Blood sugar lowering effect of *Anacardium occidentale* leaf extract in experimental rabbit model

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Abstract

**Objective:** The hypoglycaemic effect of *A. occidentale* aqueous leaf extract was evaluated on normoglycaemic and hyperglycaemic rabbits. **Materials and methods:** Antidiabetic activity of *A. occidentale* was evaluated using alloxan (80 mg/kg body weight, intraperitoneally) induced hyperglycemic rabbits. The potency of extract was compared with that of tolbutamide. The acute toxicity was determined using mice. **Results:** A dose 400 mg/kg body weight of the extracts caused maximum lowering of blood sugar levels in both normal and alloxanized rabbits. The fasting blood sugar in the normoglycemic rabbits was reduced from 184.6 mg% to 74.2 mg% in three hours; while in alloxanized rabbits, blood sugar was reduced from 447.6 mg% to 242.8 mg% in three hours. The LD₅₀ of the extract in mice was 4000 mg/kg body weight when given intraperitoneally. **Conclusion:** *A. occidentale* is almost equipotent to tolbutamide in its ability to reduce the blood sugar levels. The above observation is consistent with the use of *A. occidentale* in folklore diabetes management.

**Key words:** *Anacardium occidentale*, Hypoglycaemic activity, Alloxan.

1. Introduction

Many plant-derived preparations are used in folklore medicine in different parts of the world for the management of diabetes [1]. In Nigeria, many plants are employed by herbalists for the treatment of diabetes mellitus. Some of these plants, e.g. *Bridelia ferginea* [2], *Dioscorea dumentorum* [3-4], *Allium sativum* [5] and *Vernonia amygdalina* [6] have been found to lower blood sugar levels in experimental animals.

*Anacardium occidentale* L. (Anacardiaceae) is a shrubby tree commonly distributed in the wild and cultivated in the rain forest region [7]. The herbalists and native doctors use almost all parts of the plant in the treatment of ailments; the dark oily juice of the fruits for warts removal, leaf and bark extracts for lowering blood pressure [8] and to relieve toothache, dysentery, diarrhoea and the infusion from the bark for the thirst treatment [7]. It is a widely used local plant in Nigeria for both nutritional and therapeutic purposes. In addition to its numerous uses in folklore, the leaf decoction of the plant is traditionally employed as an antidiabetic remedy. The
aim of the study was to screen the leaf of *Anacardium occidentale* for hypoglycaemic effect in order to establish a basis for its use in Nigerian herbal practice. The effect of the extract was compared with that of tolbutamide, a standard antidiabetic agent.

2. Materials and methods

2.1 Plant material

*Anacardium occidentale* fresh leaves were collected from plants growing in Nsukka (Enugu State, Nigeria) in February 1999, botanical identity was confirmed by Mr. A.O. Ozioko of Department of Botany, University of Nigeria, Nsukka. A specimen of the plant is deposited in the department of pharmacognosy.

2.2 Preparation of the aqueous extract

The fresh leaves of *A. occidentale* were washed with water, air-dried at room temperature and then reduced to coarse powder. About 250 g of the powder was soaked in 500 ml of distilled water and left to stand for 24 h with occasional shaking. The materials were filtered and the filtrate concentrated to obtain the extract as solid residue (18.5%). The freshly prepared extract was chemically tested for the presence of different constituents using standard methods [9].

2.3 Animals

Wistar albino mice (13-30 g) bred in the animal house of Department of Pharmacology and Toxicology, University of Nigeria, Nsukka, and local strain of adult rabbits (1.8-3.0 kg) were used in the experiments. The animals were kept under standard conditions for 7 days with free access to water and food before the experiments commenced.

2.4 Acute toxicity test

The LD$_{50}$ of the extract was determined in the mice intraperitoneally using the method of Dietrich [10].

2.5 Anti-diabetic evaluation.

2.5.1 Using normoglycaemic rabbits.

The animals were fasted for 12 hours, but were allowed access to water before and throughout the duration of experiment. At the end of the fasting period, taken as zero time (0 hr.), blood was withdrawn from the marginal ear vein, blood sugar level were determined by O-toluidine method [11] and animals having blood sugar concentration of 100-200 mg% were used. Then normal rabbits were divided into 3 groups of five animals each. Group I and II received the aqueous extract of *A. occidentale* at a dose of 250 and 400 mg/kg body weight respectively. Group III received tolbutamide 200 mg/kg body weight, as the standard hypoglycaemic agent. All the administrations were through intraperitoneal route.

