Effect of Aqueous Extract of *Azadirachta indica* (Neem) Leaves on Some Indices of Pancreatic Function in Alloxan-induced Diabetic Wistar Rats

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Abstract: Aim: The effects of aqueous extract of *Azadirachta indica* on blood glucose concentration, serum α-amylase activity, body weight and pancreatic integrity of normal and alloxan-induced diabetic Wistar rats were investigated with the view to establishing possible mechanism of its antidiabetic action. Method: Thirty-two Wistar rats were randomly divided into four groups (1-4) of 8 rats each. Groups 1 and 2 rats were made diabetic by intraperitoneal administration of 150 mg kg⁻¹ alloxan monohydrate while groups 3 and 4 were normal rats. Groups 2 and 4 were treated with 400 mg kg⁻¹ of aqueous extract of *Azadirachta indica* leaves. Groups 1 and 3 rats were treated with placebo (0.5 mL distilled water). Treatments were administered to the rats by oral intubations for a period of 14 days and animals were maintained on commercial rat chow and tap water ad libitum. Results: Results showed that treatment with the extract caused a significant (p<0.05) reduction in fasting blood glucose level in the extract treated Diabetic (DT) rats by 54% but not in the extract treated Normal (NT) rats. The serum α-amylase activity was also significantly lower (p<0.05) in the extract treated Diabetic rats (DT) when compared to the placebo treated Diabetic Control (DC). However, there was no significant difference (p<0.05) in the serum α-amylase activity of the Normal Treated (NT) rats when compared to the normal control. Histological examination of pancreas of diabetic control rats showed cellular degeneration which appeared to be reversed in the animals following extract treatment. Conclusion: We concluded that the extract might have antidiabetic properties, which may be associated with enhanced islets cells regeneration.

Key words: *Azadirachta indica*, fasting blood glucose level, serum α-amylase activity, pancreatic regeneration

INTRODUCTION

*Azadirachta indica* known as neem in many countries of the world is a large evergreen tree that belongs to the family Meliaceae. It is believed to have originated from Assam and Burma in South Asia (National Research Council, 1992) and grows well in tropical and sub-tropical regions around the world (Jacobson, 1990) with ability to withstand many adverse environmental conditions such as drought, infertile soil, stony, shallow or acidic soil (Jacobson, 1990).

The major active constituents of the tree are nimbin, nimbidin and nimbinene (National Research Council, 1992; Biswas et al., 2002). The leaves yield quercetin (Flavonoid) and nimbosterol (β-sitosterol) as well as a number of limonoids (Jacobson, 1990). The trunk bark contains nimbin (0.04%), nimbine (0.08%), tannins (6.0%), while, the stem bark contains tannins (12-16%) and non-tannins (8-11%) (Biswas et al., 2002). The oil extracted from the seeds contains nimbosterol and flavonoids (Biswas et al., 2002).

*Azadirachta indica* is used for a wide range of purposes. The oil extracted from the seeds (Neem Oil) has insecticidal properties (Kraus, 1995; Jacobson, 1990). Neem cake, the residue of neem seed after oil extraction is used as fertilizer (Kraus, 1995; Jacobson, 1990) nematocide (Kraus, 1995) and to lower soil nitrogen loss. *Azadirachta indica* has been used as traditional remedies for treatment of various forms of diseases from antiquity. All parts of the plant are said to have some medicinal properties (Biswas et al., 2002). It is traditionally used for treatment of arthritis, leprosy, typhoid, respiratory disorders, constipation, chronic fatigue, cancer, chronic syphilis sores and indolent ulcer. It is also traditionally used as

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tonic and astringent for wounds, tooth decay and gum
diseases (Biswas et al., 2002) and as a general health
conditioner it is said to be a potent antimalaria, antifungal
and antibacterial agent (Van der Nat et al., 1986).

