ANTIDIABETIC AND ANTIHYPERLIPIDEMIC EFFECTS
OF AQUEOUS NEEM (AZADIRACHTA INDICA)
EXTRACT ON ALLOXAN DIABETIC RABBITS

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Abstract:
Extracts of various plants material capable of decreasing blood sugar have been tested in experimental animal models and their effects confirmed. Neem or Margose (Azadirachta Indica) is an indigenous plant believed to have antiviral, antifungal, antidiabetic and many other properties. This paper deals with a comparative study of effect of aqueous Neem leaves extract alone or in combination with glibenclamide on alloxan diabetic rabbits. Administration of crude aqueous Neem extract (CANE) alone (1.5 ml/kg/day) as well as the combination of CANE (1.5 ml/kg/day) with glibenclamide (0.25 mg/kg/day) significantly decreased (P<0.05) the concentrations of serum lipids, blood glucose and lipoprotein VLDL and LDL but significantly increased (P<0.05) the concentration of HDL. The change was observed significantly greater when the treatment was given in combination of CANE and glibenclamide than with CANE alone.

KEY WORDS: Crude Aqueous Neem Leaves Extract (CANE), hypoglycemic, cholesterol, triglycerides, phospholipids

1. Introduction
Diabetes mellitus is a chronic metabolic disorder affecting approximately 5% of the world’s population. It is characterized by dysregulation in carbohydrate, protein and fat metabolisms caused by the complete or relative insufficiency of insulin secretion and/or insulin action [1]. According to World Health Organization projections, the diabetic population is likely to increase to 300 millions or more by the year 2025[2].
In recent years, extracts from the seeds of Neem plant have emerged as a promising source of natural pesticides owing to their low mammalian toxicity [3, 4]. The medical properties has been well known in the indigenous Indian system of Ayurvedic medicine for many centuries commercially available products such as
tooth paste, bath soaps, skin care, capsules and tonics based on antibacterial[5].
Extracts of various plant material capable of decreasing blood sugar have been
tested in experimental animal models and their effects confirmed [6, 7].
Also, Neem oil and nimbidin significantly delayed the peak rise in blood sugar after
administration of glucose. Further it was found that glucose tolerance curves were
similar to that of to lithiaamide[8], it has been reported that an aqueous extract of
tradle leaves of Neem tree reduced blood sugar in dogs [9].
The present paper deals with comparative study of effects the aqueous extract of
Neem leaves alone or with glibenclamide on alloxan diabetic rabbits.

2. Materials and Methods
2.1. Plant material
Neem leaves was collected from Hodeidah, Yemen during 2003, the leaves were
removed from their mud and dried at room temperature then blended with a coffee
bean blender and stored at 4 °C.
2.2. Preparation of extract
The powder was extracted with distilled water using soxhlet at boiling temperature
(100 °C) up to 4 hrs. The extract was cooled and filtered to remove the residue. This
was designated as crude aqueous Neem extract (CANE)[10]. Administration was
affected in volume of 1.5 ml/kg, once daily for one month orally.

2.3. Animals
2.3.1 Experimental animals
A) Thirty-six adult male albino rabbits (1 and 2 kg), maintained at 25±1°C in a well
Ventilated animal house under natural photoperiod conditions were used for study.
They were provided with standard diet and water ad libitum.
B) Diabetic was induced in the rabbit by a single I.V. injection of alloxan mono
hydrated (Hopkins and Williams) in distilled water at the rate of 75mg/kg body
weight [11]. The animals were fasted for 36 hrs before injection. After fortnight
rabbis moderate diabetes having glucosuria (using urine test tab (glucose test)) for
detected on glucose in urine [Bohringer-Mannheim, Germany][12]. And
hyperglycemia (blood glucose 200-250/100ml) were divided into six groups of six
animals each as mentioned below.
Group1: control given with saline (1.5ml/kg/day).
Group2: diabetic given with saline (1.5ml/kg/day).
Group3: diabetic treated with (1.5ml CANE/kg/day).
Group4: diabetic treated with glibenclamide (0.5mg/kg/day).
Group5: diabetic treated with (1.5ml CANE/kg/day) plus glibenclamide
(0.25mg/kg/day).
Group6: diabetic treated with insulin (8 units/kg/day).
Body weight, urine sugar (qualitative) and fasting blood glucose of all rabbits were
determined before the start of the experiment. After 30 days of treatment the body
weight, urine sugar and fasting blood glucose of animal were determined. Total cholesterol, HDL-cholesterol, VLDL, triglyceride and phospholipids were analyzed from the serum by using Lab Kits (Randox, United Kingdom) whereas LDL-cholesterol concentration was calculated according to Pr Johannesburg formula:

\[
\text{LDL-cholesterol} = \text{Total cholesterol} - \left(\text{TG} / 5 + \text{HDL-cholesterol}\right)
\]

Statistical analysis: the results were presented as Mean ± SEM and included student's t-test and analysis of variance (ANOVA) test. The significance level for all tests was taken as P value < 0.05.

