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## Hypoglycemic and Antidiabetic Profile of the Aqueous Root Extracts of *Leptadenia hastata* in Albino Rats

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**Abstract:** This study was designed to evaluate the antidiabetic profile and the hypoglycaemic activity of aqueous root extracts of *L. hastata* in normal and alloxan-induced diabetic rats model. Eighty five albino rats were used for this study out of this thirty five were used subjected to experimental diabetes by the use of alloxan at a dose of 160 mg kg<sup>-1</sup> body weight. Seven experimental groups of five rats per group (A-G) were used for this study. A standard antidiabetic drug (insulin) group (B) and normal saline group (G) serves as positive control. The blood glucose lowering activity of the extract, insulin and normal saline groups were monitored at 0, 1, 3, 6, 12 and 18 h post extract administration. On the other hand the remaining fifty albino rats were used to determine the acute toxicity and the hypoglycemic activity of the extract. The blood glucose levels of the rats were monitored at 0, 7, 14, 21 and 28 days post extract administration. Oral administration of aqueous root extract at 600 and 800 mg kg<sup>-1</sup> b.wt have significantly (p<0.05) decreased the blood glucose in diabetic albino rats. On the other hand the hypoglycemic activity of the aqueous root extract on normal rats at dose of 1000 mg kg<sup>-1</sup> b.wt have significantly (p<0.05) decreases blood glucose level in normal albino rats. The results of the current study have demonstrated the antidiabetic and hypoglycaemic effects of *L. hastata* aqueous root extracts and underscore its potentials in the management of diabetes mellitus especially following prolonged use in days.

**Key words:** Hypoglycemic, antidiabetic, *Leptadenia hastata*, aqueous root extract

### INTRODUCTION

Diabetes is a chronic disorder of carbohydrate, lipid and protein metabolism that is characterized by an increase in the blood glucose level as a result of insufficient or complete synthesis of insulin by the pancreatic beta cell (Murray and Pizzorno, 1997). Insulin-Dependent Diabetes Mellitus (IDDM) or Type 1 diabetes mellitus occurs as a result of the lack of insulin and could results from the destruction of the insulin-producing beta-cells in the pancreas.

Many medicinal plants that are commonly available to the rural populace are used as a decoction in the treatment of many diseases without proper knowledge of their efficacy Lee *et al.* (2003). Ogle *et al.* (2003). Adebooye and Opabode (2004), Ayodele (2005). However, most of these plants that are being

used could be associated with varying degree of side effects (Rao *et al.*, 1999; Jaouhari *et al.*, 2000). *Leptadenia hastata* which belongs to the family Asclepiadaceae it is mostly used as vegetables in the Tropics (Burkill, 1985; Aliero *et al.*, 2001). Information gathered from traditional practitioners in the study area revealed that *L. hastata* is used in the treatment of diabetes mellitus. However, there is paucity on the hypoglycemic and antidiabetic profile of the plant. The objective of this study was to clearly define the hypoglycemic and antidiabetic profile of *L. hastata* aqueous root extracts in diabetic and normal albino rats.

### MATERIALS AND METHODS

**Plant collection and extraction:** Fresh roots of *L. hastata* were collected around Lake Alau River Bank in Maiduguri,

Borno state, Nigeria. The plant sample was authenticated at the Herbarium of Botany department of faculty of Science, university of Maiduguri where voucher specimen No. Unimaid/Vet/0012 deposited. The roots were allowed to air dry at room temperature and pulverized into fine powder. Three hundred and fifty grams of the sample were separately extracted with water for 24 h as described by Trease and Evans (1983). The extracts were filtered and concentrated to dryness under reduced pressure. The dried extracts obtained were screened for hypoglycaemic effects using alloxan induced diabetic Albino rat model.