Azadirachta indica is popularly used worldwide in
the treatment of diabetes mellitus (Yampallewar et al.,
2003; Chattopadhyay, 2003). Its use for the treatment of
diabetes in Ayurveda (India) was based on the ancient
belief that when an excess of one taste causes a disease,
the opposite taste is introduced into diet to counteract
the effect of the other (Usman et al., 2005). Both
traditional and clinical trials have supported the fact that
Azadirachta indica is a potent antidiabetic agent. Based
on the numerous successful clinical trials, the Indian
Government approved the manufacture and sales of neem
tablets by pharmaceuticals bodies for diabetes treatment
(Biswas et al., 2002). In view of the growing interest in the
use of Azadirachta indica for medicinal purpose, it would
therefore be desirable to conduct extensive clinical and
laboratory investigations on this plant to justify its
continued use as an antidiabetic agent. Previous studies
have elucidated the effect of this plant on blood glucose
levels both in humans and animal diabetic models
(Biswas et al., 2002; Halim and Hussain, 2002;
Mahdi et al., 2003; Usman et al., 2005). The mechanism of
its antidiabetic properties is not well understood. There
exist shades of opinion about its mechanism of action.
Some proposed modes of action of the plant include; the
presence of insulin-like substances or substances which
interact with carbohydrate absorption, inhibition of
insulinase activity and/or increase in number of
functional beta cells in the pancreas of the diabetics
(Jelodar et al., 2005). Furthermore, there are conflicting
reports on its impact on blood glucose level. Some authors
reported hypoglycemic effect in normal as well as
alloxan-induced diabetic rats (Biswas et al., 2002) while,
others reported a lowering of blood glucose level to
normal in diabetic treated group.

The present study was designed to determine the
effect of aqueous extract of Azadirachta indica on blood
glucose level, serum amylase activity, body weight and
histological integrity of pancreas of normal and alloxan-
induced diabetic albino Wister rats and to establish a
possible mechanism of the antidiabetic properties of
Azadirachta indica.

MATERIALS AND METHODS

Fresh and matured leaves of Azadirachta indica were
harvested from University of Calabar Botanical Garden,
Calabar, Nigeria. Specimens of these leaves were
authenticated in Botany Department of the University of
Calabar and voucher specimens deposited in the
herbarium. The harvested leaves were selected to remove
dead ones and unwanted materials. The leaves were
thoroughly washed with tap water to remove all dust
particles. The leaves were dried under shed until
completely dried and were pulverized into fine powder.
Three hundred and thirty grams of the powder was
extracted in 2 L of distill water and stored overnight for
complete extraction in a well-labelled container in a West
cool fridge at 4°C. The mixture was stirred thoroughly to
mix and filtered with a chess cloth. Thirty milliliters
aliquots were drawn and dried in beakers of known weight
at 50°C to a constant weight using rotary evaporator. The
extract concentration was determined by gravimetric
method. The extract concentrates were stored in the
refrigerator at 4°C. Three grams of the dry extract were
dissolved daily in 25 mL of distilled water for
administration to the animals. The concentration of the
redissolved extract was 120 mg mL⁻¹ and 0.5 mL of the
solution administered to 150 g rat was equivalent to
400 mg kg⁻¹ body weight. The volumes of solution for the
various weights of rats were determined accordingly.

Animal treatment: Forty albino Wistar rats of both sexes
weighing between 150-180 g were obtained from the
animal house of Department of Anatomy, University of
Calabar, Calabar, Nigeria. They were allowed to
acclimatized for 7 days at the El-camel Animal House,
College of Medical Sciences, University of Calabar,
Nigeria, where they were caged throughout the duration
of the experiment with plastic cages (North Kent Co. Ltd).
The room temperature was 28±2°C with adequate
ventilation. The animals were maintained on commercial
rat chow and tap water ad libitum throughout the
duration of the experiment except withdrawal of food on
nights preceding measurements of fasting blood glucose.

