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*Full Length Research Paper*

# Antidiabetic properties of the methanolic extract of *Bridelia grandis* (Euphorbiacae) in ob/ob and db/db mice

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***Bridelia grandis*** **is used in most parts of tropical Africa for the treatment of diabetes. The anti-diabetic properties of the methanolic stem bark extract of this plant was studied in ob/ob and db/db mice. The plant extract induced hypoglycaemic effects of long duration in the ob/ob mice, while its effects were less pronounced in the db/db mice. Twenty four hours after the cessation of drug administration, both ob/ob and db/db extract treated animals still had significantly lower fasting blood sugar levels when compared to controls, thus confirming its antidiabetic properties.**

**Key words:** *Bridelia grandis* (Euphorbiaceae), antidiabetic properties, ob/ob and db/db mice.

## INTRODUCTION

The World Health Organisation (WHO) defined diabetes mellitus as a syndrome and group of metabolic disorders characterised principally by hyperglycaemia and glucose intolerance. This could either be due to an absolute insulin deficiency (type 1 diabetes, formerly called insulin-dependent diabetes) or relative insulin deficiency (type 2 diabetes, formerly known as non-insulin-dependent diabetes), or a combination of both phenomena. It is equally known today that diabetes brings about derangements in protein and lipid metabolism. According to the WHO report for the year 2000, over 150 million people aged 20 and older are living with diabetes. It is estimated that this figure will double by the year 2025 due to late diagnosis and poor control in developing countries. In view of modern pharmacological treatment regimens for diabetes, alternative and complementary strategies are warranted (WHO, 2002). Medicinal plants represent an alternative source of medicines for rural populations and could constitute an important source of antidiabetic agents. These plants are indispensable because current treatment regimens (sulphonylureas, biguanides and insulin), even if used as drug combinations fail in the long run to control glucose homeostasis (Davidson, 2000). Moreover, in most developing countries, the cost of treatment with these pharmaceuticals is prohibitive for most patients. It is therefore important to find readily available local plants which can be useful in the management of diabetes. *Bridelia grandis* (Euphorbiacaea) is a medicinal plant which is used in tropical Africa for the treatment of diabetes-related ailments (Ngueyem et al., 2009). Bridelia species are widespread in tropical Africa and most of them have diverse ethno pharmacological properties. Previous phytochemical studies showed that extracts

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from *B. grandis* had polyphenols (Brusotti et al., 2010), glycoside indoles and tannins (Ngueyem et al., 2008). In the present study, we investigated the antidiabetic properties of extracts of *B. grandis* in order to justify their use in traditional medicine. We also isolated and determined the structure of the most abundant compounds in the extract.

## MATERIALS AND METHODS

### Plant material

Stem barks of *B. grandis* (Euphorbiacaea) were harvested in Simbock and Ebepda (Centre Province of Cameroon) in August, 2003. The botanic samples were deposited at the Cameroon National herbarium in Yaoundé where identification was done.

### Extraction

The stem barks of *B. grandis* (7 kg) were extracted with methanol at room temperature for 72 h. After evaporation of the solvent at reduced pressure using a rotary evaporator, 520 g of a red extract was obtained. This extract was partitioned between hexane and methanol, then between ethyl acetate and methanol, giving 50 and 20 g of soluble fractions in hexane and ethyl acetate, respectively. The methanol-soluble extract was mostly made up of tannins (about 90% of the crude methanol extract). The hexane-soluble fraction underwent chromatographic partitioning on silica gel, with hexane and hexane/ethyl acetate mixture as dilution solvent. Two pure substances were obtained: octadecan-1-ol and lupeol. Using the same chromatographic methods, similar results were obtained with the ethyl acetate-soluble fraction.

### Experimental animals

Obese diabetic ob/ob mice (C57BL/6J colony) in which the ob gene for leptin has been knocked out and db/db mice (C57BLKS colony) in which the gene responsible for the expression of leptin receptor has been knocked out were used in this study. These mice were purchased from Jac-Mice (USA).

