Phytochemical Composition of Cassia Alata Leaf Extract and its Effect on the Histology of the Pancreas of Diabetic Wistar Rats


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Abstract: This study intends to validate the local use of cassia alata in the management of diabetes by quantitatively analyzing the phytochemicals present and its effect on blood sugar level and the histology of destroyed pancreatic islet cells.

50g of fresh leaves of cassia alata were used for phytochemical and nutritional analyses while the remaining leaves were dried and blended into fine powder. Bioactive substances in the herb were extracted with 80% ethanol. The herb extract was reconstituted with normal saline before administration to the rats.

Twenty-four wistar rats of both sexes with average weight of 160g were used in this study. The rats were randomly divided into three parallel groups of diabetic and non-diabetic rats. The non-diabetic groups were designated A, B, and C while the diabetic groups were designated A5, B5, and C5. Rats in groups A (A5 and A), served as control for each parallel group and received 0.4ml of normal saline. Rats in the 2 parallel groups of B (B5 and B) received single daily doses of 500mg/kg body weight of ethanolic leaf extract of cassia alata via orogastric tube while rats in groups C (C5 and C) received subcutaneous injection of 5IU/kg body weight of human insulin daily. All the rats received water freely and normal rat feeds. The experiment lasted for twenty-eight days. The rats were sacrificed and pancreatic tissue taken for histological studies. Histological sections were stained with Haematoxylin and Eosin staining techniques. Experimental diabetes was induced using a single intraperitoneal injection of 65mg/kg body weight of streptozotocin. Results revealed the presence of varying quantities of saponins, flavonoids, tannins, oxalate and alkaloids. Blood sugar level also reduced in both the diabetic and non-diabetic groups and histology revealed regeneration of destroyed pancreatic islet cells. It can be inferred that cassia alata potentiates the regeneration of beta cells in the pancreas of diabetic rats and has hypoglycaemic effect. These antidiabetic effects may have been brought about by its rich phytochemical composition.

Keywords: Cassia alata, phytochemical, pancreatic islet cell.

I. Introduction

Diabetes mellitus is a chronic metabolic disease and for now there is no substantive cure. Many plant products have been used locally to bring about beneficial antidiabetic effects but most have not been screened to know the bioactive components that may be responsible for these effects. Recently the search for appropriate hypoglycaemic agents has been focused on plants used in traditional medicine[1].

Natural compounds with antidiabetic activity include complex carbon hydrates, alkaloids, glycopeptides, amines, flavonoids, sulphur compounds and inorganic ions. The antidiabetic mechanisms are numerous including activation of regeneration of functional pancreatic beta cells thereby increasing the number of insulin producing pancreatic beta cells[2], inhibition of insulinase activity[3], possession of insulin-like polypeptides[4] which will mimic the action of insulin, interference with carbohydrate absorption by the plant fibre[5], stimulation of insulin secretion in pancreatic beta cells[6] and increase in glycogen storage[7]. Cassia alata is a beautiful flowering shrub, a member of genus cassia belonging to the family of caesalpiniaceae. It is commonly known as candle bush. Different parts of the plant have been used for a wide spectrum of diseases. The leaves have been reported to possess anti-inflammatory and hypoglycaemic actions[8].

This study seeks to validate the traditional use of this herb by screening for the bioactive components of the leaves and its effect on destroyed beta cells of the pancreas.

II. Methodology

2.1 Plant material

Fresh leaves of Cassia alata were harvested locally in the month of July and taken to the botanical garden of the University of Calabar for authentication and acquisition of a voucher number. The leaves were and air dried at room temperature. 50g of the fresh leaves were used for analysis of the phytochemical composition, nutritional and mineral contents. The methods for these analysis were described by AOAC[9] and Harbone[10].

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Dried leaves of *Cassia alata* were blended to fine powder and 570g of the powder was extracted with 80% of alcohol. The oily brown extract was refrigerated until use. For administration, the extract was reconstituted with normal saline.

### 2.2 Animals

Twenty-four presumably healthy wistar rats of both sexes with average weight of 160g were bred in the animal house of the department of Anatomy, University of Calabar, Calabar. The rats were randomly divided into three parallel groups of non-diabetic (A, B and C) and diabetic (A, B and C) groups. Table 1 shows the experimental protocol. The experiment lasted for twenty-eight days.

