

Hypoglycemic Effect of *Anacardium occidentale* L. Methanol Extract and Fractions on Streptozotocin-induced Diabetic Rats

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Abstract: The effect of the methanol leaf extract, dichloromethane, ethyl acetate and *n*-hexane fractions from *Anacardium occidentale* Linn was investigated in streptozotocin-induced diabetic rats. Oral administration of methanol extract at doses of 35, 175 and 250 mg kg⁻¹ significantly reduced blood glucose levels in diabetic rats 3 h after administration. Of three different doses, maximum reduction of 37 and 35% in blood glucose levels was respectively observed with doses of 175 and 250 mg kg⁻¹. When administered repeatedly, the blood glucose reducing effect of the methanol extract at the dose of 175 mg kg⁻¹ in diabetic rats became more pronounced (48%). Fractions from the methanol extract at the dose of 175 mg kg⁻¹ also decreased the blood glucose levels in diabetic rats after repeated administration. The *n*-hexane fraction produced the maximum hypoglycemic effect (45%) and the same dose of the dichloromethane and ethyl acetate fractions respectively reduced hyperglycemia by 21 and 41% at the end of the treatment. On the other hand, a significant decrease in urine glucose levels was observed in diabetic rats after repeated administration of the methanol extract and fractions. These results suggest the hypoglycemic effect of the methanol extract and fractions of *A. occidentale* in streptozotocin-induced diabetic rats. Hexane and ethyl acetate fractions showed the most prominent actions suggesting the presence of non polar and polar hypoglycemic compounds in the plant.

Key words: *Anacardium occidentale* • diabetes mellitus • fractions • methanol extract • hypoglycemic effect • streptozotocin

INTRODUCTION

Despite considerable progress in the management of diabetes mellitus by synthetic drugs, the search for improved and safe natural antidiabetic agents is ongoing. The plant kingdom offers a wide field to look for oral hypoglycemics. More than 400 species have been reported to display hypoglycemic effects, but only few of them have been investigated [1] and the World Health Organization has recommended that this area warrants attention [2].

In this study, our attention has been focussed on *Anacardium occidentale* L. (Anacardiaceae), a plant used in South Cameroon as well as in other countries as a folk remedy for diabetes mellitus [3, 4]. The plant is an ornamental tree up to 10 m high and is widely distributed in tropical countries [5]. In previous studies, we reported the hypoglycemic effect of the aqueous leaf extract of *A. occidentale* in diabetic rats and its prophylactic

activity against the diabetogenic action of streptozotocin [4, 6].

This work was thus undertaken to evaluate the hypoglycemic effect of a methanolic extract of *A. occidentale* leaves and its fractions in streptozotocin-induced diabetic rats.

MATERIALS AND METHODS

Plant material: The leaves of *A. occidentale* were collected from Lolodorf (South Province, Cameroon). Botanical identification and authentication were performed at the National herbarium, Yaounde, Cameroon. Herbarium voucher specimen n° 41935/HNC collected by B. Satabie and R. Letouzey (413) has been deposited in Yaounde herbarium.

Preparation and fractionation of the methanol extract: Leaves were cleaned with tap water, dried at room

temperature for 2 weeks and ground into powder. The methanol extract was prepared by mixing 400 g of plant powder with 1500 ml methanol for 48 h three times at room temperature and filtering through No. 3 Whatman filter paper at room temperature. The combined extracts were evaporated under reduced pressure using a rotary evaporator to obtain 55 g of dried material yielding 13.75%. The dried sample was suspended in water and then partitioned successively with n-hexane, dichloromethane and ethyl acetate. The parent methanol extract and its fractions were tested in diabetic rats.

Animals: Adult male Wistar rats weighing 170-225 g were used. The rats were housed and raised in the animal house of the Faculty of Science, University of Yaounde, Cameroon, with a 12 h light and 12 h dark cycle. The animals were kept in the experimental animal room for 6 days for acclimatization before the experiment with free access to standard pelleted diet (LANAVET, Garoua, Cameroon) and tap water.

