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ANTIHYPERGLYCEMIC EFFECT OF *BRIDELIA NDELLENSIS* ETHANOL EXTRACT AND FRACTIONS IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

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Abstract

The effects of the ethanol extract (1.25 g/kg) and fractions (1 g/kg) of *Bridelia ndellensis* stem bark on the blood glucose levels in streptozotocin-induced types 1 and 2 diabetic rats at different prandial states were studied. The ethanol extract of *B. ndellensis* had no hypoglycemic effect in type 1 diabetic rats in fasting and postprandial glucose load conditions and, in type 2 diabetic rats in fasting condition. However, the extract, and its ethyl acetate and dichloromethane fractions significantly lowered blood glucose levels in type 2 diabetic rats when fed simultaneously with glucose. The active principles responsible for the antihyperglycaemic effect are concentrated in the ethyl acetate and dichloromethane fractions of the extract.

Keywords: *Bridelia ndellensis*; hypoglycemic effect; Streptozotocin; diabetes; rats.

Introduction

The pathogenesis of diabetes mellitus and the possibility of its management by existing therapeutic agents without any side effects have stimulated great interest in recent years (Bailey, 1999). Management of diabetes without any side effects is still a challenge

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for the medical system. This leads to an increasing search for improved antidiabetic drugs. Few of plant treatments used in traditional medicine for diabetes have received scientific scrutiny, and the World Health Organisation has recommended that this area warrants attention (WHO, 1980).

This work describes the study of *Bridelia ndellensis* Beille (Euphorbiaceae), a medicinal plant commonly used in Cameroon against fever, rheumatism, diarrhoea, and diabetes. Hypoglycemic effects of *Bridelia ferruginea* leaf water and methanol extracts have been reported in alloxan-induced diabetic rats (Addae-Mensah and Achenbach, 1985; Onunkwo et al., 1996). However, no scientific investigation has so far been conducted on the antidiabetic activity of *B. ndellensis*.

The present work was therefore undertaken to study the glucose-lowering effects of the ethanol extract and fractions of *B. ndellensis* stem bark in streptozotocin-induced types 1 and 2 diabetic rats at different prandial states.

Materials and Methods

Plant Material

B. ndellensis stem bark was collected from Ngaoundere, Adamawa Province, Cameroon. Botanical identification was performed at the national herbarium, Yaounde, Cameroon and herbarium voucher specimen number 9676/HNC has been deposited. The bark was dried under sunlight and ground into powder.

Extraction procedure and Fractionation

A 2.5 kg powdered *B. ndellensis* bark was extracted (4 times, 24 hours each time) with 80% ethanol at room temperature, filtered and concentrated *in vacuo* (40^oC) and freeze-dried to obtain a 200 g extract (8%w/w). The dried powder was suspended in water (500 ml) and then partitioned successively with CH_2Cl_2, EtOAc and 1-BuOH to give 12.92 g, 20.60 g and 30.82 g of extracts, respectively. The parent extract (80% EtOH) and its dichloromethane (DCM), ethyl acetate (EA), 1-butanol (BU) soluble parts were tested on IDDM and NIDDM model rats at different prandial states.

Animals

Male Long-Evans rats (200 - 230g) bred at BIRDEM laboratory were used for this study. The animals were maintained on 12 hours light-dark cycle at room temperature, fed on a standard laboratory pellet diet and with water supplied *ad libitum*.

Effects on the fasting blood glucose levels

Type 1 (IDDM) diabetes was induced by intraperitoneal injection of streptozotocin (stz, 65 mg/kg body weight) dissolved in citrate buffer (pH 4.5) immediately before use to 3 month old adult rats fasted for 18 hours. Diabetes state was checked 5 days after injection of stz. Type 2 diabetes (NIDDM) was induced by intraperitoneal injection of streptozotocin (90 mg/kg body weight) to 48-hour old pups rats as previously described by Bonner-Weir et al. (1981). Groups of 6 to 8 rats each were used for the experiments.

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The ethanol extract of *B. ndellensis* (1.25 g/kg body weight) were fed orally by gastric intubation to overnight fasting (12 hours) rats at 0 min and blood samples were drawn at 0, 60 and 120 min. Negative control group received only water (10 ml/kg body weight) and another group of rats received glibenclamide (5 mg/kg body weight) as a standard drug (Positive control group). The animals were kept unfed throughout the period.