<table>
<thead>
<tr>
<th>Table 1: Showing the experimental protocol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-diabetic Groups</td>
</tr>
<tr>
<td>---------------------</td>
</tr>
<tr>
<td>A&lt;sub&gt;N&lt;/sub&gt;</td>
</tr>
<tr>
<td>B&lt;sub&gt;N&lt;/sub&gt;</td>
</tr>
<tr>
<td>No. of rats</td>
</tr>
<tr>
<td>Treatment</td>
</tr>
<tr>
<td>received/Route of administration</td>
</tr>
</tbody>
</table>

Bwt: body weight, CA: *Cassia alata*, N/S: Normal saline, Subcut: Subcutaneous

Apart from the daily treatment of the animals with *cassia alata* extract, normal saline and insulin, the animals were given freely fed with normal rat feeds.

### 2.3 Induction of experimental diabetes

Experimental diabetes was induced with a single dose of 65mg/kg body weight intraperitoneal injection of streptozotocin reconstituted in normal saline after an overnight fast. Non-diabetic rats were injected with only normal saline. 72 hours post induction the fasting blood sugar (FBS) was estimated using finest glucometer. Blood was collected from the tail vein of the rats. Rats with FBS greater than 13.3mol/L were judged to be diabetic[11] and were used for this study. FBS was monitored weekly.

At the end of the 28 days, the rats were fasted overnight and then anaesthetized using chloroform inhalation. Thereafter, a midline incision was made and the peritoneum stripped open. The pancreas was carefully dissected out and fixed in 10% formal saline. Routine histological tissue processing was done and haematoxylin/eosin staining technique was employed. Statistical analysis was done using the students t-test.

### III. Results

Results are presented in the tables below

#### 3.1 Effect of *Cassia alata* on mean body weight.

From Table 2, the negative control group (A<sub>N</sub>) and the groups that received insulin (C<sub>N</sub> and C<sub>D</sub>) showed appreciable weight gain but there was significant weight loss in the groups that received *cassia alata* (B<sub>N</sub> and B<sub>D</sub>) and the diabetic control groups (A<sub>D</sub>) at P<0.05. The percentage weight gains in groups A<sub>N</sub>, C<sub>N</sub> and C<sub>D</sub> are 9.58%, 10.32% and 4.32% respectively. These were not significantly different at P<0.05. The percentage weight losses in groups B<sub>N</sub>, A<sub>D</sub> and B<sub>D</sub> are 6.52%, 20.31% and 9.23% respectively and when compared with the negative control group (A<sub>N</sub>) these weight losses are significantly different at P<0.05. A significant difference also exist between groups A<sub>D</sub> and B<sub>D</sub> at P<0.05.

<table>
<thead>
<tr>
<th>Table 2: Showing the mean body weight of the rats at the beginning and end of the experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-diabetic Groups</td>
</tr>
<tr>
<td>---------------------</td>
</tr>
<tr>
<td>A&lt;sub&gt;N&lt;/sub&gt; weight in g</td>
</tr>
<tr>
<td>---------------------</td>
</tr>
<tr>
<td>Beginning of experiment</td>
</tr>
<tr>
<td>End of experiment</td>
</tr>
</tbody>
</table>

Values are mean weight ± SEM. Significant at P<0.05

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3.2 Effect of cassia alata on the mean fasting blood sugar

From Table 3, diabetic groups B_D and C_D showed a significant drop in FBS at p<0.05 back to normal when compared with the negative control groups (A_N). The FBS of the diabetic control group (A_D) remained significantly high at p<0.05. The non-diabetic animals in group B_N that received the herb extract developed hypoglycaemia.

Table 3: Showing the mean fasting blood sugar (FBS) of the rats at the beginning and end of the experiment

<table>
<thead>
<tr>
<th></th>
<th>Non-diabetic Groups</th>
<th>Diabetic Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A_N (mmol/l)</td>
<td>B_N (mmol/l)</td>
</tr>
<tr>
<td></td>
<td>0.30</td>
<td>4.15</td>
</tr>
<tr>
<td>Beginning</td>
<td>3.91±</td>
<td>6.73±</td>
</tr>
<tr>
<td>of experiment</td>
<td></td>
<td>0.44</td>
</tr>
<tr>
<td></td>
<td>C_N (mmol/l)</td>
<td>A_D (mmol/l)</td>
</tr>
<tr>
<td></td>
<td>0.33</td>
<td>17.52±</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.73</td>
</tr>
<tr>
<td>End of</td>
<td>4.17±</td>
<td>20.80±</td>
</tr>
<tr>
<td>experiment</td>
<td>0.33</td>
<td>*3.86±</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.34</td>
</tr>
<tr>
<td></td>
<td></td>
<td>*4.53±</td>
</tr>
</tbody>
</table>

