AZADIRACHTA INDICA SEED EXTRACT AS ANTIHYPERGLYCEMIC AND ANTIHYPERLIPIDEMIC AGENT OF FUTURE

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ABSTRACT

Azadirachta indica seed extract has been shown to have antihyperglycemic and antihyperlipidemic effect as per earlier report of traditional medicine used by native people of Africa origin. In the present study, antihyperglycemic and antihyperlipidemic effect of Azadirachta Indica (family, Meliaceae) seed was evaluated in adult albino rat. Methanol extract (ME) and fractions (PEF, MF) were administered orally at the doses of 100,500,1000mg/kg body weight for duration of 30 days, and 0.5ml/kg of 3% tween 80 was administered to control group. There was significant reduction (P<0.05) in fasting blood glucose and total cholesterol levels compared to baseline and control. The reduction was not in a dose related manner. It is concluded that Azadirachta indica may have beneficial effect as antihyperglycemic and antihyperlipidemic agent of future.

INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia that could lead to mortality and morbidity (Mohammed et al 2007). Insufficient action and deficiency of insulin on peripheral tissues disrupts the metabolism of carbohydrates, fats and proteins (Abdirahman et al, 2015).

The clinical manifestation of hyperglycemia includes; polydipsia, polyuria, fatigue, weight loss and polyphagia (Njagi et al, 2015). Untreated hyperglycemia increases the risk of hyperlipidemia microvascular damage. It is associated with morbidity, reduced life expectancy, increased risk of macrovascular complications, diminished quality of life and death (Piero et al, 2015). Hyperglycemia increases diacylglycerol level and activates protein kinase C activity in the aorta of streptozotocin induced diabetic rat (Inoguchi et al, 1994). There is a tremendous increase of diabetic patients globally with a number of 170 million in the year 2000 and is projected to rise to 366 million by the year 2030 (Wild et al, 2004).

Many allopathic antidiabetic drugs used today fail to give long-term glycemic control (Singh, Lorke and Furberg, 2007). Moreover, the conventional drugs are expensive to most people who resort to alternative therapies from herbal remedies. These herbs are believed to be safe and effective (Zhu, Lew and Leung, 2002). The WHO committee on diabetes has recommended that herbal medicine be further investigated (Elizabeth, 2002). However, most herbal medicine have not been scientifically evaluated (Yanko et al, 2007).

Azadirachta indica (A. Juss), (family, meliaceae) popularly known as Neem (English) is one of the commonly used plants in traditional medicine. It is the most popular tree with great potential. The therapeutic relevance attributed to Azadirachta Indica are as diverse as antiseptic, spermicidal, antibacterial, antimalarial, antiviral, and immunodulating (Prakash, et al, 1988) antidiabetic (Gupta et al, 2004).

The aim of this research was to investigate methanol extract and its fraction of A. Indica seed as antihyperglycemic and antihyperlipidemic agent of future.
MATERIALS AND METHODS

Collection of plant material and extraction

Seeds of *Azadirachta indica* were collected from University of Nigeria Nsukka in January 2015. The seeds were identified and authenticated by pharmacognosy expert and a voucher specimen deposited at Nsukka garden. The seeds were dried under the sun for 2 weeks and reduced to coarse powder using a Hammar mill. The powdered plant (12.5kg) was extracted with 7.5 litre of methanol by continuous extraction in a soxhlet. The extract was concentrated using a rotatory evaporator to obtain dry 265g (2.12% w/w) of methanol extract (ME).

Experimental animals

Male swiss Albino mice (15-25g) and Adult Wistar albino rat (140-250g) were obtained from the laboratory animal facility of the faculty of veterinary medicine, university of Nigeria Nsukka. The animals were housed in a steel cage within the laboratory and maintained on standard pellet and water *ad libitum*. Four week was allowed for acclimatization. All animal experiment were carried out with the approval of Institutional animal ethical committee.

Phytochemical screening

Phytochemical screening of *A. Indica* seed extract and fractions were done qualitatively to determine the class of secondary metabolites present which included alkaloids, resins, carbohydrates, proteins, saponin, glycoside, terpenoid, steroids using conventional method (Harborne 1998).

Chromatographic separation of Methanol Extract (ME)

The methanol extract (ME) was subjected to a silica gel (70-200 mesh size) column eluted with petroleum ether: ethyl acetate (1:1) and methanol (100%). The silica gel column was packed dry, loaded on top with the extract (265g) mixed with silica gel and eluted with the solvents. The fractions were collected and pooled together into 2 broad fractions on the basis of solvent of elution. Concentration of the fractions in a rotary evaporator afforded 65g of petroleum ether-ethyl acetate fraction (PEF, 24.53% w/w) and 80g of methanol fraction (MF, 30.19% w/w). The PEF and MF were subject to studies.