Streptozotocin-induced hyperglycemia: Streptozotocin (STZ) purchased from Sigma-Aldrich (St. Louis, MO, USA) was dissolved in 0.1 M cold citrate buffer, pH 4.5, immediately before use. Rats were anaesthetised by sodium pentobarbital (40 mg kg⁻¹, i.p.) and diabetes was induced by intravenous administration of streptozotocin (60 mg kg⁻¹) through the right jugular vein [7]. Forty eight h after STZ injection, fasting blood glucose as well as glycosuria were assessed to confirm the diabetic state. Rats with fasting blood glucose values of at least 200 mg dl⁻¹ and a positive urine glucose level were used for the experiment.

Biological assays: The hypoglycemic activity of the plant extract and its fractions was evaluated in acute and subchronic experiments. In the first experiment, blood samples were taken for the glucose determination (A₀) from overnight fasting rats and afterwards, plant methanol extract was administered by gastric intubation to three groups of five diabetic rats each at respective doses of 35, 175 and 250 mg kg⁻¹. The dried methanol extract was dissolved in a 1% DMSO water solution (v/v) before administration. Control animals were given vehicle (1% DMSO water solution, v/v). Blood samples were then collected 1.5, 3, 5 and 8 h later (A_x) from rat tail tip. The percentage change in blood glucose was calculated by applying the following formula [8, 9]:

$$\% \text{ Change of Glycemia} = [(A_x - A_0) / A_0] \times 100$$

In the second experiment, two groups of five diabetic rats each were given the methanol extract of *A. occidentale* by gastric intubation at doses of 35 and 175 mg kg⁻¹ twice daily for 3 days. Three other groups of five diabetic rats each received respectively n-hexane, dichloromethane and ethyl acetate fractions twice daily at the dose of 175 mg kg⁻¹. Another group considered as control received distilled water instead plant extract or fractions. Blood and urine glucose was assessed at the beginning and at the end of the treatment.

Blood sampling and biochemical estimations: Blood samples (10 µl) for glucose determination were obtained from the tail tip. Blood glucose level was measured with an ACCUTREND GC blood glucose analyser (Boehringer Mannheim, Germany). Urine glucose was assessed in fresh urine using glucose indicator sticks (Boehringer Mannheim, Germany) before and after treatment.

Statistical analysis: Statistical analyses were performed using Student's *t*-test. Results are given as mean blood glucose levels ± S.E.M. or expressed as relative percentage mean blood glucose levels (r (%) mbgl) with glucose level at time 0 taken as 100%. P values of 0.05 and less were taken as significant.

RESULTS

Effect of the methanol extract of *A. occidentale* on serum glucose levels: The effect of the methanol extract of *A. occidentale* on the blood glucose levels in fasting diabetic rats is shown in Fig. 1. Oral administration with doses of 35, 175 and 250 mg kg⁻¹ of the methanol extract exhibited a decrease in blood glucose levels of treated rats compared to control rats. Maximum reduction of 37 and 35% (p<0.001) in blood glucose levels was recorded 180 min after administration respectively with doses of 175 and 250 mg kg⁻¹. Blood glucose levels thereafter gradually increased to reach the time 0 values 480 min after administration of the extract.

Effect of repeated administration of *A. occidentale* methanol extract on blood and urine glucose levels: Repeated administration of the methanol extract of *A. occidentale* to streptozotocin-induced diabetic rats significantly reduced the blood glucose levels in a dose-dependent manner when compared to untreated diabetic rats (Table 1). The highest significant (p<0.001) hypoglycemic effect (48%) was observed with the dose of 175 mg kg⁻¹. On the other hand, the same dose of the