### Study of the hypoglycaemic effects of extracts

The ob/ob mice used in this experiment were 12 weeks old weighing 40 to 45 g, and db/db mice weighing 25 to 30 g. Twenty four ob/ob and 24 db/db mice where randomly allocated to control and treatment groups. Both the ob/ob and db/db mice were divided into 4 groups of 6 mice each, respectively. The control-groups were administered the vehicle (5% DMSO), while the other groups received tolbutamide (50 mg/kg), 100 or 200 mg/kg methanolic extract of the stem bark of *B. grandis* (SEBG). These animals were treated for 7 days, during which they received 2 doses of drug daily at 8 am and 5 pm, respectively. On day 1, fasting blood sugar levels were determined for all animals using whole capillary blood obtained from the tip of the tail, after an overnight fast of 10 to 12 h. Thereafter, the different test drugs were administered. Capillary glucose measurements were done at 2, 4 and 6 h after drug administration. The fasting glycaemia of these animals was again measured on day 8 of treatment after an overnight fast. The quantitative determination of blood glucose was done by the glucose dehydrogenase method, using a specific spectrometer-analyzer, the B-glucose Hemocue. This device uses microcuvettes which serve at the same time as a reaction chamber and measuring cuvette.

### Statistical analysis

The non-parametric Mann-Whitney U test was used for all statistical analysis using SPSS software (version 10.1). The level of signifycance was set at $p < 0.05$.

## RESULTS AND DISCUSSION

### Determination of the structure of products obtained

The spectrum of RMN -$^{1}$H and -$^{13}$C ($CDCl_3$) were recorded using an AC-300 Bruker spectrophotometer operating respectively, at 300 and 75 MHz. Octadecan-1-ol: 11 mg, thin sheet-like appearance (in ethanol); melting point (MP) = 59 to 60°C. Its structure was obtained from data using the RMN spectrum. Lupeol: 9 mg; needle-like ($Me_2CO$/MeOH); MP = 215 to 216°C. Its structure was determined using physical and RMN-$^{1}$H and -$^{13}$C spectral data, and from comparison with data in literature.

### Hypoglycaemic effects of SEBG in ob/ob mice

The methanolic extract of SEBG (100 mg/kg) induced a significant decrease in blood sugar level from 169.0 ± 6.4 mg/dl to 134.0 ± 8.3 mg/dl, 2 h after administration of plant extract. This decrease remained significant through the 4, 6 h and beyond (Figure 1). On the other hand, the 200 mg/kg dose of SEBG significantly decreased glycaemia during the 4 h ($p < 0.05$) and 6 h ($p < 0.01$) after treatment and even on day 8 when animals received no treatment ($p < 0.05$). Tolbutamide (50 mg/kg) significantly ($p < 0.05$) decreased glycaemia only during the 6th hour after administration. On day 8 when animals received no treatment, animals previously treated with the SEBG 200 mg/kg still had significantly lower blood sugar levels when compared to the tolbutamide treated and control animals.

### Hypoglycaemic effects of SEBG in db/db mice

In db/db mice, SEBG showed significant hypoglycaemic effects during the 4 and 6 h post treatment, while tolbutamide had an earlier onset of activity (2 h post treatment). However, after one week of treatment, SEBG still showed significantly ($p < 0.01$) decreased glycaemia (41.58%), when compared to the mean glycaemia on day 1 of treatment (Figure 2). Tolbutamide on the other hand, significantly decreased glycaemia from the 2nd hour after administration.