Induction of experimental diabetes: After the 7 days of
acclimatization, 22 rats were randomly selected for
induction of diabetes. Diabetes was induced by
intraperitoneal injection of freshly prepared alloxan
monohydrates (Sigma Louise, MD, USA) solution at a
dosage of 150 mg kg⁻¹ body weight after an overnight
fast. A week after administration, fasting blood was
collected from tail prick of surviving rats using lancet and
dropped on the reagent pad of One Touch strip (Life Scan
Inc. Militas, California, USA), which was inserted into a
One Touch Brand Glucometer (Life Scan, USA) to obtain
the glucose concentration. Rats with consistent fasting
blood glucose level between 200-400 mg dL⁻¹ for three
days were considered diabetic and were recruited in the
study.
Animal grouping and experimental protocol: There were four groups of experimental animals (group 1 to 4) of 8 rats each. Groups 1 and 2 were diabetic animals while group 3 and 4 consisted of normal animals. Group 1 was Diabetic Control (DC) receiving placebo treatment (0.5 mL distill water). Group 2 was Diabetic Treated (DT), receiving aqueous extract of Azadirachta indica at 400 mg kg⁻¹ body weight. Group 3 was Normal Control (NC), receiving placebo (0.5 mL distill water), while group 4 was Normal Treated (NT) and receiving aqueous extract of Azadirachta indica at 400 mg kg⁻¹ b wt. Treatments were administered to the rats by oral intubations for a period of 14 days.

Collection of sample for analysis: Fasting blood samples for determination of blood glucose concentration were collected every three days and were collected before commencement of the day's feeding. At the end of the experiment, rats were sacrificed using chloroform vapour anesthesia and were dissected medio -ventrally to expose the heart and the pancreas. Blood samples were obtained from the heart by cardiac puncture using syringe and needle and collected into non-heparinized tubes, allowed to clot for two hours and then centrifuged at 3,000 g for 10 min. Serum were collected into clean tubes and kept in a refrigerator at -4°C and analyzed within 2 days. The pancreas were removed and fixed in 10% neutral formalin. Body weight of animals in each group was also noted before, during and after treatment.

Biochemical assays: Blood glucose concentrations were determined using a One Touch Glucometer (Life Scan, USA) based on method of Trinder (1972). Serum amylase activity was determined using commercial kit from Dialab Ventrieck von Chemisch, Wien based on method of Lorentz (1988).

Histological studies: Histological studies were carried out in the Histology unit, Department of Anatomy, University of Calabar. The pancreas were fixed in 10% neutral formalin for 48 h and dehydrated in grades of alcohol 70, 80, 95% and then absolute alcohol for 1 h per two changes each. There were then cleared in two changes of xylene for 1 h each, infiltrated in molten paraffin wax at 60°C and embedded in pure paraffin wax to make block for sectioning. Sections of the tissues were made at 5 microns and attached on slides and stained with haematoxylin and eosin (Gomori, 1950). Sections were dewaxed in xylene and passed through absolute alcohol 95, 70% and then in water. The section were stained in haematoxylin for 15 min and rinsed in water. They were differentiated in 1% acid alcohol and left in water to turn blue for 3 min. The sections were counterstained in 1% alcoholic eosin for 3 min, dehydrated in 70 and 95% alcohol, then absolute alcohol, cleared in xylene and mounted with DPX mountant.

Statistical analysis: Data obtained were expressed as Mean±Standard deviation. Students' t-test was employed to test for variation of means between treatment groups and control. The differences between the mean of two groups were considered significant at p<0.05. Correlation analysis was also employed to test the relationship between blood glucose concentration and serum amylase activity.

RESULTS

Table 1 shows the blood glucose level and body weight of the experimental groups of rats before treatment with either Azadirachta indica or placebo while Table 2 shows the blood glucose level, serum amylase activity and body weight of the rats after 14 days of treatment with either Azadirachta indica or placebo. Figure 1 shows the blood glucose levels of the experimental groups of Albino Wistar rats during treatment with either Azadirachta indica or placebo monitored at interval of 3 days while Fig. 2 to 4 show the histological architecture of the pancreas of the experimental group of the animal after treatment with either Azadirachta indica or placebo. Diabetic control animals had significant higher (p<0.05) blood levels of glucose (306.5±20.46 mg dL⁻¹) when compared to the normal control (NC: 104.80±29.10 mg dL⁻¹) and the Diabetic Treated (DT) rats (140.27±4.25 mg dL⁻¹) (Table 2). The blood glucose levels of diabetic treated rats (140.27±29.10 mg dL⁻¹) were significantly higher (p<0.05) when compared to those of normal control (104.80±29.10 mg dL⁻¹) and normal treated (104.80±16.50 mg dL⁻¹) after 14 days of treatment. There was a gradual reduction of glucose level from 301.00±22.10 on day 1 to 140.27±24.25 on day 14 (Fig. 1) in diabetic rats treated with extract.

