, with terminal and axillary efflorescences, at the opposite side from leaves, developing well in loose and humid soil by the tree shadows[13]. In folk medicine, this species is employed on abscesses, furuncles, and skin sores, as well eye inflammation (conjunctivitis). Literature data confirmed the antimicrobial and analgesic effects including other activities, such as anti-inflammatory effect[14,15]. Phytochemical studies revealed the presence of dill-apiol and pellucidin A, in P. pellucida[16]. The present study aims at investigating the antidiabetic and antioxidant effect of P. pellucida in alloxan induced diabetic rats.

2. Materials and methods

2.1. Preparation of plant material

Fresh leaves of P. pellucida were collected from the environs of the University of Ibadan, Ibadan, Nigeria. They were authenticated at the Herbarium, Botany Department, University of Ibadan, Ibadan, Nigeria. The leaves were air dried under laboratory conditions and grinded to powdering form. The fine powder was stored in airtight containers at room temperature until use. 100 g and 200 g of P. pellucida were compounded with 900 g and 800 g of standard rat feed (Ladokun feeds, Ibadan) to get 10% and 20% w/w of the plant-supplemented diet respectively.

2.2. Chemicals

Alloxan was obtained from were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals and drugs used in this experiment were of analytical grade and the purest quality available.

2.3. Animals

Male albino rats of Wistar strain weighing about 150–200 g obtained from the faculty of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria were used for the study. They were fed on standard rat pellet diet (Ladokun Feeds, Nigeria) and water was provided ad libitum. They were maintained under standard laboratory conditions and were subjected to natural photoperiod of 12 h light: dark cycle. Group one animals were administered with physiological saline at 10 mL/kg body weight and served as control. Experimental protocols complied with the “Principle of Laboratory Animal Care” (NIH publication No 85–23) guidelines.

2.4. Alloxan Induced diabetes

Diabetes was induced by a single (I.P) injection of 100 mg/kg of alloxan monohydrate. After 72 h of alloxan injection, the diabetic rats (glucose level > 250 mg/dL) were separated and used for the study.

2.5. Preparation of reference drug

The reference drug, Glibenclamide (Clamide by hovid, Malaysia) was purchased from Danax Pharmaceuticals, a local chemist in Ibadan, Nigeria. It was administered orally to the group on standard drug daily. The drug was dissolved freshly in normal saline and appropriate volumes were given to the animals depending on their weight. The animals were given 600 µg/kg body weight of the active ingredient.

2.6. Experimental design

A total of 35 rats were used. The rats were randomly distributed into seven groups of five rats each.

Group A – received water; standard feed and served as Normal control (10 mL/kg bwt)

Group B – Normal + 10%w/w of P. pellucida

Group C – Normal + 20%w/w of P. pellucida

Group D – Diabetic control

Group E – Diabetic + 600 µg/kg body weight glibenclamide (standard drug)

Group F – Diabetic + 10%w/w P. pellucida

Group G – Diabetic + 20%w/w P. pellucida

The rats were treated for four weeks after which they were sacrificed by cervical decapitation. Plasma was collected after centrifugation of collected blood samples at 3 000 g for 10 min in MSC bench centrifuge (Beckman and Hirsch, Burlington, 10, USA).

The livers from animals were removed and rinsed in ice – cold isotonic, 1.15% KCl solution. The liver samples were homogenized in four times of ice – cold isotonic phosphate buffer, pH 7.4 and centrifuged at 10 000 g for 15 min to obtain the post mitochondria fraction (PMF). Both plasma and PMF aliquots were stored at −4 °C until use.
2.7. Biochemical analysis

Blood glucose was determined by the Glucose Oxidase method described by NCCLS[17]. Total plasma cholesterol was determined by Roeschlau et al[18], plasma triglycerides by enzymatic colorimetric method using Randox Kit[19]. Plasma HDL was assayed by the method of Lopes–Virella et al[20] plasma LDL[21] plasma alanine aminotransferase (ALT) and aspartate aminotransferase activity was determined by the optimized DGKC method using Randox kit. The supernatant obtained from the centrifuged liver homogenate was used for the following biochemical assays: superoxide dismutase (SOD), catalase, reduced glutathione (GSH) and lipid peroxidation (LPO)[23–26].

2.8. Statistical analysis

All values are expressed as mean±S.E.M. Data was analyzed by one–way analysis of variance (ANOVA) followed by Newman–Keuls multiple comparison tests. Differences of means were considered significant at P<0.05 using Graph–Pad Prism software Prism version 4.00 for Windows, GraphPad Software, San Diego California USA, “www.graphpad.com.”

3. Results

The hypoglycemic effect of *P. pellucida* supplemented diet is shown in Figure 1.