## DISCUSSION

The present study reports on the anti-diabetic effects of

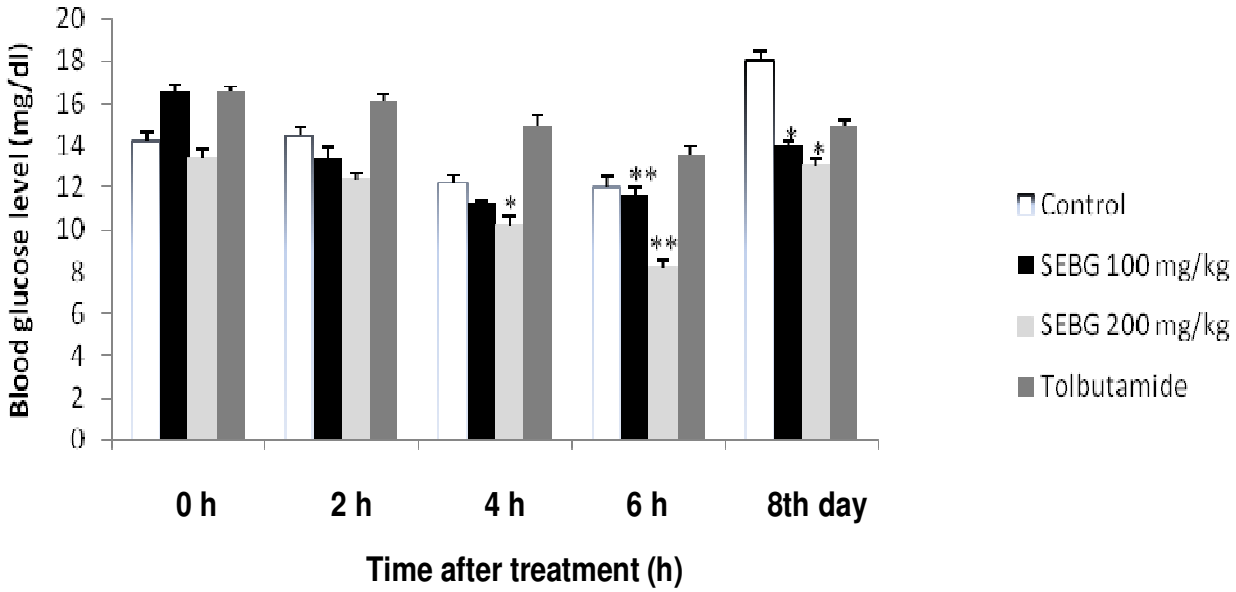


**Figure 1.** Hypoglycaemic effects of the methanolic extracts of *B. grandis* and tolbutamide in ob/ob mice. *p < 0.05; **p < 0.01; n = 5. p was obtained by comparing glycaemia in treated with control animals at the same time period, and also with baseline values.