Acute toxicity and lethality test

The acute toxicity and lethality LD50 of ME in mice (n = 12) was estimated using the method (Lorke, 1983). Animals received oral administration of one of 10, 100 or 1000mg/kg (n=3) of ME and were observed for 24hr for number of death, since no death occurred in any of the groups in the first stage of the test, another doses of 1600, 2900 and 5000mg/kg were administered in a fresh batch of animal (n=1) and no death was recorded within 24hrs. Thus the oral LD50 of ME in mice was found to be greater than 5000mg/kg.

Biochemical studies

Thirty Six (36) adult albino rats of either sex were used for the study. The animals were divided into 6 groups of 6 animals each; group I-III received one of 100,500 or 1000mg/kg of ME respectively. Group IV was the control received 0.5ml/kg of vehicle, 3% tween 80. Group V-VI received 100mg/kg of PEF and MF received respectively.

Extract and fractions were administered orally once for 30days. On day 10, 20 and 30, 3ml of blood was withdrawn from each rat by ocular puncture. The blood samples were collected for estimation of serum cholesterol (Allian 1974) and fasting blood sugar (Trinder, 1969). The weight was checked before and after the treatment.

Data Analysis

Data obtained were analyzed using student t-test and presented as mean ± SD P. values P<0.05 were accepted to be significant.

RESULTS

Extraction

Extraction process yield 265g (2.12% w/w) of methanol extract.

Acute toxicity and lethality studies

Oral administration of methanol extract to mice caused no death at doses ranging from 10-5000mg/kg. Therefore the LD50 of the extract in mice was estimated to be greater than 5000mg/kg.

Phytochemical of the extract and fraction

The ME gave positive reaction for alkaloids, glycosides, resins, carbohydrate, reducing substances, steroid, and terpenoids. PEF gave positive reaction for alkaloids, glucosides, resin, saponins, terpenoids.

MF gave positive reaction for alkaloids, glycosides, resin, saponins, terroid and terpenoids (table 1).

| Table 1 The phytochemical constituents of extract and fraction |
|---------------------------------|-------|-------|-------|
| **Relative presence**             | **ME** | **PEF** | **MF** |
| Alkaloids                        | ++    | +     | +     |
| Carbohydrates                    | +     | +     | +     |
| Glycosides                       | ++    | +     | +     |
| Resins                           | ++    | +     | +     |
| Saponin                          | -     | ++    | +     |
| Steroid                          | ++    | +     | +     |
| Terpenoid                        | +     | +     | +     |
| Reducing substances              | ++    | +     | +     |

ME = Methanol Extract; PEF = Pet ether: ethyl acetate fraction, MF=Methanol Fraction  
*+ = present in low quality, ++ = Present in moderate quantity*

The methanol extract (ME) and the fractions significantly (P<0.05) reduced fasting blood sugar (FBS) concentration in normal rats. The methanol (MF) and petether-ethyl acetate (PEF) fractions caused a moderate reduction in the concentration of FBS (Table 2 & 3).

| Table 2 Effect of ME on fasting blood sugar of normal rats |
|---------------------------------|-------|-------|-------|
| **Treatment**                  | **Dose** | **Glucose Concentration (mmol/l)** |
|                                | (mg/kg) | **Baseline** | **Day 10** | **Day 20** | **Day 30** |
| ME                             | 100    | 4.96 ± 0.59  | 3.96 ± 0.73 | 3.70 ± 0.45a | 3.50 ± 0.45a |
| 500                             | 5.10 ± 1.50 | 4.15 ± 0.24a | 3.20 ± 0.47 | 3.70 ± 0.28b |
| 1000                            | 5.75 ± 0.89  | 4.30 ± 0.48a  | 3.58 ± 0.43ab | 3.50 ± 0.37a |

*a*Compared to baseline; *a*Compared to 10 days using Student t-test
The methanol extract (ME) and its fractions caused varying levels of significant (P<0.05) reduction in serum cholesterol concentration of treated rats. The methanol extract (ME) caused an initial reduction with subsequent increase. The cholesterol level, however, did not reach baseline levels by day 30. The methanol (MF) and pet ether/ethyl acetate (PEF) fractions caused a mild reduction in serum cholesterol. However, PEF evoked a greater reduction than ME (Table 4 & 5).

Table 4 Effect of the ME on serum cholesterol concentration

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Glucose Concentration (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Day 10</td>
</tr>
<tr>
<td>ME 100</td>
<td>3.48 ± 0.23</td>
<td>2.56 ± 0.30</td>
</tr>
<tr>
<td>500</td>
<td>3.40 ± 0.44</td>
<td>2.93 ± 0.23</td>
</tr>
<tr>
<td>1000</td>
<td>3.53 ± 0.47</td>
<td>2.95 ± 0.44</td>
</tr>
</tbody>
</table>

*compared to baseline; †compared to 10 days using Student t-test

The extract and fractions increased body weight in treated rat abnormal (Table 6).