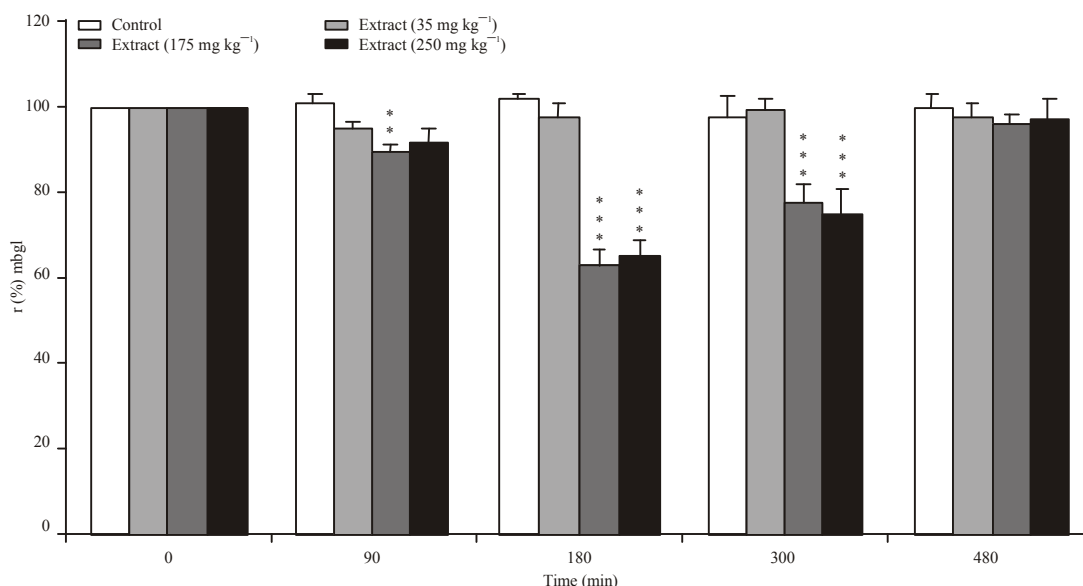


Fig. 1: Effect of the methanol extract of *Anacardium occidentale* on blood glucose levels in streptozotocin-induced diabetic rats

Values are relative percentage of the mean blood glucose level±SEM (r(%)mbgl), n = 5 for each group. Glucose at time 0 min (mg dl⁻¹) is taken as 100%, Significant difference from the 0 min value of the respective groups at each time point: *p<0.05, **p<0.01 and ***p<0.001

Table 1: Effect of repeated administration of *A. occidentale* methanol extract on blood and urine glucose levels in streptozotocin-induced diabetic rats

Treatment group	Dose (mg kg ⁻¹)	Blood glucose (mg dl ⁻¹)		Urine glucose (%) ^s	
		Before	after	Before	after
Control	-	297.40±11.25	309.60±10.36	4.6±0.4	4.8±0.2
Extract	35	289.80±12.16	269.51±9.60*	4.2±0.4	3.8±0.4
Extract	175	295.20±15.56	164.80±7.67***	4.6±0.4	1.0±0.5**

§: Qualitative test. Values are expressed as means±SEM (Standard Error of the Mean), n = 5, Significantly different from control group: *p<0.05, **p<0.01, ***p<0.001

Table 2: Effect of repeated administration of fractions from *A. occidentale* leaf (175 mg kg⁻¹) on blood and urine glucose levels in streptozotocin-induced diabetic rats

Treatment group	Blood glucose (mg dl ⁻¹)		Urine glucose (%) ^s	
	Before	After	Before	After
Control	301.10±08.35	312.37±11.02	4.5±0.4	4.7±0.3
H. fraction	293.20±12.43	172.99±6.13***	4.5±0.4	2.0±0.6**
DCM fraction	289.25±09.93	240.07±5.99*	4.2±0.3	3.0±0.2*
EA fraction	285.04±10.99	179.57±6.03***	4.3±0.5	1.5±0.3**

§: Qualitative test. Values are expressed as Means±SEM (Standard Error of the Mean), n = 5. Hexane fraction (H), Dichloromethane fraction (DCM), Ethyl acetate fraction (EA). Significantly different from control group: *p<0.05, **p<0.01 and ***p<0.001

extract significantly (p<0.01) decreased the urine glucose in diabetic treated rats compared to their control.