2.5.2 Using hyperglycaemic rabbits

Normal rabbits having blood sugar concentrations of 100-120 mg% after 12 h fast were used. Rabbits were made diabetic by injecting alloxan monohydrate (Sigma, USA) intravenously at a dose of 80 mg/kg body weight. The animals were fed for 7 days. On

<table>
<thead>
<tr>
<th>Drug dose (mg/kg)</th>
<th>0 h</th>
<th>1 h</th>
<th>2 h</th>
<th>3 h</th>
<th>6 h</th>
<th>Percentage max. reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extract 250</td>
<td>199.2 ± 14.2</td>
<td>198.1 ± 20.1</td>
<td>178.3 ± 10.3</td>
<td>168.6 ± 9.2</td>
<td>180.2 ± 11.1</td>
<td>15.4</td>
</tr>
<tr>
<td>Extract 400</td>
<td>184.7 ± 17.8</td>
<td>160.5 ±21.3</td>
<td>152.4 ± 13.4</td>
<td>74.2 ± 18.9*</td>
<td>90.2 ± 15.8*</td>
<td>59.8</td>
</tr>
<tr>
<td>Tolbutamide 200</td>
<td>167.7 ± 10.9</td>
<td>54.0 ± 13.7**</td>
<td>45.2 ± 9.8**</td>
<td>34.2 ± 14.3**</td>
<td>30.5 ± 12.6**</td>
<td>85.6</td>
</tr>
</tbody>
</table>

Values are expressed as mean ±SEM; * p <0.05 vs respective control; **p <0.01 vs respective control; n=5.
the day 8 the survivors were fasted for 12 h and their blood sugar levels were determined as before. Only the animals with blood sugar levels of upto 400 mg% were used for the experiment.

The diabetic rabbits were divided into 3 groups of five animals each and treatment was on the same pattern as normoglycaemic animals except the treatment were given on 9th day.

2.6 Collection of blood and blood glucose estimation

At fixed time intervals (0, 1, 2, 3, 6 h) after treatment, blood samples were withdrawn from the marginal ear vein of the rabbits and blood sugar levels were determined as before [11].

2.7 Statistical analysis

Mean blood sugar levels were expressed in mg% ± SEM, and the significance of difference between the blood sugar levels at time zero (zero hour) and other time intervals in each treatment group, and the extract treated groups and the tolbutamide control group were analysed using student’s t-test (p<0.05).

3. Results

3.1 Chemical constituents of extract

The fresh extract of A. occidentale gave positive chemical reactions for glycosides, saponins, tannins, flavonoids proteins and carbohydrates.

3.2 Acute toxicity test

Administered intraperitoneally, the LD₅₀ of the extract in mice was 4000 ± 75.1 mg/kg.

3.3 Hypoglycaemic effect of extract

From the effect of different doses of the extract on the fasting blood sugar levels of both normal and alloxanized rabbits, the reductions were obtained between 250 mg/kg to 400 mg/kg doses. In the normal rabbits, the 400 mg/kg dose significantly (p<0.05) lowered the mean fasting blood sugar value than 250 mg/kg, from 184.7 mg% at time zero hour to 74.2 mg% at time three hour (table 1), while beyond that hour, the blood sugar levels rose continually.

In alloxanized rabbits, 400 mg/kg dose of extract lowered the mean fasting blood sugar levels from 447.6 mg% in 0 hr to 242.8 mg% at three hours (table 2). The effect of tolbutamide as the control, follows the same pattern with its maximum reduction at time three hour in both the normal and alloxanized rabbits. The percentage maximum reduction in blood sugar levels produced by extract at 400 mg/kg dose in the fasted normal and alloxan treated rabbits were 59.8% and 56.4% respectively.

4. Discussion

The studies on the aqueous leaf extract of the plant A. occidentale revealed that the plant extract caused significant reductions in the blood sugar levels in rabbits. In both the normoglycaemic and alloxan-induced hyperglycaemic rabbits, the extract was found to produce marked reduction in blood sugar between 2-3 hours of administration, but became significant at exactly three hours after administration. The hypoglycaemic effect did not persist beyond the first three hours.
When compared with tolbutamide treated animals, the extract caused noticeable percentage maximal reduction in the mean testing blood sugar levels in both classes of animals except that the effect of tolbutamide was at the first one hour while that of the extract occurred in three hours. The comparable effect of the *A. occidentale* extract with tolbutamide on both normoglycaemic and hyperglycaemic animals may suggest similar modes of action. Alloxan permanently destroys the pancreatic B-cells [12] and the extract lowered blood sugar levels in alloxanized rabbits, an indication that the extract has extrapancreatic effects. The exact biological active constituent(s) has neither been known nor the exact mode of action of the hypoglycaemic effect determined. Nonetheless, this observation is consistent with the use of *A. occidentale* in folklore diabetes management.

**References**