Proximate Composition, Antioxidant and Hypoglycaemic Potential of Aqueous Extracts of Seeds of Delonix regia on High Fat Diet and Streptozotocin-Induced Diabetes in Female Wistar Rats

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Abstract

The proximate composition, antioxidant and hypoglycaemic potential of aqueous extracts of seeds of Delonix regia on high fat diet (HFD) streptozotocin (STZ)-induced diabetes in female wistar rats were investigated. Forty eight (48) rats were grouped into eight. Diabetes was induced in all the groups except for group 1 which was used as normal control (NC) and was fed with normal diet. Diabetic state was achieved by feeding the rats with HFD which contained 20% sucrose, 20% lard and 60% grower mash for six weeks, followed by 40mg/kg body weight (BW) of a single dose intraperitoneal injection of STZ. Seven days after induction of diabetes, treatment with cooked D. regia seeds (A) and uncooked D. regia seeds (B) extracts commenced and this lasted for six weeks. Rats in groups 3(MET) and 4(MET+VDG) received metformin 100mg/kg and metformin/vildagliptin 50/25mg/kg (BW) respectively as standard drugs. While the rats in groups, 5(A1), 6(A2) and 7(B1), 8(B2) were induced and treated with A and B extracts respectively by receiving 150 and 300mg/kg (BW) orally and daily. Blood was obtained through cardiac puncture after the rats were anaesthetized and sacrificed. Histological evalu-
tions of the pancreas was done. Proximate analysis showed that the seeds contained, moisture, ash, lipid, protein, carbohydrate and fiber contents of $3.83 \pm 0.07$, $3.50 \pm 0.00$, $7.82 \pm 0.02$, $17.50 \pm 0.00$, $21.79 \pm 0.21$ and $45.57 \pm 0.26$ respectively. The treatment with both extracts A and B significantly decreased ($p<0.05$) the fasting blood glucose concentration and pancreatic amylase activities in dose and time dependent manner, when compared to the DC. Similarly, activities of superoxide dismutase (SOD) in both groups A and B were significantly ($p<0.05$) increased in a time dependent manner when compared to DC. Conclusively, $D. \text{regia}$ seed extract exhibited very impressive potency and promise in the management of diabetes and its complications and hence a potential source for the discovery of new orally active anti-diabetic drugs and alternative source for food nutrients.

**Keywords:** Proximate composition, $D. \text{regia}$, streptozotocin, pancreatic amylase, pancreas

**Introduction**

Dietary supplement is a product intended to supplement the diet by increasing the intake, which might consist of, but not limited to: a vitamin, mineral, herb or botanicals, an amino acid, a concentrate, metabolite, extract, enzymes or a combination of these ingredients. Out of the 250,000 high plant species on earth, more than 80,000 species are reported to have at least some medicinal values and around 5000 species have specific therapeutic value. It has been known that about 25% of all the drugs prescribed today are from plants (Raskin and Ripoll, 2004). This implies that plant-derived drugs make up significant part of natural product-based pharmaceuticals. In recent years, medicinal plants are receiving recognition as being beneficial in diabetes, cancer, obesity, osteoporosis and other chronic and degenerative diseases e.g. Alzheimer’s disease. The world health organization (WHO) estimates indicates that 80% of about 4 billion population of the developing countries relies on herbal medicine for some part of their primary healthcare (WHO, 2001). There are insufficient scientific data to support folkloric medicine despite the ancestral use of medicinal plants in the treatment of diabetes mellitus. To this effect a lot of medicinal plants and herbs have been studied and validated for their hypoglycaemic potentials using experimental animal models of diabetes (Kesari et al., 2005), including clinical studies involving diabetic patients (Herra-Arellano et al., 2004). Notwithstanding, it is necessary to study more anti-diabetic plants and mechanisms of actions by which most of these plants or products exert blood glucose lowering effects on tissue or organs. $D. \text{regia}$ commonly called royal ponciana, flyboyant or flame tree used for the management of diseases such as diabetes, wound healing, ulcer, obesity and heart problems, was selected for the present study in respect of the ethno-medical/tribal information and earlier studies. The seed extract of $D. \text{regia}$ at lethal dose LD$_{50}$, index for acute toxicity, is not toxic (Kaga et al., 2015). Plants have been relied upon by people for nourishment and medicine for treatment control and management of varieties of
diseases that have threatened their existence and survival dated back since the existence of human kind (Philipeon, 2001). Diabetes mellitus especially type 2 diabetes is prevalent in the world, and it is strongly associated with high fat diet, obesity and insulin resistance. Synthetic oral hypoglycaemic drugs, which are the main form of treatment for type 2 diabetes mellitus have been shown to have undesirable side effects, high secondary failure rates and are expensive (Bailey, 2000; Erasto et al., 2005). Due to these shortcomings, researchers all over the world have continued to search for anti-diabetic remedies with the expectation of finding new natural products that could be used or developed into harmless, cheap and efficient anti-diabetic remedies. Oxidative stress markers can be objectively measured and elevated as indicators, when there is alteration in major biomolecules in the cell and status of plasma antioxidant potentials during diabetes mellitus. This study was aimed to scientifically evaluate the proximate composition, antioxidant and hypoglycaemic potential of the aqueous extracts of Delonix regia seeds on high fat diet and streptozotocin-induced diabetic female wistar rats.