*Significant at P<0.05

3.3 Proximate Composition of cassia alata

Table 4 showed that cassia alata leaves contain 68.80 ± 0.32% moisture content and 31.20 ± 0.17% dry matter out of the percentage dry matter, the carbohydrate, protein, fat, fibre and ash contents were 51.47 ± 0.29, 13.65 ± 0.02, 7.28 ± 0.01, 21.38 ± 0.14 and 6.22 ± 0.01 respectively.

Table 4: Showing percentage proximate composition of cassia alata leaves.

<table>
<thead>
<tr>
<th>Carbohydrate</th>
<th>Protein</th>
<th>Fat</th>
<th>Fibre</th>
<th>Moisture content</th>
<th>Dry matter</th>
<th>Ash</th>
</tr>
</thead>
<tbody>
<tr>
<td>51.47±</td>
<td>13.65±</td>
<td>7.28±</td>
<td>21.38±</td>
<td>68.80±</td>
<td>31.20±</td>
<td>6.22±</td>
</tr>
<tr>
<td>0.29</td>
<td>0.02</td>
<td>0.01</td>
<td>0.14</td>
<td>0.32</td>
<td>0.17</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Values are mean±S.E.M; n=3.

3.4 Phytochemical Composition of cassia alata

Quantitative phytochemical analysis(table 5) revealed the presence of alkaloids, flavonoids, saponins, tannins, phenols, oxalates and phytates. These bioactive substances have been known to exert their actions on the biochemical activities of the body and by so doing may alter certain body functions.

Table 5: Showing the percentage Phytochemical Composition of cassia alata leaves

<table>
<thead>
<tr>
<th>Alkaloids</th>
<th>Flavonoids</th>
<th>Saponin</th>
<th>Tannin</th>
<th>Phenol</th>
<th>Oxalate</th>
<th>Phytate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.14±</td>
<td>0.36±</td>
<td>1.14±</td>
<td>0.34±</td>
<td>0.28±</td>
<td>0.26±</td>
<td>0.34±</td>
</tr>
<tr>
<td>0.12</td>
<td>0.02</td>
<td>0.12</td>
<td>0.02</td>
<td>0.01</td>
<td>0.02</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Values are mean±S.E.M; n=3.

3.5 Quantitative Mineral Composition of cassia alata leaves

From table 6, cassia alata leaves contain potassium, calcium, sodium, magnesium and phosphorus in the following proportions 428.40 ± 4.25, 62.40 ± 0.35, 22.30 ± 0.14, 36.80 ± 0.18, 498.60 ± 4.27mg/100g

Table 6: Showing the quantitative mineral composition of cassia alata leaves

<table>
<thead>
<tr>
<th>Potassium (mg/100g)</th>
<th>Calcium (mg/100g)</th>
<th>Sodium (mg/100g)</th>
<th>Magnesium (mg/100g)</th>
<th>Phosphorus (mg/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>428.40 ± 4.25</td>
<td>62.40 ± 0.35</td>
<td>22.30 ± 0.14</td>
<td>36.80 ± 0.18</td>
<td>498.60 ± 4.27</td>
</tr>
</tbody>
</table>

Values represent mean ± S.E.M; n=3.

3.6 Effect of cassia alata leaf extract on the pancreas

Photomicrographs reveal the effect of cassia alata extract on the pancreas.

Plate 1 (Group A_N Negative Control)
Phytochemical Composition of Cassia Alata Leaf Extract and its Effect on the Histology of the

Stain: H/E magnification x 400
Photomicrograph reveals:
Numerous pancreatic acini
Islet of Langerhans (arrow) with a distinct capsule

Plate 2 (Group BN – received 500mg/kg bwt of cassia alata leaf)

Stain: H/E magnification x 400
Photomicrograph reveals:
Normal islet of Langerhans(arrow)

Plate 3 (Group CN received 5IU/kg bwt of insulin)

Stain: H/E magnification x 400
Photomicrograph reveals:
Numerous pancreatic acini
Islet of Langerhans(arrow) with clusters of cell

Plate 4 (Group AD – diabetic Control)