**Table 1**

<table>
<thead>
<tr>
<th>Group No</th>
<th>Treatment</th>
<th>Total cholesterol (mg/dL)</th>
<th>TG (mg/dL)</th>
<th>HDL (mg/dL)</th>
<th>LDL (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>Normal control</td>
<td>17.60±4.51</td>
<td>9.60±0.89</td>
<td>6.40±2.19</td>
<td>8.80±4.64</td>
</tr>
<tr>
<td>Group B</td>
<td>Normal + 10%w/w <em>P. pellucida</em></td>
<td>15.00±3.81</td>
<td>8.00±7.18</td>
<td>8.60±2.51</td>
<td>5.70±2.52</td>
</tr>
<tr>
<td>Group C</td>
<td>Normal + 20%w/w <em>P. pellucida</em></td>
<td>14.40±5.60</td>
<td>7.20±3.70</td>
<td>9.80±3.49</td>
<td>4.36±2.65</td>
</tr>
<tr>
<td>Group D</td>
<td>Diabetic control</td>
<td>27.60±7.83</td>
<td>21.00±3.67</td>
<td>3.40±1.34</td>
<td>20.52±7.15</td>
</tr>
<tr>
<td>Group E</td>
<td>Diabetic + 600 µg/kg Gilben.</td>
<td>19.00±6.09**</td>
<td>12.40±5.86**</td>
<td>5.80±1.92**</td>
<td>10.52±3.52**</td>
</tr>
<tr>
<td>Group F</td>
<td>Diabetic + 10% w/w <em>P. pellucida</em></td>
<td>17.50±5.30**</td>
<td>10.75±5.60**</td>
<td>6.75±2.36**</td>
<td>9.30±5.67**</td>
</tr>
<tr>
<td>Group G</td>
<td>Diabetic + 20% w/w <em>P. pellucida</em></td>
<td>15.00±5.77**</td>
<td>8.75±5.69**</td>
<td>8.25±1.26**</td>
<td>8.75±1.54**</td>
</tr>
</tbody>
</table>

Result are expressed as Mean±Standard deviation (n = 5). *P<0.05 All groups compared with Normal control; **P<0.05 All diabetic treated compared with Diabetic control; *P<0.05 the two normal treated with *P. pellucida* compared; †P<0.05 Diabetic treated with *P. pellucida* compared with Diabetic + Glibencamide.

**Table 2**

<table>
<thead>
<tr>
<th>Group No</th>
<th>Treatment</th>
<th>AST (mg/dL)</th>
<th>ALT (mg/dL)</th>
<th>ALP (unit/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>Normal control</td>
<td>16.20±7.05</td>
<td>8.60±6.91</td>
<td>10.80±4.32</td>
</tr>
<tr>
<td>Group B</td>
<td>Normal + 10%w/w <em>P. pellucida</em></td>
<td>11.20±5.26</td>
<td>6.40±3.78</td>
<td>8.80±2.95</td>
</tr>
<tr>
<td>Group C</td>
<td>Normal + 20%w/w <em>P. pellucida</em></td>
<td>17.80±9.23</td>
<td>9.20±5.54</td>
<td>13.40±3.78</td>
</tr>
<tr>
<td>Group D</td>
<td>Diabetic control</td>
<td>31.20±14.53*</td>
<td>18.20±3.27*</td>
<td>26.20±4.34*</td>
</tr>
<tr>
<td>Group E</td>
<td>Diabetic + 600 µg/kg Gilben.</td>
<td>22.80±10.18</td>
<td>13.00±5.24**</td>
<td>15.00±9.51**</td>
</tr>
<tr>
<td>Group F</td>
<td>Diabetic + 10% w/w <em>P. pellucida</em></td>
<td>18.25±9.09</td>
<td>11.25±5.06**</td>
<td>12.25±3.30**</td>
</tr>
<tr>
<td>Group G</td>
<td>Diabetic + 20% w/w <em>P. pellucida</em></td>
<td>17.50±8.50</td>
<td>10.00±6.78**</td>
<td>10.75±1.30**</td>
</tr>
</tbody>
</table>
in blood glucose level respectively.

It was observed that the levels of total cholesterol, triglycerides and LDL—cholesterol except HDL—cholesterol were significantly ($P<0.05$) higher in case of alloxan induced hyperglycemic animals (Diabetic control) when compared with normal control (group 1) animals while the values of the above mentioned plasma lipid parameters were near to normal in case of animals receiving glibenclamide and diet supplemented with 10% and 20% P. pellucida. However, HDL—cholesterol increased significantly in these groups compared to the diabetic control (Table 1).

Table 2 shows the activities of AST, ALT and ALP of experimental rats. Diabetic rats showed significantly more activities of plasma AST, ALT and ALP respectively compared to normal control. Treatment with all plant supplemented diet and glibenclamide significantly reduced the activity of AST, ALT and ALP in the diabetic control rats ($P<0.05$).

Table 3 show the effect of P. pellucida on lipid peroxidation, GSH concentration and stimulation of Ca$^{2+}$ influx, an initial key step in insulin secretion by closure of K$^{+}$−ATP channels, membrane depolarization and stimulation of Ca$^{2+}$ influx, an initial key step in insulin secretion.[29,30]

It is reported that the derangement of glucose, fat and protein metabolism during diabetes, results into the development of hyperlipidemia.[31,32] Diabetic rats were observed to have increased plasma lipids, which are responsible for several cardiovascular disorders.[33] The higher lipid levels seen in diabetic rats may be due to increased mobilization of free fatty acids from peripheral depots and also due to lipolysis caused by hormones.[34]

Supplementation with both supplements of P. pellucida produced significant beneficial effects in the lipid profile in alloxan—induced diabetic rats, reducing triglycerides, total cholesterol, LDL, and increasing HDL, significantly. Thus, it can be concluded from this findings that the levels of total plasma cholesterol, triglycerides and LDL—cholesterol which are actually raised in diabetics can be lowered with P. pellucida supplementation; this antihyperlipidemic effect could represent a protective mechanism against the development of cardiovascular disorder such as atherosclerosis in diabetic patients.