**Materials and Methods**

**Reagent and chemicals**
Streptozotocin (Sigma-Aldrich, Germany), Agappe assay kits (Agappe diagnostics LTD, Ernakulan Kerala, India) Formaldehyde (90%), Chloroform (90%). All other reagents and apparatus were of analytical grades.

**Collection of Delonix regia Seeds and Sample preparation**
Seeds of Delonix regia were collected at the premises of the University of Port Harcourt Teaching Hospital (UPTH) Port Harcourt, Rivers State. The pods from D. regia tree were collected and split open in order to abstract its seeds; the hard seeds were collected and ground into a fine powder. The ground fine powder for the uncooked sample was weighed and freshly soaked in distilled water for 24 hours after which it was filtered. The powder for the cooked sample was also weighed, soaked in distilled water, cooked for 15mins and was filtered after cooling. The extracts were quantified by drying 1ml of the homogeneous filtrate in an oven at 40°C in a pre-weighed watch glass. This is based on the fact that most preparations used in traditional medicines are formulated in cold or hot water (Asano, 2008).

**Procurement of animal**
Forty eight (48) female wistar rats weighing 100-130g were bought from the animal house of the Department of Biochemistry, University of Port Harcourt Choba, Rivers State. The rats were weighed and divided into eight (8) groups of six (6) rats each and were housed differently in plastic cage covered with wire gauze. They were left to acclimatize for one week and fed with grower mash and access to clean water, ad libitum.
Experimental design

Forty eight (48) female wistar rats weighing 100-130g were used for the study. The rats were weighed and divided into eight (8) groups of six (6) rats each and were housed differently in plastic cage covered with wire gauze. They were left to acclimatize for one week and fed with grower mash and access to clean water, *ad libitum*. Rats in group 2 to 8 were fed with high fat diet (20% sucrose+ 20% Lard + 60% grower mash) for six weeks and afterwards injected with 40mg/kg body weight (BW) streptozotocin in distilled water to induce diabetes (Srinivasan *et al.*, 2005). After seven days of induction of diabetes, groups 5 and 6 were treated respectively with 150 and 300mg/kg BW cooked *D. regia* seeds (A) while groups 7 and 8 were treated respectively with 150 and 300mg/kg BW with uncooked *D. regia* seeds (B) extracts for 6 weeks. Rats in groups 1 received normal saline, 2 HFD + streptozotocin (40mg/kg), 3 HFD + streptozotocin (40mg/kg) + Met (100mg/kg) and 4 HFD + streptozotocin (40mg/kg) + Met +VDG (50mg+25mg)/kg. Three rats from each group were sacrificed at the end of every 3 weeks. Histological evaluations of the pancreas and heart were done after six (6) weeks. Blood was collected through cardiac puncture into plain bottles and the organs preserved with formalin for histological analysis. The blood samples in plain bottles were centrifuged, after which the supernatants were collected and designated plasma stored in the refrigerator (4°C) for further analyses.