The Diabetic Control rats (DC) (Table 2) also had significant (p<0.05) higher serum amylase activity

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Blood glucose level (mg dL⁻¹)</th>
<th>Body weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetic control (DC)</td>
<td>306.20±20.21</td>
<td>179.2±5.10</td>
</tr>
<tr>
<td>Diabetic treated (DT)</td>
<td>301.00±22.01</td>
<td>178.5±6.20</td>
</tr>
<tr>
<td>Normal control (NC)</td>
<td>104.80±26.10</td>
<td>180.0±5.15</td>
</tr>
<tr>
<td>Normal treated (NT)</td>
<td>104.50±26.25</td>
<td>179.4±6.25</td>
</tr>
</tbody>
</table>

NB: results are expressed as Mean±SD. Means within a column with different superscript are significantly different (p<0.05)
Table 2: Blood glucose level, serum amylase activity and body weight of experimental groups of albino Wistar rats after 14 days of treatment with either *Azadirachta indica* or placebo

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Serum glucose level (mg dL⁻¹)</th>
<th>Serum amylase activity (U L⁻¹)</th>
<th>Body weight (g)</th>
<th>%Total wt. gain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetic control (DC)</td>
<td>306±50±20.46</td>
<td>195±10±49.69</td>
<td>181±6±8.25</td>
<td>1.00</td>
</tr>
<tr>
<td>Diabetic Treated (DT)</td>
<td>140±25±24.25</td>
<td>100±18±30.90</td>
<td>181±6±9.50</td>
<td>1.38</td>
</tr>
<tr>
<td>Normal Control (NC)</td>
<td>104±80±29.10</td>
<td>119±26±36.5</td>
<td>183±5±7.12</td>
<td>1.91</td>
</tr>
<tr>
<td>Normal Treated (NT)</td>
<td>104±50±16.50</td>
<td>119±50±26.25</td>
<td>185±4±8.20</td>
<td>3.24</td>
</tr>
</tbody>
</table>

N/B results are expressed as Mean±SD. Means within a column with different superscript are significantly different (p<0.05)

![Graph showing blood glucose concentration over days of treatment](image)

Fig. 1: Blood glucose concentrations of rats monitored at 3 days intervals during the period of treatment

Fig. 2: Photomicrograph of section of pancreas of the Normal Control (NC) stained with H and E (X 40). All the cells of the islets and acini with their nuclei were well stained. The interlobule and Intralobule were closely associated with the acini cells

(195±10±49.69 U L⁻¹) compared to the Normal Control (NC: 119±20±36.5 U L⁻¹) and the Diabetic Treated group (DT: 100±18±30.0 U L⁻¹). The enzyme activity however, reduced significantly (p<0.05) following extract treatment. The amylase activity of the diabetic treated group (DT: 100±18±30.00 U L⁻¹) was not significantly different (p<0.05) when compared to the Normal Control (NC: 119±20±36.50 U L⁻¹) and the Normal Treated (NT: 119±50±26.25 U L⁻¹). This result shows that aqueous extract of *Azadirachta indica* when administered to alloxan-induced diabetic rat significantly lowered serum amylase activity. There was a strong positive correlation (r = 0.939) between blood glucose level and serum amylase activity in the diabetic groups.

The photomicrograph of pancreas of the experimental animals showed that pancreas of the Normal Control (NC) (Fig. 2) had secretory acini and islet cells with distinct nuclei. The pancreatic cells were well stained and consistent with normal tissue histology. But in the Diabetic Control (DC) (Fig. 3), there were areas of marked necrosis particularly on the pancreatic islets and some cells of the acini. It showed cellular oedema and fluid filled spaces consistent with cellular inflammation. In the Diabetic Treated group (DT) (Fig. 4), the pancreatic islet and acini cells, although with some necrotic areas, showed signs of cellular regeneration. Arterioles were fairly distinct. The results show that treatment with the extract may enhance cellular regeneration of the pancreatic islet as well as acini cells.