Effects on blood glucose levels at concomitant administration of the fractions and glucose

The *B. ndellensis* (1.25 g/kg body weight) extract and its fractions (1 g/kg body weight) were fed simultaneously with glucose (2.5 g/kg body weight) by gastric intubation to overnight fasting (12 hours) rats at 0 min and blood samples were drawn at 0, 30 and 75 min. Negative control group received only glucose (2.5 g/kg body weight) and the positive control group of rats received glibenclamide (5 mg/kg body weight) along with glucose (2.5 g/kg body weight). The animals were kept unfed throughout the period.

Blood collection and biochemical analysis

Blood samples were collected from the tail tip under mild ether anaesthesia. The serum was separated by centrifugation and the glucose level was measured immediately by the glucose-oxidase method (Sera Pak, USA). The absorbance was measured at 490 nm using a microplate ELISA reader (Bio-Tek EL-340, USA).

Statistical analysis

Results were expressed as mean blood glucose levels ± S.E.M. Data were analysed using One-way ANOVA followed by Dunnett's test. The level of significance was set at 0.05.

Results

The ethanol extract of *B. ndellensis* had no hypoglycemic effect in IDDM and NIDDM rats on fasting condition (Table 1). However, glibenclamide showed a significant ($P<0.05$) decrease of blood glucose levels in NIDDM rats on fasting state (36% and 38% reduction respectively 60 and 120 min after administration).

Figure 1 shows the effect of *B. ndellensis* 80% ethanol extract on postprandial blood glucose levels of IDDM rats when fed simultaneously with glucose (2.5 g/kg body weight). The extract showed a tendency to oppose the blood glucose rise but this effect was not significant compared to control (14% reduction 30 min after glucose load). Glibenclamide treated rats showed the same tendency.

However, in NIDDM diabetic model rats (Figure 2), the extract had a strong and significant ($p<0.001$) opposing effect on the rise of serum glucose level when fed simultaneously with glucose (32% and 35% inhibition of glucose rise respectively 30 and 60 min after glucose load). Glibenclamide also opposed the rise (20% inhibition, $p<0.05$) of serum glucose 30 min after simultaneous administration with glucose.

As shown in Figure 3, the ethyl acetate fraction showed a significant ($P < 0.001$) opposing effect in serum glucose rise 30 min after administration (36% inhibition of

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Table 1: Effect of *Bridelia ndellensis* ethanolic extract on fasting serum glucose levels of IDDM and NIDDM diabetic model rats

	Blood glucose levels, mmol/l (percent of 0 min value)		
Group	**0 min**	**60 min**	**120 min**
a. IDDM model rats (n=6)			
Control	35.35 ± 1.30	31.97 ± 0.40	30.20 ± 1.37
	(100)	(91.12 ± 3.81)	(86.42 ± 6.13)
Extract (1.25g/kg)	34.46 ± 1.66	29.29 ± 1.77	29.22 ± 1.95
	(100)	(85.30 ± 4.20)	(85.20 ± 5.24)
Glibenclamide (5 mg/kg)	35.46 ± 1.21	32.93 ± 1.23	28.73 ± 1.41
	(100)	(93.54 ± 5.03)	(82.15 ± 6.88)
b. NIDDM model rats (n=8)			
Control	9.45 ± 0.40	8.90 ± 0.36	9.97 ± 0.43
	(100)	(95.24 ± 5.10)	(107.38 ± 7.98)
Extract (1.25g/kg)	10.03 ± 0.41	8.88 ± 0.45	9.26 ± 0.33
	(100)	(89.96 ± 6.44)	(93.97 ± 6.59)
Glibenclamide (5 mg/kg)	9.75 ± 0.42	6.24 ± 0.47***	6.02 ± 0.44***
	(100)	(64.34 ± 4.62)**	(61.70 ± 3.24)***

Values are mean blood glucose levels ± S.E.M. (n=number of rats per group). Δ, Sum of increments over basal value. Significantly different from control at identical periods: **p<0.01 and ***p<0.001

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glucose rise). Compared to control rats, the ethyl acetate fraction showed a significant reduction (32%, $P < 0.01$) of serum glucose level 75 min after simultaneous administration with glucose. The dichloromethane extract also showed a significant ($P < 0.05$) reduction in serum glucose level compared to control (29% and 28% respectively 30 and 60 min after administration). In contrast, when compared to the control group, the butanol fraction was, however, devoid of this activity.

