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HYPOGLYCAEMIC ACTIVITY OF THE LEAVES EXTRACTS OF *BERSAMA ENGLERIANA* IN RATS.

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Abstract

The hypoglycaemic properties of the aqueous and methanol extracts of the leaves of *Bersama engleriana* were evaluated in normoglycaemic rats in order to scientifically validate its traditional therapeutic use. With a dose of 300 mg/kg b.w. only the aqueous extract appeared to be significantly effective while at high dose, 600 mg/kg b.w., the aqueous and methanol extracts of *Bersama engleriana* reduced the blood glucose level by 37.7% and 49.11% after 8h of treatment of rats respectively. These results confirm the hypoglycaemic properties of some extracts of the leaves of *Bersama engleriana* with the aqueous extract appearing more active.

Keywords: *Bersama engleriana*; Hypoglycaemic effect; Leaf extract.

Introduction

During the last two decades, traditional systems of medicine and medicinal plant research have become topic of global interest and importance. In many developing nations of the world, large numbers of people still rely heavily on traditional healers and medicinal plants to meet their daily primary healthcare needs. Because of their perceived effectiveness, minimal side effects in clinical experience and relatively low cost, herbal drugs are prescribed widely even when their biologically active compounds are unknown (Valiathan, 1998). In Africa, hundreds of plants are used traditionally for the management of diabetes mellitus. To date, however, only a few of these African medicine plants have received scientific scrutiny, despite the fact that the World Health Organization has recommended that medical and scientific examinations of such plants should be undertaken (WHO, 1980).

Plants like , *Momordica cymbalaria, Urtica dioica, Suzigium cumini, Acosmium panamense* etc have shown a great potential as antidiabetics and they have been included in various polyherbal preparations (Sokeng et al., 2001*;* Ojewole, 2003;

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Kameswararao et al., 2003; Bnouham et al., 2003; Mainzen et al., 2004; Andrade-cetto and Wiedenfeld, 2004). *Bersama engleriana* of the Melianthaceae family is a small tree with spikes of pink and white flowers and, brown capsule containing red seeds with yellow arils; the leaf-rhachides are slightly winged; it is widespread in Africa (Hutchinson and Dalziel, 1958). In the Western part of Cameroon, the bark and the leaves of this plant are used by tradipractitioners to treat diabetes Mellitus. However, this report is based on subjective opinion rather than its scientific verification. The present study was undertaken to verify this indigenous claim and to provide some rationale for the use of *Bersama engleriana* as an oral antidiabetic drug by evaluating the hypoglycaemic activity of the aqueous and methanolic extracts of its leaves.

Materials and Methods

Animals

Thirty male albino Wistar rats (150 – 200 g, body weight) were maintained on standard laboratory diet and tap water ad *libitum* at the Animal house of the Faculty of Science, University of Dschang, Cameroon. Prior to the experiment, the animals were subjected to fasting for 12 h but allowed free access to water.

Collection and preparation of plant material

The leaves of *Bersama engleriana* were collected during the month of July 2004 in Kumba, South-west province, Cameroon. Botanical identification was performed at the National Herbarium, Yaounde, Cameroon in comparison with the voucher specimen n°32427/HNC collected by Mbenkum. The leaves were shade-dried and ground into powder. The powder was used for the extraction of potential hypoglycaemic principle/s into different solvents (water, methanol).

Extraction of the aqueous plant material

Four-hundred grams of powdered *Bersama engleriana* leaves were boiled in 5 litters of distilled water for 15 min. The decoction was taken and allowed to cool for 30 min at room temperature (24 ± 2° C). The decoction was filtered twice and the filtrate was dried in an oven (56°C) for 3 days. The resulting material yielded 112 g (28.0 % w/w).

Extraction of the methanolic plant material

Six-hundred grams of the leaves powder were soaked with 2 litters of methanol for 3 days. The filtrate was concentrated to dryness in a rotary evaporator under reduced pressure at a temperature of 40°C. The extract yielded was 16.5 g (2.75 % w/w, based on the dried starting weight).

Extracts administration

Rats were divided at random into 6 groups (5 animals/group) treated orally by the extracts as follows: Group I served as the untreated control, receiving distilled water (5 ml/kg b.w.). Group II treated with glibenclamide as a standard oral

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hypoglycaemic agent (2 mg/kg) and served as positive control. Groups III and IV treated with two different doses, 300 and 600 mg/kg body weight, of the aqueous extract respectively. Groups V and VI treated with 300 and 600 mg/kg body weight of the methanol extract respectively.