The hepatic enzymes AST, ALT and ALP were used as biomarkers to check for early acute hepatic damage. The activities of AST and ALT are cytosolic marker enzymes reflecting hepatocellular necrosis as they are released into the blood after cell membrane damage. Therefore,
elevated activities of ALT and AST in the circulation serve as indicators of hepatic damage. ALP acts as a marker for biliary function and cholestasis. In this study, all treatment groups with experimental plant preparation effectively reduced plasma AST, ALT and ALP activities in diabetic rats better than the standard drug, suggesting that the plants may prevent hepatic injury associated with diabetes.

Oxidative stress resulting from enhanced free radical formation and/or defects in anti-oxidants defense causes severe tissue damage which may lead to a number of diseases like coronary artery disease, atherosclerosis, cancer and diabetes. Increased oxidative stress in streptozotocin diabetic rats has been reported[35]. This oxidative stress is also implicated in the development of diabetic complications[36]. Increased oxidative stress as measured by indices of lipid peroxidation and protein oxidation has been shown to be elevated in both type 1 and type 2 diabetes even in patients without complications[37,38].

The results showed increased lipid peroxidation in the liver of diabetic control group. This may be because the tissues contain relatively high concentration of easily peroxidizable fatty acids. The increase in oxygen free radicals in diabetes could be primarily due to increase in blood glucose levels, which upon auto-oxidation generate free radicals and secondarily due to the effects of diabetogenic agent of alloxan[39]. In diabetes, hypoinsulinaemia increases the activity of the enzyme, fatty acyl coenzyme, coenzyme A oxidase, which initiates β-oxidation of fatty acids resulting in lipid peroxidation[8]. Increased lipid peroxidation impairs membrane functions by decreasing membrane fluidity, and changing the activity of membrane–bound enzymes[36]. Its products (lipid radicals and lipid peroxide) are harmful to the cells in the body and are associated with various disease condition, atherosclerosis and brain damage[36]. Supplementation with P. pellucida and glibenclamide reduced the extent of lipid peroxidation in the diabetic treated group. This indicates that P. pellucida supplement may inhibit lipid peroxidation and thereby oxidative damage to tissues in diabetes.

Decreased glutathione (GSH) concentration in diabetes mellitus under in vivo concentration has been reported[40]. Supplementation with P. pellucida supplement significantly increased the glutathione content compared to diabetic control rats where the levels were significantly decreased. This depletion of GSH in diabetic rats can be attributed to GSH consumption by glutathione transferase for metabolic conjugation and to GSH oxidation for defense against the produced oxidative stress[6]. Elevated level of GSH treatment with P. pellucida therefore indicates recovery from oxidative damage done by alloxan diabetes on tissues.

SOD and CAT are the two scavenging enzymes that remove the toxic free radicals[41]. Superoxide dismutase is present in essentially every cell in the body and has been shown to play an important role in protecting cells and tissues against oxidative stress. Superoxide dismutases (SOD) remove the superoxide radical $O_{2}^{−}$ by accelerating its conversion to H$_2$O$_2$. Catalase is ubiquitous to most aerobic cells in animals and is especially concentrated in the liver and erythrocytes. It is known to decompose hydrogen peroxide into water and oxygen as shown in the equation:

$$2H_{2}O_{2} \rightarrow 2H_{2}O + O_{2}$$ (1)

The result showed that there was a significant reduction in SOD and CAT activity in diabetic rats compared to normal control while all treated groups showed significant increase in activity with 20% w/w supplement showing greater increment compared to either group treated with the drug and the 10% w/w supplement. This decrease in the activity of SOD observed in diabetic rats is consistent with various reports already documented[40,41]. As proposed by Wohaib and Godin[41], the reduction in SOD activity might be due to the direct damaging effect of free radicals on the enzyme. Therefore the activity of SOD, which is to remove the superoxide radical $O_{2}^{−}$ by accelerating its conversion to H$_2$O$_2$, is inactivated. The ability of the plant preparation to increase catalase activity may be due to the induction of the enzyme. Catalase is haemoprotein and phytochemical screening of P. pellucida has also shown the presence of iron (Fe)[42]. Iron is essential for the function of catalase.

The results of the present study show that diet supplemented P. pellucida have an antidiabetic and antioxidant property in alloxan induced diabetic rats. This effect may be due to the presence of tannin, saponin, flavonoid and other constituents in the plant, which could act synergistically or independently in enhancing the activity of glycolytic and antioxidant enzymes. Therefore the acclaimed traditional antidiabetic use of this plant is justified in this study.

Conflict of Interest

There was no conflict of interest among the authors.

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