**Experimental animals:** Adult albino rats (Wister strains) of both sexes were used for this study. Apparently healthy rats were purchased from the Animal House of Department of Veterinary Physiology, Pharmacology and Biochemistry, University of Maiduguri. The rats were allowed to acclimatize to the Laboratory environment and were maintained on standard laboratory diet and distilled water *ad libitum* for a period of one week. The study was conducted with strict adherence to the ethical procedure on the use of animals for experiment.

**Acute toxicity study:** Eighty-five Adult albino rats (Wister strains) of both sexes and body weight of 200-250 g were used for this study. Out of this 25 rats were used for the acute toxicity study. They were divided into five groups of five rats each. Following an arbitrary choice of 200, 400, 800 and 1600 mg kg<sup>-1</sup> doses, groups one to four were administered with these doses of the extract, respectively and group five serves as control and was administered distilled and observed for number of deaths. The LD<sub>50</sub> was calculated as described by (Aliu and Nwude, 1982).

**Diabetes induction and extract administration:** Thirty-five rats out of the 85 rats were used for this study. Thirty rats were made diabetic by single intra-peritoneal administration of Alloxan Monohydrate (Sigma Chemical Co., St. Louis, U.S.A.) at a dose rate of 160 mg kg<sup>-1</sup> dissolved in 0.1 M freshly prepared citrate buffer at a pH 4.5 as described by Al-Shamaony *et al.* (1994). Baseline blood glucose levels were determined using glucose oxidase method, blood glucose level of more than 200 mg dL<sup>-1</sup> was considered as diabetic. Stable hyperglycemia was confirmed on the fifth day post Alloxan administration when the fasting blood glucose levels of the rats were found to be greater than 200 mg dL<sup>-1</sup>. The diabetic rats were divided into six groups of five rats each. Group A (diabetic untreated rats) were administered distilled water while Group B received the standard drug (insulin) at 0.1 mg kg<sup>-1</sup> b.wt and groups

C-F were administered 200, 400, 600 and 800 mg kg<sup>-1</sup> b.wt of the extract, respectively. While group G serve as normal control rats and was orally administered 0.25 mL of distilled water. Blood glucose was determined by glucose oxidase method of Trinder (1969), using One Torch Basic Glucose monitoring system (Life Scan Inc. Milpitas, California, USA) at 0, 1, 3, 6, 12 and 18 h post extract administration.

**Determination of hypoglycemic activity:** Twenty-five rats out of the 85 rats were used for the study. They were divided into five groups of five rats each labelled A-E. The rats in group A were orally administered 0.25 mL of distilled water each serving as a negative control. Groups B-E were orally administered 400, 600, 800 and 1000 mg kg<sup>-1</sup> b.wt of the aqueous root extract of *L. hastata*, respectively.

**Statistical analysis:** Data obtained in the study were presented as Mean±SD. Difference between mean were assessed using Two-way analysis of variance (ANOVA) and post-test using Turkey Kramer multiple comparison test (GraphpadInstat, 2003).

## RESULTS

The acute toxicity calculated value (LD<sub>50</sub>) was 1440 mg kg<sup>-1</sup>. The effect of *Leptadenia hastata* root aqueous extract on mean fasting blood glucose levels of diabetic rats is presented in Table 1. The mean blood glucose levels of rats treated with 200, 400, 600 and 800 mg kg<sup>-1</sup> b.wt of the extract at 0 h were 342.0±36.0, 383.4±12.5, 386.8±36.2 and 410.8±26.7 mg dL<sup>-1</sup>, respectively. After 1 h, the blood glucose level of rats treated with 200 mg kg<sup>-1</sup> b.wt of the extract showed no statistically significant decrease compared to zero hour blood glucose value. The value significantly (p<0.05) decreased to 305.4±41.0, 282.4±44.7, 192.8±92.7 and 132.8±40.9 at 3, 6, 12 and 18 h post extract administration, respectively. The groups treated with 400 mg kg<sup>-1</sup> b.wt of the extract had their blood glucose level significantly (p<0.05) decreased to 259.4±47.1, 230.0±82.6, 203.0±95.2, 145.0±56.3 and 101.8±7.1 at 1, 3, 6, 12 and 18 h post-extract administration, respectively. The blood glucose levels of rats administered with 600 mg kg<sup>-1</sup> also significantly (p<0.05) decreased from zero hour value of 386.8±36.2-259.4±47.1, 173.6±43.5, 131.2±31.5, 105.2±6.6 and 99.0±2.6 at 1, 3, 6, 12 and 18 h after extract administration.

