ANTIDIABETIC POTENTIALS OF LEAVES, STEM-BARK AND ROOTS EXTRACT OF LEPTADENIA HASTATA IN ALBINO RATS.

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Key words
Hexane extract
Alloxan
Leptadenia hastata
Insulin
Albino rats

Abstract
This study was designed to evaluate the hypoglycaemic and diabetic potential of Leptadenia hastate Ariel parts in normal and alloxan-induced diabetic in albino rats as acclaimed by traditional herbal practitioner, that the plant possesses diabetic healing property. 120 white albino rats were used for this study over a period of 21 days. Standard drugs (Insulin) were used. The albino rats were randomly divided into eight groups, this include normal group, negative and positive control group while five groups for extracts dosage, except the normal all the groups were induced with diabetic. Those in group one served as control group, group two negative control (Alloxan) Diabetic induced with no treatment, while group three positive control (Insulin) and groups four to eight are dosed groups ranged from; 100mg/kg 200mg/kg, 300mg/kg, 400mg/kg and 500mg/kg extracts respectively. The blood glucose levels of the rats were monitored at 0, 7, 14 and 21 days post extract administration. Oral administration of these extracts at 300and 500mg/kg bwt have significantly (P<0.05) decreased the blood glucose level in the induced diabetic albino rats. The results of the current study have demonstrated the antidiabetic potential of the Ariel parts of Leptadenia hastata.

1. INTRODUCTION
Diabetes Mellitus (DM) is a chronic metabolic disorder, characterized by a state of insulin deficiency that leads to a rise in glycaemia[1], initially involving changes in carbohydrate metabolism and secondarily of lipids and proteins [2,3].

It 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 [4]. Insulin-Dependent Diabetes Mellitus known as type 1 diabetes mellitus occurs as a result of lack of insulin and could result from the destruction of the insulin producing beta-cells in the pancreas. The most common symptoms observed in type I diabetes patients are polydipsy, polyuria, glycosuria, weakness with no apparent cause, and slow healing of wounds [3]. The levels of glycemia and insulinemia must be controlled in order to avoid later complications of diabetes, such as atherosclerosis, hypertension, hypertriglyceridemia, hypercholesterolemia, myocardial infarction, ischemic attacks, impotence, retinopathy, nephropathy [5].

Many hypoglycemiant agents, such as the biguanides and sulfonylureas, are used alone or together with insulin to treat this disease. However, these medications can cause serious side effects [6]. these bring about a search for safer, more efficacious agents to control this monster (diabetes).

Many medicinal plants have proved to successfully aid in various ailments leading to mass screening for their therapeutic components. Today, the search for Medicinal plant rich in wound healing and antimicrobial properties is escalating due to their medicinal importance in controlling many related chronic disorders such as ulcer, cancer and diabetic diseases [7].

The majority of the plants that are used in popular medicine for treatment of diabetes have been shown to possess biologically active chemical constituents (alkaloids, carbohydrates, cumarins, flavonoids,
terpenoids, phenolic substances, Saponin and other constituents) that can be used as new hypoglycemic agents [1,8,3]. *Leptadenia hastata*, a plant that is widely distributed throughout the world. This plant species is used in various applications especially for medicinal purposes. They are significant element of the world cultural heritage; they resort for treating health problems. This knowledge is passed down from generation to the next generation with or without little written information was available on the active, safety and effectiveness of this medicine. It is a perennial plant of the family of Asclepiaceae, the plant is edible non-domesticated vegetable and it is collected in wild throughout Africa. It is a volatile herb with creeping latex stems, glabescent leaves, glomerulus and racemes flowers as well as follicle fruits. The leaves are up to 10cm long, mostly ovate and light green. The flowers are cream or yellowish green [9].

2. MATERIAL AND METHODS

Plant material: Freshly leaves of *Leptadenia hastata* were collected from the uncultivated farm land of the Federal University Wukari Taraba State, Nigeria and was authenticated at Ahmadu Bello University Zaria and Voucher No PU: 2 ABU Herbarium No 900220. The plant *Leptadenia hastata* (yadiya) was dried under room temperature.

2.1 Preparation of Plant Material

The plant *Leptadenia hastata* were washed with distilled water to remove the soil and dust particles they were thoroughly air dried and powdered using laboratory grinder machine (FGR-350, Quest Scientific) extraction using methanol by placing 150g of the powdered samples into an Erlenmeyer flask and methanol three times the weight of the extracts was added, the solution was covered and shaken at an interval of an hour and then allowed at room temperature to stand for 7days, the mixture were then filtered using what man filter paper No.4 and the solvent was evaporated using a rotary evaporator (Heidolph Laborato 400) under reduced pressure below 50EC. It was then stored under a frozen condition until required.