3. Results

3.1. Blood glucose

In alloxan diabetic rabbits there were a significant increase (P< 0.05) in fasting blood glucose and urine sugar and there was a significant decrease (P< 0.05) in body weight and total hemoglobin content. A significant increase in body weight and hemoglobin level were observed, and significant decrease fasting blood glucose (FBG) and urine sugar (Table 1) in diabetic rabbits treated with CANE, glibenclamide, insulin and in combination of CANE and glibenclamide. Though all the antidiabetic drugs used significantly decreased the FBG level, combination therapy of CANE (1.5 ml/kg) and glibenclamide (0.25 mg/kg) produced greater reduction in FBG as compared to all the other groups. There was a significant (P<0.05) a melioration of body weight and total hemoglobin content in the diabetic rabbits given with combination therapy of glibenclamide with CANE.

Table 1: The effect of crude aqueous Neem extract (CANE) on blood glucose, change in body weight and urine sugar in normal and diabetic rabbits. (Values are mean±SEM of six rabbits in each group).

<table>
<thead>
<tr>
<th>Group</th>
<th>FBG (mg/dl)</th>
<th>Change in body weight (g)</th>
<th>Total Hb content</th>
<th>Urine sugar</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>13±1.1</td>
<td>14±1.3</td>
<td>34±5.2</td>
<td>13±1.2</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>230±16.9</td>
<td>290±5.9</td>
<td>-16±3.4</td>
<td>16±2.9</td>
</tr>
<tr>
<td>Diabetic + CANE</td>
<td>223±8.2</td>
<td>152±3.1</td>
<td>5±0.1</td>
<td>12±0.2</td>
</tr>
<tr>
<td>Diabetic + Glibenclamide</td>
<td>22±12.3</td>
<td>15±14.2</td>
<td>9±2.4</td>
<td>12±1.7</td>
</tr>
<tr>
<td>Diabetic + (CANE) + Glibenclamide</td>
<td>229±145</td>
<td>154±10.2</td>
<td>16±2.1</td>
<td>14±1.8</td>
</tr>
<tr>
<td>Diabetic + Insulin</td>
<td>22±9.1</td>
<td>12±8.4</td>
<td>11±0.1</td>
<td>13±1.2</td>
</tr>
</tbody>
</table>

Group 2 is compared to group 1, Group 3, 4, 5 and 6 are compared to Group 2.
*P Value < 0.05.
3.2. Serum cholesterol and triglycerides

Serum cholesterol and triglyceride levels in all groups of animals are given in Table 2. In alloxan diabetic rabbits there was a significant (P<0.05) increase of total cholesterol, triglycerides, phospholipids, LDL-cholesterol and VLDL and the significant decrease (P<0.05) in HDL-cholesterol in serum compared to that of normal control. All antidiabetic agents used in the experiments significantly decreased (Table 2) the levels of cholesterol, triglycerides, phospholipids, LDL and VLDL. The significant (P<0.05) fall of the serum lipid parameters was observed in the combination therapy of CANE with glibenclamide when compared with diabetic control, CANE or glibenclamide.

**Table 2.** The effect of crude aqueous Neem extract (CANE) on serum lipid of normal and diabetic rabbits.

(Value expressed as mg/100 ml serum are mean±SEM of six rabbits in each group)

<table>
<thead>
<tr>
<th>Group</th>
<th>Total cholesterol</th>
<th>Triglycerides</th>
<th>Phospholipids</th>
<th>VLDL</th>
<th>HDL</th>
<th>LDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>112.6±5.5</td>
<td>72.4±5.5</td>
<td>162.5±7.7</td>
<td>13.2±0.8</td>
<td>40.3±5.8</td>
<td>50.1±6.36</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>200.5±12.9*</td>
<td>168±12.1*</td>
<td>260±3.5*</td>
<td>17.5±1.2*</td>
<td>50.2±7.0*</td>
<td>68±6.6*</td>
</tr>
<tr>
<td>Diabetic + CANE</td>
<td>200.5±12.9*</td>
<td>168±12.1*</td>
<td>260±3.5*</td>
<td>17.5±1.2*</td>
<td>50.2±7.0*</td>
<td>68±6.6*</td>
</tr>
<tr>
<td>Diabetic + Glibenclamide</td>
<td>182.2±11.2*</td>
<td>99.2±10.2*</td>
<td>182.0±12.2*</td>
<td>22.5±2.4*</td>
<td>39.5±1.4*</td>
<td>68.5±2.4*</td>
</tr>
<tr>
<td>Diabetic + CANE + Glibenclamide</td>
<td>179.2±11.1*</td>
<td>95.6±11.5*</td>
<td>180.8±7.3*</td>
<td>15.5±1.3*</td>
<td>44.5±2.5*</td>
<td>48.9±5.5*</td>
</tr>
<tr>
<td>Diabetic + Insulin</td>
<td>205.8±10.2*</td>
<td>106.8±11.2*</td>
<td>188.3±7.4*</td>
<td>20.2±2.9*</td>
<td>37.5±4.8*</td>
<td>66.2±0.8*</td>
</tr>
</tbody>
</table>

Group 2 is compared to group 1, Group 3, 4, 5 and 6 are compared to Group 2.