In 800 mg kg<sup>-1</sup> b.wt treated group, all the values of blood glucose levels were also significantly (p<0.05) reduced from zero hour value of

Table 1: Effect of *L. hastata* root aqueous extract on mean blood glucose level of diabetic rats

Treatment groups	Dose mg kg <sup>-1</sup>	Mean fasting blood glucose level post treatment (h)					
		0	1	3	6	12	18
A	-	352.4±64.8	378.8±55.6	413.6±17.8	451.2±32.3 <sup>a</sup>	473.4±30.6 <sup>a</sup>	492.0±5.1 <sup>a*</sup>
B	1.0	397.4±46.2	198.2±61.9 <sup>b*</sup>	118.6±7.0 <sup>b*</sup>	107.0±3.8 <sup>b*</sup>	094.2±7.5 <sup>b*</sup>	088.2±9.1 <sup>b*</sup>
C	200	342.0±36.0	326.8±42.2	305.4±41.0 <sup>*</sup>	282.4±44.7 <sup>*</sup>	192.8±92.7 <sup>ab</sup>	132.8±40.9 <sup>ab*</sup>
D	400	383.4±12.5	259.4±47.1 <sup>*</sup>	230.0±82.6 <sup>b*</sup>	203.0±95.2 <sup>b*</sup>	145.0±56.3 <sup>b*</sup>	101.8±7.1 <sup>b*</sup>
E	600	386.8±36.2	259.4±47.1 <sup>b*</sup>	173.6±43.5 <sup>b*</sup>	131.2±31.5 <sup>b*</sup>	105.2±6.6 <sup>b*</sup>	099.0±2.6 <sup>b*</sup>
F	800	410.8±26.7	246.0±60.1 <sup>b*</sup>	130.0±44.9 <sup>b*</sup>	102.0±10.1 <sup>b*</sup>	089.8±6.1 <sup>b*</sup>	079.6±11.9 <sup>b*</sup>
G	2.5	117.8±11.4 <sup>*</sup>	121.8±6.1 <sup>*</sup>	123.4±5.5 <sup>*</sup>	123.8±7.6 <sup>*</sup>	125.0±6.9 <sup>*</sup>	126.3±7.2 <sup>*</sup>

Values with Superscript<sup>a</sup> within a group along the row is significantly (p<0.05) higher than zero hour blood glucose value, Values with Superscript<sup>b</sup> within groups along the row are significantly (p<0.05) lower than zero hour blood glucose value, Values with Superscript<sup>\*</sup> between groups along the column are significantly (p<0.05) lower than blood glucose value in the diabetic control group

Table 2: Effect of *Leptadenia hastata* aqueous root extract on mean glucose levels of normal rats

Dosage group (mg kg <sup>-1</sup> )	Mean fasting blood glucose level post treatment (day)				
	Day 0	Mean±SD	(mg dL <sup>-1</sup> )	Day 21	Day 28
Control	117.8±11.4	121.8±6.1	123.4±5.5	123.8±7.6	125.0±6.9
400	118.8±6.5	104.4±3.9	104.4±6.6 <sup>*</sup>	102.4±6.6 <sup>ab</sup>	101.0±4.0 <sup>ab</sup>
600	120.6±10.9	112.2±8.5 <sup>ab</sup>	107.2±12.4 <sup>ab</sup>	102.6±2.5 <sup>ab</sup>	97.6±1.7 <sup>ab</sup>
800	128.8±5.9	108.2±2.5 <sup>ab</sup>	107.0±10.2 <sup>ab</sup>	101.6±4.8 <sup>ab</sup>	101.8±3.7 <sup>ab</sup>
1000	138.2±3.1	97.8±8.3 <sup>ab</sup>	95.4±4.5 <sup>ab</sup>	96.4±4.5 <sup>ab</sup>	92.2±5.9 <sup>ab</sup>