Stain: H/E magnification x 400
Photomicrograph reveals:
Pale stained nuclei in the islet of Langerhan(arrow)
Hypocellular and atrophic islets of Langerhan.
Phytochemical Composition of Cassia Alata Leaf Extract and its Effect on the Histology of the

Plate 5 (Group B received 500mg/kg bwt of cassia alata leaf extract)

Stain: H/E magnification x 400
Photomicrograph reveals:
Increase in islet cell mass with distinct capsule
Increase in number of cells islet of Langerhan(arrow)

Plate 6 (Group C – received 5IU/kg/bw of insulin)

Stain: H/E magnification x 400
Photomicrograph reveals:
Numerous pancreatic acini (A)
Small volume islet of Langerhan(arrow)

IV. Discussion

Weight changes is one of the features of diabetes. The hallmark of a poorly controlled diabetes is weight loss. It has been noted that patients with pervious poor glycaemic control who started newly on insulin had increase in weight[12],[13]. Weight loss was observed in the diabetic control group in this study which has also been reported by other researchers[14],[15],[16].

The groups treated with cassia alata had significant weight losses when compared with the non-diabetic control even with good glycaemic control so contradicting the relationship between glycaemic control and weight gain. Previous studies with cassia alata isolated Naringenin which stimulated a significantly higher glucose uptake activity and causes lipolysis in adipocytes both in the presence and absence of insulin[17]. This weight reduction effect of cassia alata may be good in the management of type II diabetes.

In this study, cassia alata exerted a hypoglycaemic and antihyperglycaemic effect. These effects have been reported in other studies using other herbs[18],[19],[20],[21]. Several mechanisms have been proposed for these effects. Nelson et al.,[5] reported that plants exert their antihyperglycaemic effect by interfering with carbohydrate absorption by plant. In this study cassia alata was found to have high fibre content. Another proposed mechanism is by the action of phytocchemicals of plants. Earlier studies have established that saponin and flavonoids isolated from plants exhibit hypoglycaemic effect by increasing insulin release from pancreatic beta cells, increasing peripheral glucose uptake and by reducing glucose absorption[22],[23],[24]. Bolkent et al.,[25] and Diatawa et al.,[26] have reported that alkaloids and some saponins isolated from various medicinal plants have blood glucose reduction effect. Saponins have also been reported to enhance natural resistance and recuperative powers of the body[27]. Tannins and phenol are insulin – like substances[28],[29] and mimic the effect of insulin on glucose metabolism and enhanced insulin secretion. These substances have been isolated from cassia alata leaf in this study. Another possible way by which cassia alata may have exerted its hypoglycaemic and antihyperglycaemic effect may be by attenuating the body antioxidant system. Flavonoids, saponins and alkaloids have potent antioxidant activities. Hyperglycaemia generates glucose auto-oxidation and auto-oxidative glycosylation of proteins which leads to increased oxidative stress by increasing reactive oxygen species[30]. The increased oxidative stress results to depletion of majority of plasma antioxidants[31],[32],[33]. Expression of antioxidant enzymes is known to be very low in islet cells compared with other tissues and cells[34], therefore, once beta cells face oxidative stress they may be rather sensitive to it suggesting that oxidative stress may in part mediate toxicity of hyperglycaemia. It is not surprising therefore, that the blood
sugar of the diabetic control group increased instead of reducing while the group that was treated with cassia alata leaf extract achieved good glycemic control.

The presence of magnesium in cassia alata may have also contributed to the glycemic control. Magnesium is a co-factor in various enzyme pathways involved in glucose oxidation and it modulates glucose transport across cell membrane. Its deficiency is common in patients with diabetes[7]. It may increase insulin secretion[35],[36]. A decrease in fasting blood glucose and increase in postprandial insulin have been reported with magnesium supplementation[37],[38]. Calcium, which was also isolated from the plant material also counteracts the autoimmune deficiency abnormality that is associated with type 1 diabetes and helps to normalize blood sugar levels in type II disease[39]. The changes seen in diabetic islet cells have also been reported by other researchers who have worked with other plant materials[21],[40],[41],[42],[43].

V. Conclusion

Cassia alata leaf has been found to have great beneficial antidiabetic effects which is brought about by its wide array of proximate, mineral and phytochemical constituents. Results from this study therefore validates the traditional use of this plant material for the management of diabetes. Purification and standardization of this herb for clinical trails will be an advancement towards providing a cheap, accessible and effective therapy for the management of both type I and type II diabetes.

Reference

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