**Proximate composition** was analysed using the method described by (AOAC, 2006)

**Determination of blood biochemical indices**

Blood glucose concentration, pancreatic amylase, superoxide dismutase and catalase activities were analyzed using, ACCU-CHEK, Agappe assay kits (Agappe diagnostics LTD, Ernakulan Kerala, India) and methods described by (Beauchamp and Fridovich, 1971; Aebi 1974) respectively.

**Slide preparation and Slide examination**

Histopathological slides were prepared at Anatomical Pathology Laboratory, University of Port Harcourt Teaching Hospital (UPTH), Port Harcourt, Rivers State. Small tissues of the pancreas was collected in 10% formalin for proper fixation. This was processed and embedded in paraffin wax. Section of 5-6µm in thickness were cut, mounted on slide and stained with hexatoxylin and eosin. The photomicrographs were taken and analysed at Anatomy Department Laboratory, Faculty of Basic Medical Science, Madonna University, Nigeria, Elele, Rivers State. The prepared slides were examined with a Motic™ compound light microscope using x4, x10 and x40 objective lenses. The photomicrographs were taken using a Motic™ 9.0 megapixels microscope camera at x200 and x400 magnifications.
Statistical analysis

Data were expressed as Mean ± S.E.M. (Standard error of the mean) and graphs were drawn using Microsoft Office Excel and Software 2007. The data were analysed for statistical differences from test control groups of animals by One-Way Analysis of Variance (ANOVA). At p<0.05, differences between groups were considered statistically significant.

Results and Discussion

Results

Table 1: Proximate composition of hard seeds of Delonix regia

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Compositions (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>3.83 ± .07</td>
</tr>
<tr>
<td>Ash</td>
<td>3.50 ± 0.00</td>
</tr>
<tr>
<td>Lipid</td>
<td>7.82 ± 0.02</td>
</tr>
<tr>
<td>Protein</td>
<td>17.50 ± 0.00</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>21.79 ± .21</td>
</tr>
<tr>
<td>Fibre</td>
<td>45.57 ± 0.26</td>
</tr>
</tbody>
</table>

Values represent Mean ± S.E.M. of triplicate sample

Results obtained after administration of high fat diet streptozotocin-induced diabetes showed significant elevation (p<0.05) of fasting blood glucose concentration and pancreatic amylase activities of the rats. Treatments with cooked and uncooked extracts of Delonix regia seeds significantly reduced (p<0.05) these parameters when compared to negative control and the efficacy is comparably same to metformin and metformin/vildagliptin standard hypoglycemic drugs treated groups (3 and 4) respectively.

There was observed decrease in superoxide dismutase and catalase enzyme activities of the diabetic untreated rats compared to the normal control but following the oral administration of cooked and uncooked sample extracts, they significantly (p<0.05) increased the level of SOD activities only. There were observed high values of crude fibres and carbohydrate. Damaged organs of study were also reverted.

Data represent Mean ± S.E.M., n = 3 per group. Superscripts “a, b” indicate significant differences (p<0.05) when the normal and negative control groups are compared to the induced and treated groups respectively. Superscripts “c, d” indicate significant differences (p<0.05) when the positive and second positive control groups are compared to the groups treated with extracts respectively.
Fig 1: Effect of aqueous extracts of *Delonix regia* seeds on blood glucose concentration of high fat diet and streptozotocin induced diabetic female wistar rats after 3 and 6 weeks of treatments respectively.

Fig 2: Effect of aqueous extracts of *Delonix regia* seeds on pancreatic amylase of high fat diet and streptozotocin induced diabetic female wistar rats after 3 and 6 weeks of treatments respectively.
Fig 3: Effect of aqueous extracts of Delonix regia seeds on superoxide dismutase activities of high fat diet and streptozotocin induced diabetic female wistar rats after 3 and 6 weeks of treatments respectively.