* P Value < 0.05.

4. Discussion

Rabbits were used as animal models and alloxan was used a diabetogenic agents. Alloxan administration has been found to lead to long-last in diabetes in many animals species. A number of studies have shown that alloxan disrupts the integrity of the beta cell plasma membrane. Some evidences indicate that alloxan acts at the site of sugar transport in to the cell [14].

Bequest has postulated that alloxan acts by inhibiting a mitochondrial transport system for inorganic phosphate leading to fall intracellular pH and cell death [15].
It has also been proposed that alloxan leads to mitochondrial dysfunction and interferes with intracellular glucose oxidation [16].

Most early evidences were based on the fact that alloxan can produce D.M. in laboratory animals by destroying beta-cells of the pancreas through oxidative stress [17].

Inductions of diabetes in these animals were confirmed by significant rise in blood glucose and fall in the liver glycogen level [18]. The lowering activity of the glycogenic activity of various isoenzymes of LDH within single metabolic pathway possible points to decrease supply of glucose to the cell during alloxan induced diabetes. Excess of fatty acids in plasma produced by the alloxan induced diabetes promotes the liver conversion of some fatty acids into phospholipids and cholesterol. These two substances along with excess triglycerides formed at the same time in the liver may be discharge into the blood in the lipoproteins [19].

In the present study CANE alone or in combination with glibenclamide produced a marked fall of serum glucose, cholesterol, triglycerides, phospholipids, LDL cholesterol, VLDL cholesterol and increase serum HDL cholesterol. The effect was more pronounced with combination the therapy of CANE and glibenclamide compared to CANE alone which is less pronounced. The hypoglycemic and anti hyperlipaemic effect of CANE with glibenclamide may be a synergistic combination. The maximum effect of reduction was observed on LDL cholesterol and increase in HDL cholesterol after treatment with CANE plus glibenclamide. High LDL and low HDL levels are usually associated with atherosclerosis [20]. High HDL levels reduced the risk. The possible mechanism of the CANE may be due to increase in the liver LDL receptor activity[21]and decreased triglycerides synthesis[22]. This would be useful in disease like diabetes mellitus and coronary heart disease (CHD) because of their inverse relationship[23].

Hypertriglyceridermia is one of the risk factors in coronary heart disease. CANE alone or in combination with glibenclamide reduced triglycerides in alloxan induced diabetic rabbits and may be prevent the progression of CHD. Sharma, et al have found higher levels of serum lipids in both insulin dependent diabetes mellitus (IDDM) and non insulin dependent diabetes mellitus (NIDDM)[24]. CANE used alone or in combination with glibenclamide may be used to counter the high serum lipids produced by the above conditions.

The anti hyperlipaemic effect of CANE may be due to the down regulation of NADPH and NADH a cofactor in fat metabolism. When glycolysis slows down because of cellular inactivity, the pentose phosphate pathways still remains active in liver to breakdown glucose that continuously provides NADPH which converts acyl radicals into long fatty acid chains. CANE may be capable of oxidizing NADPH and enhancing the turnover of HDL ratio. Serum glucose level was reduced in rabbits treated with CANE suggests greater uptake of glucose from the blood by the liver cells. The reduction of serum lipids may be due to decreased synthesis or increased
excretion through intestinal tract by HMGCoA reductase, which is the important enzyme in the formation of cholesterol. One of the possible actions of CANE may be its inhibition of endogenous synthesis of cholesterol and enhancement of the degradation of formed cholesterol by increasing the excretion through intestinal tract. The reduction of serum glucose may be due to enhancing greater uptake of glucose from the blood by liver cells.

5. Conclusions

From this study we can conclusively state that CANE alone or in combination with glibenclamide significantly reduces the levels of serum glucose, lipids and lipoproteins which are actually raised in alloxan diabetic rabbits. CANE alone or in combination with glibenclamide has beneficial effect on serum HDL-cholesterol concentration. Moreover its antihyperlipaemic effect could represent a protective mechanism against the development of atherosclerosis. It is well known that hyperlipidemia has an association with atherosclerosis and the incidence of atherosclerosis is vastly increased in diabetics [25]. CANE in combination with glibenclamide may be utilized for the prevention or management of diabetic induced atherosclerosis in diabetic mellitus patients.
6. References
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