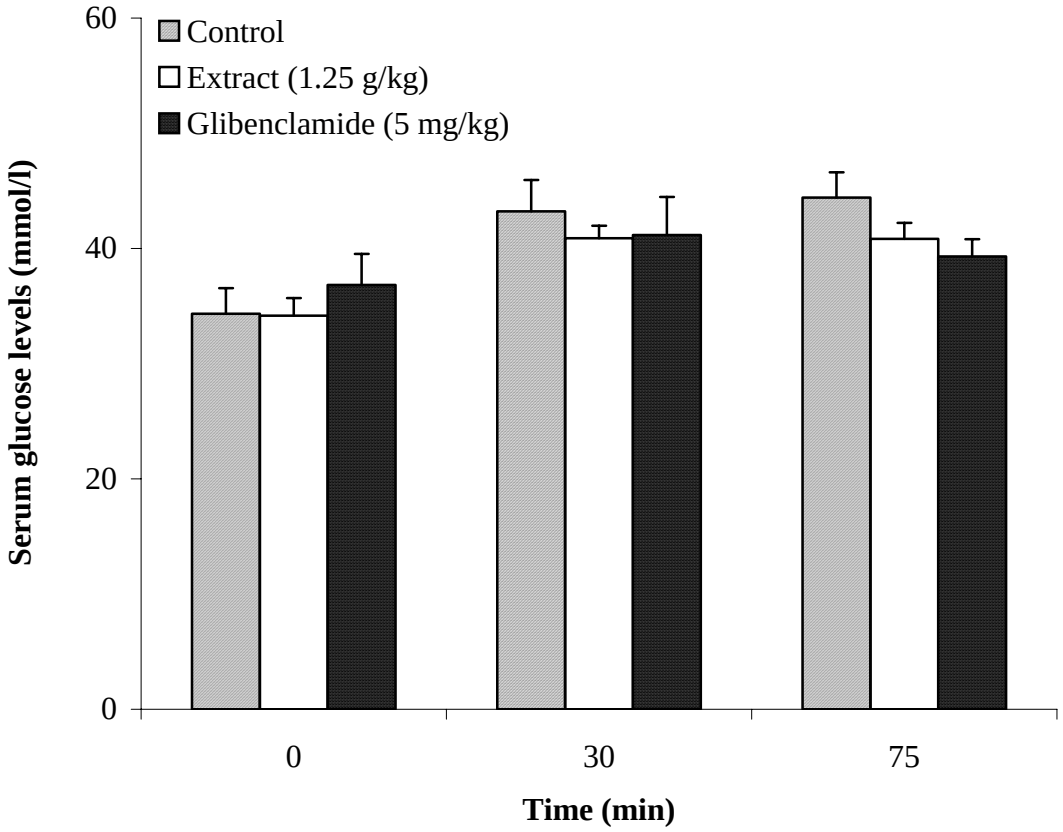

Figure 1: Effect of *Bridelia ndellensis* ethanolic extract on serum glucose levels of IDDM diabetic model rats when fed simultaneously with glucose load.Values are mean blood glucose levels ± S.E.M. (n = 6, number of rats per group).

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Discussion

In adult animals, streptozotocin selectively destroys the pancreatic insulin-secreting β-cells, leaving less active cells and resulting in type 1 diabetic state (Ledoux et al., 1986; Kamtchouing et al., 1998). Our results show that neither the 80% ethanol extract of *B. ndellensis* nor glibenclamide demonstrated hypoglycemic or antihyperglycemic effects in IDDM rats in both fasting and glucose load states respectively. The main mechanism of action of glibenclamide is by the stimulation of insulin release. It has been described that glibenclamide is effective in moderate diabetic state, and ineffective in severe diabetic animals where pancreatic β-cells are almost totally destroyed (Ivorra et al., 1989; Suba et al., 2004). The similar inactivity of the extract and glibenclamide may indicate that the extract also act by stimulation of the Islet cells and thus requires functional pancreatic β-cells for its action. This was confirmed by the inactivity of the extracts on fasted IDDM rats (Table 1). In the NIDDM diabetic model rats, the ethanol extract of *B. ndellensis* showed an anytihyperglycemic effect comparable to that of glibenclamide when fed simultaneously with glucose. Thus, the extract may act on β-cells like sulfonylurea drugs to stimulate insulin secretion. Similar results have been reported with *Anacardium occidentale* aqueous leaf extract (Sokeng et al., 2001). As the ethanol extract of *B. ndellensis* did not show any hypoglycemic effect in NIDDM rats on fasting condition, it can be assumed that this extract like tetraethylammonium (TEA), may stimulate insulin secretion in a glucose-dependent manner (MacDonald and Wheeler, 2003). On the other hand, the hypoglycemic effect of the extract in the glucose-fed rats may be accounted in part, by an inhibition of intestinal glucose absorption and the stimulation of the glucagon-like peptide (GLP-1) which is also a glucose-dependent insulin secretagogue (Goke et al., 1995).