Histology Result
Normal control group (A), showed intact intercalated disc, acinus and acinar cells which are arranged in lobules with prominent nuclei and islet of Langerhan with large proportion of islet cells. In negative control group (B), pancreas with connective tissue stroma were observed to have solid masses which appeared to be little tumour cells inside the connective tissue with presence of inflammatory cells. Groups 3 and 7 (C and G), showed regenerated islet cells with more prominent stable cells and onset of regeneration of islet cells and the connective tissue stroma.
contains more lymphocytes and macrophages which are observed in the periphery and within the islets respectively. Group 4 (D), showed moderate atrophied acini which decreased in sizes followed by loss of acinar cells. Group 5, (E) showed major degeneration of the acini especially (clumped and elongated) with hypoplasia of acinar cells. Group 6 (F), showed intact pancreas while group 8 (H), showed acini degeneration and which are not uniformly arranged but are sparsely separated from each other. Lymphocytic infiltrates are scanty.

Plates 1: Photomicrograph of the pancreas at week 6 showing Normal control group (A), Negative control group (B), Positive control (C), Second positive control (D), A1 group - treated with 150mg/kg (E), A2 group - treated with 300mg/kg (F), B1 group - treated with 150mg/kg (G), B2 group - treated with 300mg/kg (H)

**Discussion**

the proximate composition of the seed sample of *Delonix regia* presented in Table 1 showed that the moisture content of the sample which was 3.83% was found to be lower than 4.24% for brebra (*Millettia ferruginea*) seed (Ekop, 2007; Berhanu and Amare, 2014), 31.60% for *Gnetum africanum*. The moisture content for the sample, falls within the recommended storage range of 0–13% as reported by (James 1995) and therefore, highly recommended for storage stability (Oyebode et al., 2007). The ash and crude fibre contents were higher than those of *Gnetum africanum* and brebra which were 1.20% and 2.41% respectively while the carbohydrate content was lower than 87.62% of *Gnetum africanum* and higher than 11.9% of brebra seed (Berhanu and Amare, 2014; Ekop, 2007). The ash value content is a clear indication of high mineral content in the sample. The sample is rich in crude fibre, which represents the amount of indigestible sugar, and higher than most of other seeds as well as *Sphenostylis stenocarpa* obtained in eastern Nigeria (Ojiako et al., 2010; Adegunwa et al., 2012) used for the management of
Proximate composition, antioxidant and hypoglycaemic potential...

diabetes. Adequate intake of dietary fibre can lower serum cholesterol level, diabetes and heart diseases (Ishida et al., 2000). Reports have shown that diets that are low in fibre are undesirable as they could cause constipation and such diets have been associated with diseases of colon like piles, appendicitis, and cancer. Both the protein contents of Delonix regia seed and that of Gnetum africanaum were comparably same as well as, 17.80% reported for bambara groundnut by (Adewusi and Osuntogun, 1991) but a bit lower compared to the report of 18.25% for cream coloured Vigna subterranean (Ojo et al., 2014). The lipid content was within recommended range and higher than 3.15% of Gnetum africanaum. The crude lipid is low compared to reported values, 8.3-27.0% (Ifon and Bassir, 1980). This makes it an interesting healthy food; because high fat diets lead to increased blood cholesterol levels, diabetes, cancer and ageing (Anita et al., 2006). Plant food that provides more than 12% of its caloric value from protein is considered good source of protein (Pearson, 1976). The values of carbohydrate and protein are suitable for compounding of animal feeds and carbohydrate promotes the utilization of dietary fats and reduces wastage of proteins (Balogun and Olatidoye, 2012).