Table 6 Effect of the ME, PEF and MF on body weight of treated rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Body weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Final</td>
</tr>
<tr>
<td>ME 100</td>
<td>130.0 ± 8.1</td>
<td>225.0 ± 13.67</td>
</tr>
<tr>
<td>500</td>
<td>138.0 ± 35.0</td>
<td>256.0 ± 20.0</td>
</tr>
<tr>
<td>1000</td>
<td>223.0 ± 24.0</td>
<td>254.0 ± 15.6</td>
</tr>
<tr>
<td>PEF 100</td>
<td>125.6 ± 16.7</td>
<td>144.5 ± 13.4</td>
</tr>
<tr>
<td>MF 100</td>
<td>142.4 ± 12.5</td>
<td>164.9 ± 23.0</td>
</tr>
</tbody>
</table>

*P=0.05 compared to baseline values; Values of body weight shown are Mean ± SD; ME = Methanol extract; PEF = Petroleum ether-ethyl acetate fraction; MF = Methanol fraction.

DISCUSSION

In the present study, we report the effect of *A. indica* seed extract and fractions (ME, PEF and MF) on biochemical parameters with respect to their antihyperglycemic and antihyperlipidemic activities in normoglycemic rat. This study shows that the methanol extract and fractions of *Azadirachta Indica* seed produce a marked decrease in fasting blood glucose and total cholesterol concentration. *Azadirachta Indica* has been shown to possess antihyperglycemic activity (Sharm et al., 1983) and antihyperlipidemic effect in diabetes (Laakso, 1996). However chronic oral administration of the extract and fractions of *A. indica* seed at 100,500 and 1000mg/kg after 10,20 and 30 days showed pronounced reduction in blood glucose level in treated normoglycemic rat acquiring good glycemic control. The onset of antihyperglycemic activity was seen within 10 days of treatment though maximum reduction was observed after 30 days but not in a dose related manner. The decline in blood glucose is steady and follows a uniform trend throughout the study period. The study was in agreement with Khosla et al who used neem oil extract and Pilla et al (1981) who used leaf extract and seed oil in normal and diabetic rabbit.

This has also been reported for the leaf extract and seed oil in normal and diabetic rabbit (Pilla et al, 1981). The mechanism of action is still unclear but in a study by Khosla et al, neem has been shown to be protective against diabetes induced by alloxan. It can be said that the seed extract of *A. indica* protects beta cells probably through its antioxidant action. Hypoglycemic effect could be linked to potentiation of insulin release from the pancreas. Bajaj and Srinivasan (1999) suggested that the extract could have increased the uptake of glucose peripherally, due to its blood glucose reducing action. Several other mechanisms have been suggested like decreased synthesis by the liver, inhibition of proximal tubular absorption of glucose in kidney (Sharma et al, 1983). The antihyperglycemic could be attributed to the presence of phytochemicals constituents. The alkaloids promote the regeneration of pancreas thereby restoring insulin secretion (Elliot et al, 2000), saponins have been shown to have hypoglycemic effect (Broadhurst et al, 2000). The terpenoids which are heart-friendly because they reduce diastolic blood pressure and lower the sugar level in the blood (Hawkins and Ehrch, 2006). There was increased body weight in rat. This may suggest a direct stimulant effect on muscle and liver glycogen metabolism which prevent loss of muscle mass. The extract and fractions also showed the marked reduction in total cholesterol. Since alterations in serum lipid are known in diabetic (Laakso, 1996). Elsewhere, an extract of this plant has been demonstrated antilipidemic effect in clinical trial in human (Njoku et al, 2001) and may have a therapeutic relevance. The mechanism of this antihyperlipidemic is yet to be elucidated. Chemical analysis revealed the *A. indica* leaf extract contains the following six compounds which may be seen in seed extract.

Quercetin-3-0-B-D-glucoside, myricetin-3-0-rutinoside, Kaempferol-3-0-rutinoside, kaempferol-3,0-B-D-glucoside, Quercetin-3-O-L-rhamnoside ( Chattopadhyay, 1999). It is presumed that these compound ether wholly or partly may be responsible for antihyperlipidemic activity.

In spite of all these explanations, the exact mode of action needs be extensively studied in both human and animal models and it is necessary to know the effect of each of the active principles.

Acute toxicity of the extract in mice using oral route revealed an LD₅₀ value greater than 5000mg/kg suggesting the extract may be generally regarded as safe (Lorke, 1983). The present study has drawbacks. Only fasting blood sugar and total cholesterol were estimated in this study which does not give a clear picture of about the effect of neem seed on other
parameters of diabetes mellitus. Chronic toxicity studies to evaluate the effect of need on various hematological parameters electrolyte profile needs to be evaluated.

CONCLUSION

It holds the scope of a new generation of antidiabetic drugs. However, there is need for further studies, using various active principles, to establish mode of action.

Acknowledgement

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References


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