Values with superscript<sup>a</sup> within groups along the row are significantly (p<0.05) lower than zero hour blood glucose value, Values with superscript<sup>\*</sup> between groups along the column are significantly (p<0.05) lower than blood glucose value in the control group

410.8±26.7-246.0±60.1, 130.0±44.9, 102.0±10.1, 89.8±6.1 and 79.6±11.9 at 1, 3, 6, 12 and 18 h post extract administration, respectively. The diabetic group treated with insulin (standard drug) had their blood glucose level significantly (p<0.05) reduced from zero hour value of 397.4±46.2-198.2±61.9, 118.6±7.0, 107.0±3.8, 94.2±7.5 and 88.2±9.1 at 1, 3, 6, 12 and 18 h, respectively. The diabetic untreated group had their blood glucose levels increased significantly (p<0.05) from 352.4±64.8 at 0 h-492.0±5.1 at 18 h of the experiment. The normal control group had their blood glucose levels maintained between 117.8±11.4 and 126.3±7.2. In comparing blood glucose values between the test groups (insulin treated, 200, 400, 600, 800 mg kg<sup>-1</sup>) with the diabetic control group, all the groups showed no difference between their values with that of the diabetic control at zero hour. Similarly result obtained for all the test groups at 1 h when compared with the diabetic control was insignificant except in 800 mg kg<sup>-1</sup> group where the blood glucose value was significantly 246.0±60.1 (p<0.05) lower. All the test groups had their blood glucose values significantly (p<0.05) lower at 3, 6, 12 and 18 h than the diabetic control group at the respective h. Blood glucose level in the non-diabetic normal group was significantly (p<0.05) lower than diabetic control group throughout the study period. The effect of *Leptadenia hastata* root aqueous extract on mean fasting blood glucose levels of normal rats is presented in Table 2.

After day 7 as well as day 14 post-extract administration, the group treated with 400 mg kg<sup>-1</sup> b.wt of the extract showed no significant decrease in blood glucose level compared with the day zero value. However, between day 14-28 there was significant (p<0.05)

reduction in blood glucose level to 102.4±6.6 (15%) which persisted up to day 28 with glucose level of 101.0±4.0 (15%) In 600 mg kg<sup>-1</sup> b.wt treated group, even at day seven, there was significant (p<0.05) decrease in blood glucose level compared with day zero value of 120±10.9-112±8.5 (19%). At day 14 also, the value significantly (p<0.05) decrease to 107±12.4 while at day 21 and 28, it further drop to 102±2.5 and 97.6±1.7, respectively, which were significantly (p<0.05) lower in comparison with the day zero blood glucose value. In 800 mg kg<sup>-1</sup> b.wt treated group, zero hour blood glucose level of 128±5.9 was significantly (p<0.05) reduced to 108±2.5 after day 7 of extract administration, and further decreased significantly to 107.0±10.2, 101.6±4.8 and 101±3.7 at day 14, 21 and 28, respectively. Similarly, after day 7 post-extract administration, blood glucose level decreased significantly (p<0.05) from 138±3.1-97.8±8.3 in 1000 mg kg<sup>-1</sup> treated group and after day 14, 21 and 28 it further significantly (p<0.05) reduce to 95.4±4.5, 96.4±4.5 and 92.2±5.9, respectively. The control group has their blood glucose level maintained between 117.8±11.4 and 125.0±6.9 without any significant difference between the two extremes. Blood glucose values between the test groups (400, 600, 800 and 1000 mg kg<sup>-1</sup>) and that of the normal control group showed significant (p<0.05) difference in terms of reduction in all the days except at day zero values where there were no significant difference was observed.