In this study, the ethyl acetate and dichloromethane fractions obtained from the ethanol extract, produced important hypoglycemic effects in NIDDM rats when fed simultaneously with glucose, indicating that the hypoglycemic components of the plant are concentrated in these two fractions. β-sitosterol, quercetin, quercetin-3-glycoside and epigallocatechin isolated from *B. ferruginea* (Addae-Mensah and Achenbach, 1985), have demonstrated hypoglycemic activity. *B. ndellensis*, which belongs to the same genus is likely to contain such compounds responsible for the observed antihyperglycemic and hypoglycemic effects.

Further chemical and pharmacological investigations are in progress to elucidate in detail the active principles and the real mechanism of action of this plant extract.

Acknowledgement

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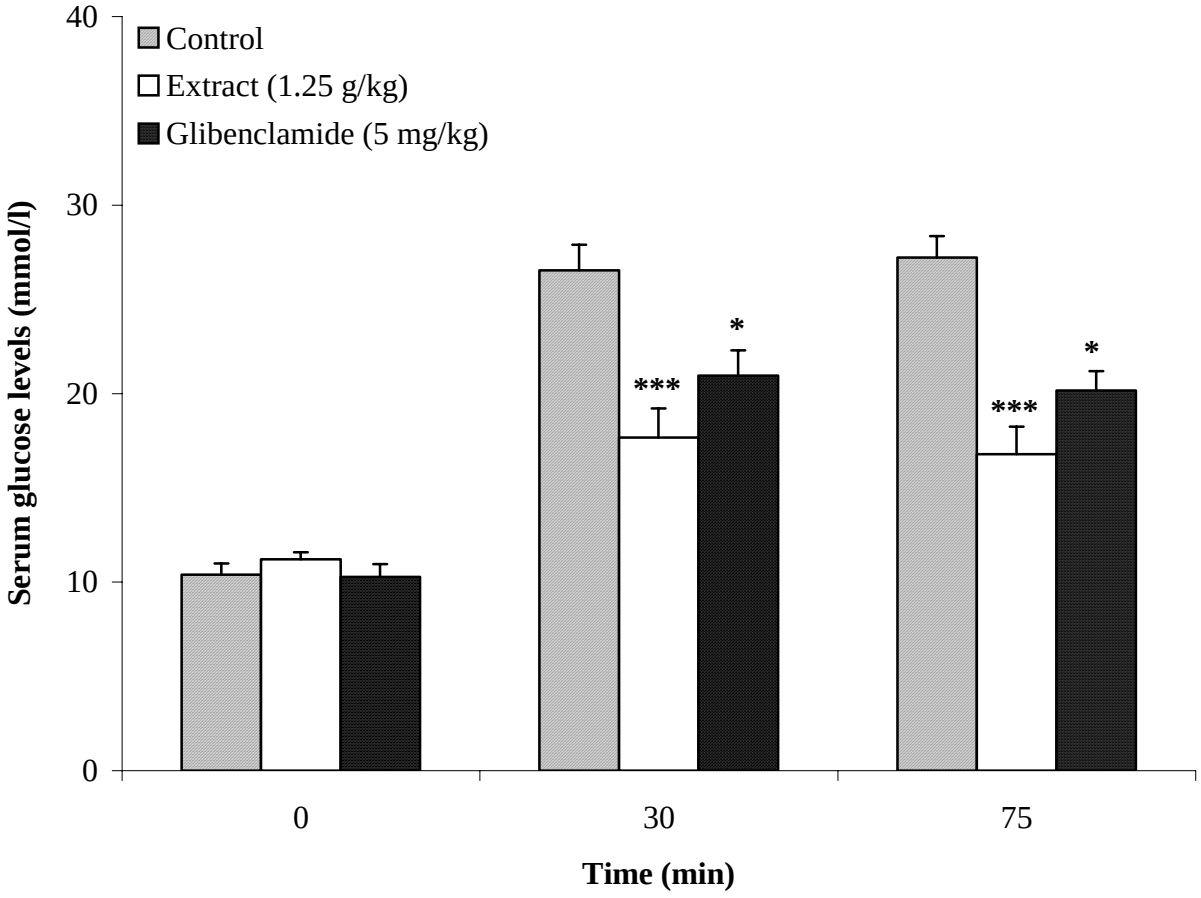

Figure 2: Effect of *Bridelia ndellensis* ethanolic extract on serum glucose levels of NIDDM diabetic model rats when fed simultaneously with glucose load.