Effect of repeated administration of fractions from *A. occidentale* on blood and urine glucose levels: Results obtained with diabetic rats treated with the methanol

extract (175 mg kg⁻¹) of *A. occidentale* prompted us to perform a study with fractions of this extract in view to determine the nature of active principles. Table 2 shows the effect of fractions from *A. occidentale* in streptozotocin-induced diabetic rats. Fractions from *A. occidentale* at the dose of 175 mg kg⁻¹ decreased the

blood glucose levels in streptozotocin-induced diabetic rats after three days repeated administration. The n-hexane fraction produced the maximum hypoglycemic effect (45%) and the same dose of the dichloromethane and ethyl acetate fractions reduced the hyperglycemia by 21 and 41% at the end of the treatment. On the other hand, a significant decrease in urine glucose levels was observed in diabetic rats receiving plant fractions compared to untreated diabetic rats.

DISCUSSION

Streptozotocin-induced diabetes provides a condition of insulinopenia and has been described as a useful experimental model to evaluate the activity of hypoglycemic agents [10]. In the present study, a single administration of the methanol extract of *A. occidentale* effectively lowered the blood glucose level in streptozotocin-diabetic rats compared to their control. Streptozotocin selectively damaged the pancreatic insulin secreting β -cells, leaving less active cells and resulting in a diabetic state. Since the methanol extract of *A. occidentale* reduced the blood glucose potently in the streptozotocin-diabetic rats, it may be assumed that the diabetic state is not severe. Thus, the extract may act by a direct stimulation of insulin secretion in remaining β -cells. This effect could be attributed to compounds like (-)-epicatechin [11], kampferol, quercetol rhamnosides [12] and β -sitosterol 3- β -D glucoside [13], previously isolated from *A. occidentale*. Such compounds have been reported to stimulate insulin secretion [13, 14]. On the other hand, the action of the extract may involve insulin-like extrapancreatic mechanisms such as the stimulation of glucose utilisation and the reduction of hepatic gluconeogenesis [15, 16]. Similar results have been reported with the aqueous extract of *A. occidentale* [6]. In this study, repeated administration of the methanol extract of *A. occidentale* and its fractions showed hypoglycaemic effects as reflected by a decrease in blood and urine glucose levels. The dichloromethane fraction had a very low hypoglycaemic activity, whereas directly previous (n-hexane) and subsequent (ethyl acetate) fractions were more active. This result suggests that at least two main types of hypoglycemic compounds are present in the methanol extract of *A. occidentale*: non-polar and polar compounds respectively soluble in n-hexane and ethyl acetate. Such observations have been reported by Niyonzima *et al.* [17] with the stem bark extracts of *Spathodea campanulata*. Moreover the hypoglycemic activity of the methanol extract of *A.*

occidentale was shown to be more pronounced in repeated treatment compared to the acute test. This plant extract may act by stimulating the revitalisation process in the residual pancreatic β -cells in treated animals. An increase in β -cells mass due to the proliferation or the anti-apoptosis process in the remnant islet was reported by Rømer *et al.* [18] after 4 days administration of NN2211 (a long-acting hypoglycemic GLP-1 derivative) in pancreatectomized rats. The retardation of membrane-bound α -glucosidase reaction and/or inhibition of passive glucose transport would successfully flatten the postprandial blood glucose excursions or reduce hyperglycaemia [19]. The presence of α -glucosidase inhibitors have already been reported in *A. Occidentale* nut shell liquids [20]. Thus, the hypoglycemic activity of *A. occidentale* may also be attributed to the inhibition of α -glucosidase in the gastro-intestinal tract.

In conclusion, this study has shown that the methanol leaf extract of *A. occidentale* and its fractions had hypoglycemic effect in streptozotocin-induced diabetic rats. Hexane and ethyl acetate fractions showed the most prominent actions suggesting the presence of non polar and polar hypoglycemic compounds in the plant. Further investigations are in progress to isolate the active principles.

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