## DISCUSSION

*Leptadenia hastate* has been popularly used locally in the treatment of diabetes in certain parts of West Africa

(Iwu, 1981). The results of this study validate the hypoglycemic effects of this plant as earlier asserted (Ampofo, 1979). The experimental hypoglycemia exhibited following alloxan administration increase from groups A-F show that alloxan, at a dose of 160 mg kg<sup>-1</sup>, is capable of inducing diabetes 4-5 days after administration. This study is in conformity with the work of Highet and West (1949) and Anaga (2003). Alloxan, which induces chemical diabetes in animal species by destructing the insulin secreting beta cells in the pancreas, causing a decrease total secretion in insulin production hence, produces sustained hyperglycemia. There are reports of increase in fasting blood glucose in albino rats after 5 days of treatment with alloxan (Karageuzyan *et al.*, 1998; Anaga, 2003). In this study, hyperglycemia was observed, on the 5th day post alloxan administration. The results of this experiment clearly shows that fasting blood glucose could be brought to normal from an increased diabetic level (experimentally induced) at any time between 3-18 h following the administration of the aqueous root extract of *Leptadenia hastata* especially at a dose of 400 mg kg<sup>-1</sup> (group D). At this dose the extract reduced the diabetic blood glucose level (230.0±82.6 mg 100 mL<sup>-1</sup>) to a significantly non-diabetic level (101.8±7.1 mg 100 mL<sup>-1</sup>) at 18 and 79.6±11.9 mg 100 mL<sup>-1</sup> at a dose of 800 mg kg<sup>-1</sup> at 18 h post extract administration. These activity values were comparable to that of insulin which gave 118.6±7.0 and 88.2±9.1 mg 100 mL<sup>-1</sup> between 3 and 18 h, respectively, from a pre-treatment diabetic level of 397.4±46.2 mg 100 mL<sup>-1</sup>. This observation agrees with the folkloric claims that the aqueous root extract of *Leptadenia hastata* decreases glycosuria after 1-2 weeks of therapy (Iwu, 1993). Since alloxan is known to destroy pancreatic beta cells, these findings suggest that the extract may have extra pancreatic anti-hyperglycemic mechanism of action secondary to their insulin secretion. (Mott *et al.*, 1985). Though, there is very little biologic evidence on the specific modes of action of this extracts in the treatment of diabetes. At 400 mg kg<sup>-1</sup> the extract had minimal effect on blood glucose even up till day 12 h. However, at 600 mg kg<sup>-1</sup> the activity was very significantly different from that observed at the 400 mg kg<sup>-1</sup> dose as well as the control, commencing from 12 h. Interestingly, however, at 800 mg kg<sup>-1</sup> there was further improvement in the blood glucose lowering effect of the extract. The reduction was significantly different from all treatment groups and control as from 1 h post-treatments. This difference was progressive up till 18 h. The activity of the extract at 800 mg kg<sup>-1</sup> which mimics that of insulin at 1.0 mg kg<sup>-1</sup> and as buttressed by the fasting blood glucose reduction of the extract (at 800 mg kg<sup>-1</sup>) with reference to insulin suggests that

the aqueous root extract of *Leptadenia hastata* used in this study probably is impure and if purified may exert same activity at a lesser concentration. In conclusion, this study, not only confirms the importance of the folkloric use of *Leptadenia hastata* in the treatment of diabetes mellitus in man (Ogle *et al.*, 2003), but has gone further to establish that excellent hypoglycemic activity is obtainable with the extract at about 3-12 h when administered daily particularly at a dose of 600-800 mg kg<sup>-1</sup>. However, before this can be accepted as an unequivocal scientific assertion, the possible pathologies associated with the administration of this extract at this dose (800 mg kg<sup>-1</sup>) should be examined as this was not performed in this study and studies on the purification and characterization of the active ingredients should be carried out for the exploitation of the potentials of *Leptadenia hastata* root extract as a valuable medicinal resource especially as an antidiabetic agent.

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