Values are mean blood glucose levels ± S.E.M. (n=8, number of rats per group). Significantly different from control at identical periods: *$p<0.05$ and ***$p<0.001$

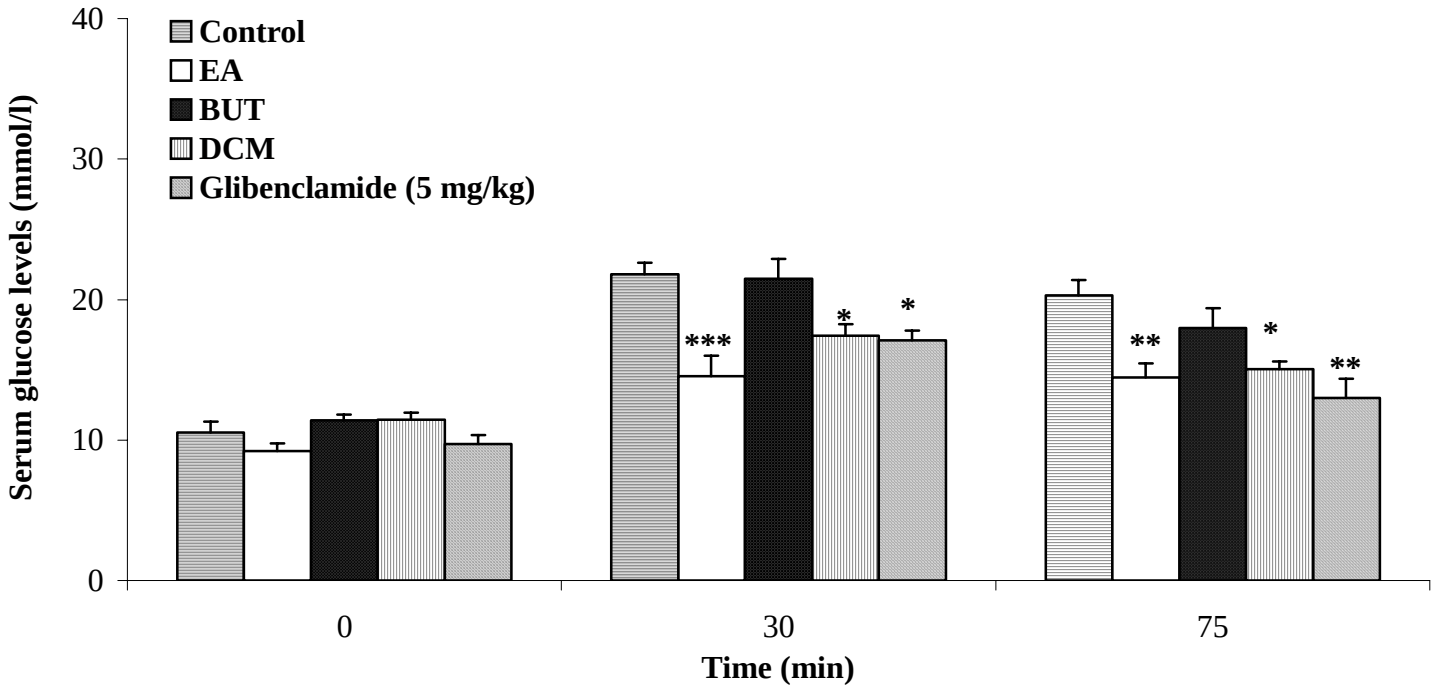

Figure 3: Effect of fractions obtained from the ethanol extract of *Bridelia ndellensis* (1 g/kg) on serum glucose levels in NIDDM rats when fed simultaneously with glucose. Values are mean blood glucose levels ± S.E.M. (n=6). EA: Ethyl Acetate, DCM: Dichloromethane, BUT: Buthanol extract. Significantly different from control at identical periods: *p<0.05; **p<0.01 and ***p<0.001

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