Foods and nutrients play vital roles in the normal functioning of the body. Inclusion of dietary active compounds in human nutrition has been demonstrated to have tremendous health benefits and reduce the risk of chronic diseases such as colon cancer, obesity, diabetes, and diverticulosis. Their uses have also been reported in the treatment and management of these chronic diseases (Slavin, 2005; Slavin, 2008). In this study results obtained after administration of high fat diet streptozotocin-induced diabetes showed that all the rats became diabetic after 7 days. Treatments with cooked and uncooked extracts of Delonix regia seeds significantly reduced (p<0.05) fasting blood glucose level after 6 weeks compared to negative control and the efficacy is comparably same to metformin and metformin/vildagliptin standard hypoglycemic drugs treated groups (3 and 4) respectively. Similar studies were also reported using Aegle marmelos and date seeds extracts in managing glucose utilization in diabetic rats (Kesari et al., 2006; Marghoob and Abdelmarouf, 2016). By enhancing glucose uptake, the extracts must have exerted their anti-hyperglycaemic activity, stimulating insulin secretion from pancreatic β-cells and insulin-like activity or alternatively by inhibiting pancreatic amylose and hepatic gluconeogenesis.

It is believed that oxidative stress plays important role in the development of vascular complications in diabetes particularly type 2 diabetes (Pham-Huy et al., 2008). ROS level elevation in diabetes may be due to decrease in destruction or/and increase in the production by catalase (CAT—enzymatic/non-enzymatic) and superoxide dismutase (SOD) antioxidants. The variation in the levels of these enzymes makes the tissues susceptible to oxidative stress leading to the development of diabetic complications (Lipinski, 2001). In this study, there was observed decrease in superoxide dismutase and catalase enzyme activities of the diabetic untreated rats compared to the normal control but following the oral administration of cooked and uncooked sample extracts of 300mg/kg and 150mg/kg (BW) respectively, they significantly (p<0.05) increased the level of SOD activities only, while other diabetic treated rats effectively, also increased the levels. This is
similar to the report made by (Sailaja and Krishna, 2017) using *Momordica dioica* seeds in streptozotocin-induced oxidative stress in diabetic rats. HFD/STZ induced diabetes increased significantly (p<0.05) the level of pancreatic amylase activities of the untreated diabetic rats which possibly led to the increase of the blood glucose level in the diabetic rats. The administration of cooked and uncooked sample extracts significantly lowered the activity of the enzyme of treated diabetic rats and the efficacy is comparably same to metformin and metformin/vildagliptin standard hypoglycaemic drugs treated groups. The inhibition of the activity of alpha amylase delays the degradation of carbohydrate, which by implication causes a decrease in absorption of glucose, as a result of the postprandial blood glucose level elevation (Rhabaso and Chiasson, 2004). These findings correlate the evidence that the inhibitory effect of pancreatic amylase would have limited the process of carbohydrate hydrolysis and absorption in the intestine, which led to a decrease in serum glucose levels (Tormo et al., 2012; Vaquero et al., 2012). There was observed significant damage in the pancreas of diabetic untreated rats supporting the biochemical changes, which showed solid masses which appeared to be little tumour cells inside the connective tissue with presence of inflammatory cells compared to intact pancreas of the normal control rats. Pancreas also showed major degeneration of the acini especially with hypoplasia of acinar cells in diabetic rats treated with MET+VDG, A1 and B2. Whereas A2 treated rats showed intact pancreas, and onset of regeneration of islet cells by B1 treated rats. There was no significant alteration in the histology of the heart of untreated and treated diabetic rats when compared to the normal rats.

**Conclusion**

This present study showed that, the values of proximate composition of *Delonix regia* seeds indicate its suitability in the compounding of feeds and alternative source of food nutrients for both humans and animals. The extracts of the *Delonix regia* seeds were able to lower the fasting blood glucose, pancreatic amylase activities and showed antioxidant properties in treated diabetic rats, and also exhibited a greater effectiveness in resolving adverse effects in the pancreas and heart of high fat diet streptozotocin-induced diabetic rats. It further suggests that the crude extract of the plant exhibited very impressive potency and promise in the management of diabetes and its complications and hence a potential source for the discovery of new orally active anti-diabetic drugs and alternative source for food nutrients.

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