Oral Glucose Tolerance Test

An oral glucose tolerance test (OGTT) was performed on normal rats. Animals were deprived of food for 16 h before and during the experiment but were allowed free access to water. The extract was administered orally at doses of 300 and 600 mg/kg of aqueous extract to two groups of 5 rats each, 1 h before glucose load (1.5 g/kg). Two other groups of 5 rats each, considered as controls, were taken into the experiment and received either, 1.5 g/kg b.w. of glucose solution or distilled water only (5 ml/kg). Blood samples were taken before administration of the extract and glucose and subsequently at 1, 2, 4 and 6 h after.

Collection of blood and measurement of blood glucose

Blood samples for glucose determination were obtained from tip of tail of the rats before administration of drugs and at times 1, 2, 4, 6 and 8 h thereafter. Blood glucose level was determined using a glucometer Accu-chek sensor (Roche, USA). The percentage of reduction of blood glucose level was determined.

Statistical analysis

Results are given as mean blood glucose levels ± SEM (standard error of mean). One way ANOVA with post hoc Dunnett's multiple comparison tests were performed using GraphPad Prism version 3.00 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com. P values of 0.05 and less were taken to imply statistical significance between the means.

Results

Hypoglycaemic activities of the extracts of *Bersama engleriana* **leaves.**

Oral administration of the aqueous extract of *Bersama engleriana* leaves at doses equivalent to 300 and 600 mg/kg body weight produced significant ($P<0.001$) hypoglycaemic effects in normal fasted animals after 2-8 h and 6-8 h respectively (Table 1).

The oral administration of methanolic extract at a dose of 300 mg/kg body weight produced no significant hypoglycaemia, whereas the administration of 600 mg/kg body weight resulted in a time-dependent reduction in the blood glucose levels in fasted normal rats. The most pronounced effect was observed at 8 h ($p<0.001$) when the dose 600 mg/kg was administrated. This dose of extract reduced the blood concentration of the fasted normal rats from an initial mean value of 90.00 ± 1.22 at 0h to a mean value of 45.80 ± 8.13 (49.11 %) at the end of the 8 h hour (Table 1). It is worthy to mentioned that animals treated with glibenclamide (2 mg/kg) showed a significant reduction in blood glucose level after 1 h ($p<0.05$) and up to 8 h ($p<0.001$).

Table 1: Effect of different doses of the aqueous and methanol extracts of *Bersama engleriana* on fasting blood glucose levels (mg/dl) of normoglycaemic rats (mean ± SEM).

Treatment groups	**Blood glucose at different hours after treatment (mg/dl)**					
	0h	1h	2h	4h	6h	8h
Distilled water 5 ml/kg b.w (negative control)	90.40 ± 0.75	93.00 ± 7.27	99.80 ± 5.95	88.20 ± 3.79	92.80 ± 2.13	91.40 ± 2.04
Glibenclamide 2 mg/kg b.w (positive control)	59.20 ± 0.20	49.60 ± 2.94* (16. 22 %)	45.60 ± 3.28** (22.97 %)	33.80 ± 2.35** (42.90 %)	27.80 ± 1.93** (53.04 %)	27.00 ± 1.22** (54.39 %)
Aqueous extract 300 mg/kg b.w	66.80 ± 1.07	64.20 ± 2.44 (3.89 %)	58.80 ± 2.03* (11.98 %)	55.00 ± 2.28** (17.66 %)	52.60 ± 1.86** (21.26 %)	46.40 ± 2.01** (30.54 %)
Aqueous extract 600 mg/kg b.w	82.20 ± 1.68	79.60 ± 5.27 (3.16 %)	66.60 ± 6.70 (18.98 %)	65.60 ± 6.18 (20.19 %)	62.40 ± 2.60* (24.09 %)	51.20 ± 4.26** (37.71 %)
Methanol extract 300 mg/kg b.w	71.00 ± 2.24	68.60 ± 5.71 (3.38 %)	65.60 ± 5.84 (7.60 %)	56.00 ± 8.38 (21.13 %)	56.00 ± 9.03 (21.13 %)	45.40 ± 7.84 (36.06 %)
Methanol extract 600 mg/kg b.w	90.00 ± 1.22	72.20 ± 4.85 (19.78 %)	66.60 ± 7.15* (26.67 %)	64.40 ± 5.29* (28.44 %)	52.00 ± 6.61** (37.35 %)	45.80 ± 8.13** (49.11 %)

The values given in parentheses represent the percentage reduction in blood glucose.
Number of rats per group = 5
* $p<0.05$ & ** $p<0.001$ compared with the initial level of blood glucose of the rats (0 h) in the respective group.