**DISCUSSION**

The result of this investigation clearly indicates that aqueous extract of *Azadirachta indica* administered orally to alloxan-induced diabetic albino Wistar rats substantially reduced blood glucose level. This result agrees with the reports of Biswas *et al.* (2002) and Khosla *et al.* (2000). The mechanism of the antidiabetic properties of the extract is not well known. Jeelodar *et al.* (2005) had suggested that the antidiabetic properties of the extract may be related to the ability of the extract to stimulate sufficient production of insulin by the pancreas, that aided in the peripheral utilization of glucose in the cells, or a possible ability of the extract to regenerate the β-cells to carry out its functions.
Fig. 3: Photomicrograph of section of pancreas of the Diabetic Control (DC) stained with H and E (X 100).
The pancreatic islets (Pi) and its cells were degenerated or affected, with the islet sclerosis (iS).
The serous acini cells (Sa) were not evenly distributed in the photomicrograph, some may have been replaced with fatty deposits (F) or lipids.

Present study supports the fact that the aqueous extract rebuilt the destroyed islet and acini cells enabling them to perform their responsibilities. Alloxan used for the induction of diabetes is known to mediate the destruction of β-cells by establishing redox-cycles resulting in the formation of reactive oxygen species which constitute the major inducer agents for cell damage and hence diabetes in the rats (Szkudelski, 2001). The photomicrograph of the pancreas of Diabetic Treated (DT) rats (Fig. 4) showed regeneration of the β-islet and acini cells. The pancreatic β-islet cells are involved in insulin synthesis while the acini produce α-amylase enzyme (Guyton and Hall, 2000, Szkudelski, 2001), which is normally channeled into the gastrointestinal tract as part of pancreatic juice for the digestion of polysaccharide in diets. Damage to acini cells results in leakage of the enzyme into blood and may thus raise its serum concentration as we observed in the diabetic control group. There was a significant reduction in serum amylase activity (p<0.05) in the diabetic treated rat (100.18±30.00 U L⁻¹) compared to diabetic control rat (195.1±49.69 U L⁻¹).

Evidence had shown that the extract of Azadirachta indica leaves does not inhibit alpha-amylase activity in vitro (Kotwaroo et al., 2006) but however, reduces in vivo lipid peroxidation activities and cellular damage associated with oxidative stress (Elaidem et al., 2007). Therefore, reduction of serum amylase activity in vivo following extract administration may not be associated with the extract inhibitory activity but rather on its ability to reduced enzyme protein release into circulation. Our findings suggest that healing of the cells prevent significant leakage of the enzyme from the acini cells into the blood. The healing enhancing effect on the acini and β-cell were perhaps responsible in part, for the antidiabetic properties of aqueous leaf extract of Azadirachta indica.

Furthermore, extract treatment on normal animals did not cause any significant reduction in the levels of blood glucose. This indicates that the mechanism of antidiabetic effect of the extract may be unconnected with the stimulation of insulin synthesis and release in normal islet β-cells as suggested by Jelodar et al. (2005) since, increased insulin production and release into circulation is known to cause hypoglycemia in normal subjects. Its mechanism of antidiabetic action may therefore be more likely associated with enhanced repairs of damaged β-cells and regeneration of new ones to increase the number of functional β-cells for appropriate synthesis of insulin.
It is possible that some constituents of the extract may have mimicked or stimulated the actions of growth factors hence its ability to enhance the repair and regeneration of damaged pancreatic tissue. This position is strongly supported considering the fact that significant (p<0.05) increase in growth rate was obtained for normal rats receiving treatment (Table 2) compared with those without extract treatment. The study supports the use of *Azadirachta indica* leaf extract in the management of diabetes mellitus particularly the type 1 diabetes mellitus.

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