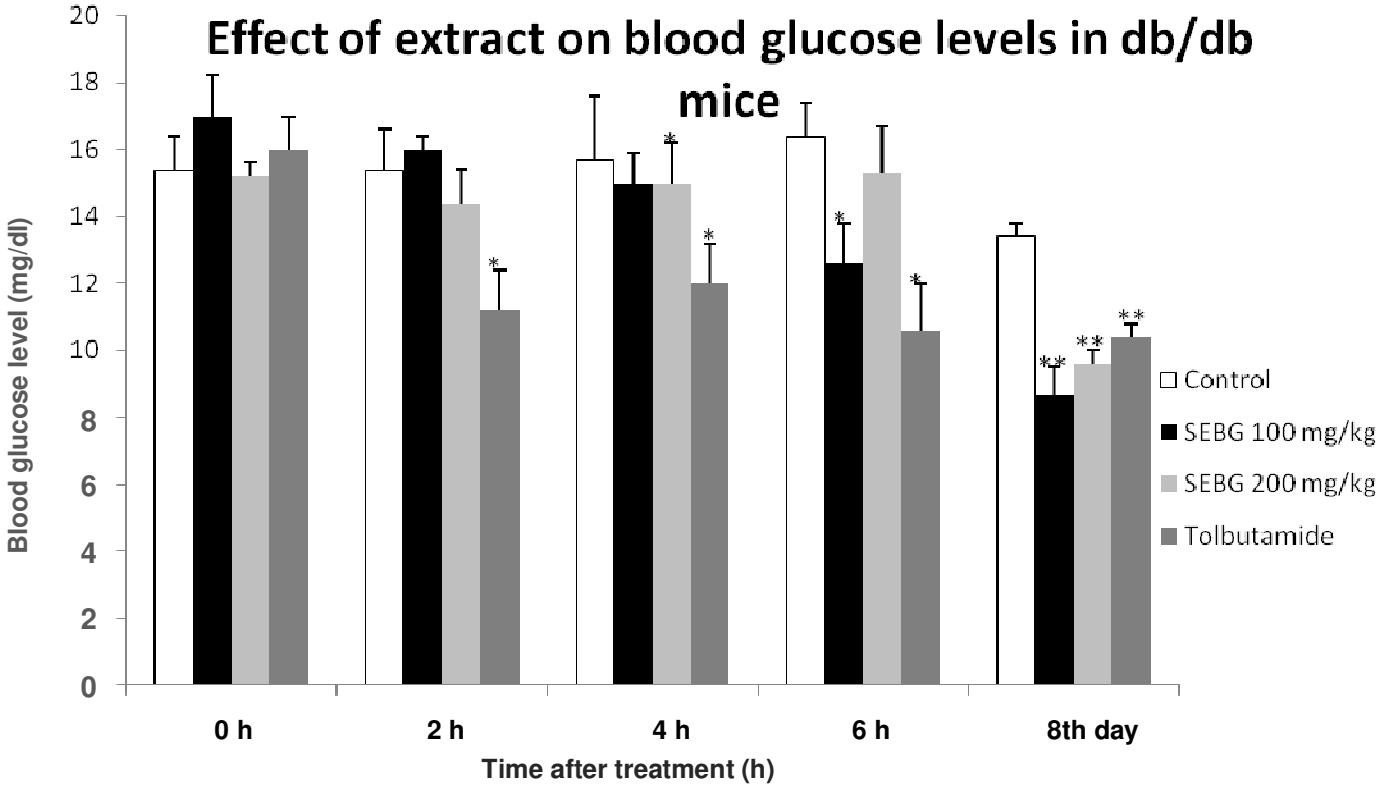


**Figure 2.** Hypoglycaemic effects of the methanolic extracts of *B. grandis* in db/db mice. *p < 0.05; **p < 0.01; n = 6. p was obtained by comparing glycaemia in treated animals with control animals at the same time period, and also with baseline values.

*B. grandis* in ob/ob (leptin deficient) and db/db mice (leptin receptor deficient) mice. We showed that the response to SEBG differed with respect to the gene knocked out. In ob/ob mice, SEBG 100 mg/kg caused a

significant and rapid decrease in glycaemia from the 2nd hour after administration. This rapid onset of hypoglycaemic effects suggested that this extract may have secretagogue actions. Tolbutamide in these animals caused a significant reduction in blood sugar levels only after 6 h. These observations suggest that 50 mg/kg tolbutamide might not have been sufficient to induce insulin secretion form islet cells. These results are similar with those obtained by Gustavsson et al. (2006) which showed that the pancreatic β cell response to a tolbutamide stimulus was impaired in ob/ob mice. In addition to the insulin-secreting actions of SEBG, there might be an extra-pancreatic action which brings about an increase in insulin sensitivity. Because of the fact that these mice were obese, the extra pancreatic action of SEBG extract could be manifesting at the level of the adipocytes, causing a release of insulin-sensitising adipocytokines such as adiponectin (Guerre-Millo, 2004). These observations were different in db/db mice where, the therapeutic dose of tolbutamide (50 mg/kg) brought about a significant decrease in glycaemia from the 2nd hour after administration (28.6%). This hypoglycemaemic effect remained significant after 6 h (30.9%) and after a week of treatment. In this case, the insulin secretion induced by tolbutamide was sufficient to combat the degree of insulin resistance in the db/db mice. Importantly, the hypoglycaemic effects obtained in both ob/ob and db/db mice was still significant after one week of treatment. This "late" effect in db/db mice suggests an insulin sensitive effect, which might be measurable at the level of the liver, skeletal muscles and adipose tissue (Cusi and DeFronzo, 1998).

The quantities of octadecan-1-ol and lupeol obtained in this study were too small for use in our *in vivo* studies. Of these two components, lupeol has been isolated from several plant species and though extensively studied (Sudhahar et al., 2005, 2006), no hypoglycaemic effects have yet been reported on it. The noted blood sugar lowering effects of SEBG might be due to the presence of either octadecan-1-ol and/or lupeol in SEBG.

## Conclusion

Results from this study showed that the methanolic stem bark extract of *B. grandis*, has hypoglycaemic effects in type 2 diabetic ob/ob and db/db mice.

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