Table 2: Effect of the aqueous extract of *Bersama engleriana* on blood glucose level after a glucose load (1,5 g/Kg b.w) in normoglyceamic rats (mean ± SEM).

Treatment groups	**Blood glucose at different hours after treatment (mg/dl)**				
	0 h	**1 h**	**2 h**	**4 h**	**6 h**
Distilled water (negative control) 5 ml/kg b.w	72.00 ± 0.71	72.60 ± 1.63	72.60 ± 1.08	73.60 ± 1.03	72.80 ± 1.32
Glucose (positive control) 1.5 g/kg b.w	50.00 ± 0.95	106.20 ± 6.32	74.40 ± 2.31	62.40 ± 1.69	53.80 ± 6.12
Aqueous extract 300 mg/kg + glucose 1.5 g/kg b.w	54.00 ± 0.55	103.20 ± 11.00 (2.82 %)	74.20 ± 3.65 (0.27 %)	61.60 ± 1.96 (1.28 %)	53.40 ± 3.67 (0.74 %)
Aqueous extract 600 mg/kg + glucose 1.5 g/kg b.w	62.20 ± 0.73	88.80 ± 6.13 (16.38 %)	69.00 ± 1.41 (7.26 %)	55.40 ± 1.36* (11.22 %)	47.00 ± 2.24 (12.64 %)

The values given in parentheses represent the percentage reduction in blood glucose vs control (positive control).
Number of rats per group = 5
* $p<0.05$ compared with the positive control (glucose).

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Hypoglycaemic effect on oral glucose tolerance test

Effects of *Bersama engleriana's* aqueous extract on blood glucose levels of normal fasted rats after a glucose load (1.5 g/kg) are outlined in Table 2. The blood glucose increased rapidly 1 h after administration of glucose, and thereafter decreased gradually. When two different doses of the extract (300, and 600 mg/kg b.w.) were given orally before the glucose administration, only the highest dose of the extract caused a significant ($P < 0.05$) attenuation in the rise of blood glucose at 4 h when compared to the control group.

Discussion

Results obtained in the present study showed that administration of the fasted normal rats with the aqueous extract (300 and 600 mg/kg b.w.) and methanol extract (600 mg/kg) of *Bersama engleriana* leaves resulted in a significant decrease in blood glucose level. The maximum hypoglycaemic activity of the plant was observed with the methanol extract at the dose of 600 mg/kg b.w. with a reduced percentage of blood glucose of 49.11% after 8 h of treatment compared to 37.71% obtained with the aqueous extract at the same time and dose. This observation suggests that the substances responsible for the hypoglycaemic activity of *Bersama engleriana* are probably polar in nature and more soluble in methanol than in water. Methanol extractives of a plant are usually known to contain many chemical compounds each of which is capable of producing definite biological activities (Ojewole, 2003). It is known that in Diabetes Mellitus, the sites and mechanism of pharmacological (drug) intervention in the attendant biochemical processes are diverse (Akah and Okafor, 1992; Marles and Farnsworth, 1995). It is likely that this possibility of diversity in the hypoglycaemic mechanism of the action of drugs may also apply to the aqueous and methanol extracts of *Bersama engleriana* leaves. Bioactive molecules present in these extracts of *Bersama engleriana* may probably possess an insulin–like effect or stimulate the pancreatic β cells to produce insulin which in turn lowers the blood glucose level. Similar observations have been reported by Fuentes et al. (2004), Hemalatha et al. (2004) and Sepici et al. (2004). Like the plant extract, glibenclamide also produced a significant reduction in the blood glucose level of fasted normal rats. The present findings appear to be in consonance with the earlier suggestion of Jackson and Bressler (1981) that sulphonylureas such as glibenclamide have extra-pancreatic hypoglycaemic mechanism of action secondary to their causing insulin secretion and the attendant glucose uptake into and utilization by the tissues.

In the oral glucose tolerance test, normal fasted rats treated with *Bersama engleriana* extract at a dose of 600 mg/kg showed an inhibition of glucose increase compared with the vehicle control. This action could be due to a direct stimulation of insulin secretion.

Results of this work indicate the hypoglycaemic activity of *Bersama engleriana* aqueous and methanol extracts in normal fasted rats. This activity could be attributed to certain compounds of different nature present in *Bersama engleriana*. Further investigations are in progress to isolate these active principles and to determine their mechanism of action.

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