2.2 Chemical and Reference Drug

All chemicals and Drugs (Alloxan Monohydrate) used in this investigation were of analytical grade and were obtained from Sigma Chemical Co., St Louis, U.S.A) insulin (reference drug) was obtained from a pharmacy shop in yola Adamawa State, Nigeria. The drug is an anti-diabetic.

2.3 Experimental Protocol

- **Group 1**: Normal control (diet/water)
- **Group 2**: Rats (induced Diabetic Alloxan 160mg/kg/bwt +diet /water)
- **Group 3**: Rats (induced Diabetic Alloxan 160mg/kg/bwt +diet /water + Insulin)
- **Group 4**: Rats (induced Diabetic Alloxan 160mg/kg/bwt +diet /water +100mg/kg/bwt extracts).
- **Group 5**: Rats (induced Diabetic Alloxan 160mg/kg/bwt +diet /water +200mg/kg/bwt extracts)
- **Group 6**: Rats (induced Diabetic Alloxan 160mg/kg/bwt +diet /water +300mg/kg/bwt extracts)
- **Group 7**: Rats (induced Diabetic Alloxan 160mg/kg/bwt +diet /water +400mg/kg/bwt extracts)
- **Group 8**: Rats (induced Diabetic Alloxan 160mg/kg/bwt +diet /water +500mg/kg/bwt extracts)

2.4 Experimental Animals

Adult albino rats (Wister strains) weighing about 160-200g body weight were used for this study. They were put in cages at room temperature (20-27°C) under 12/12 night/dark. They were maintained on a standard animal pellets (vital feeds, Grands cereals and oil meal Jos) and water ad libitum for a period of one week. All the experiment was conducted based on the adherence to the ethical procedure on the use of animals for experiment.

2.5 Toxicity Of The Plant Extracts

Following the studies and reports of toxicity of the plant *Leptadenia hastata*, Umaruet al., [9] in their studies; Cytotoxicity Brine Shrimp Activity of *Leptadenia hastata*(Pers) Decne Leaves, Stem-Bark and Root Extract, they reported base on the fact that lethality concentration of LC50 was assessed at 95% confidence using probit analysis. it has been observed LC50 value of less than 1000μg/mL is toxic while LC50 value of greater than 1000μg/mL is non-toxic [1]. They reported hexane extract of the leaves, stem-bark and roots LC 50 are; 9421.49,4657.358,4657.358 respectively, exhibited cytotoxic activity against brine shrimp greater than 1000 ppm (μg/mL). From this result, it is evident that the leaves, stem-bark and roots is not toxic. [9].

2.6 Diabetes Induction and Extract Administration

The experimental rats were induced and made diabetic by single intraperitoneal administration of Alloxanmonohydrate at a dose rate of 160mg/kg dissolved in 0.1M freshly prepared citrate buffer at a base linepH 4.5 as reported by Al-Shamaonyet al., [10]. Baseline blood glucose was determined using glucose oxidase method, blood glucose level of more than 200mg/kg was considered as diabetic. Animals were divided into eight groups of 5 rats each that had fasted for 24 h prior to receiving an oral dose of saline, (insulin, 0.1 mg/kg) and extract of *Leptadenia hastata* (100, 200, 300,400 and 500 mg/kg). Group A (normal rats) were administered distilled water, Group B (diabetic untreated rats) were administered distilled water, while Group C received the standard drug (Insulin) at 0.1mg/kg and the rest of the groups D-H were administered the hexane extracts. Blood glucose was determined by glucose oxidase method of Trinder [11] using one Torch Basic Glucose monitoring system with little modification at 0, 3, 7, 14, 21, 28 days’ post extract of *Leptadenia hastata* administration.

3. STATISTICAL ANALYSIS

Data were expressed as Mean±standard deviation for three determinations of each experiment. The analysis was done using the software-SPSS one-way ANOVA.
Table 1: Effect of Leptadenia hastata Leaves extract on Mean blood glucose Level of diabetic rats

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Dose mg/kg</th>
<th>0</th>
<th>3</th>
<th>7</th>
<th>14</th>
<th>21</th>
<th>28</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (Normal)</td>
<td>0.25ml</td>
<td>112.3±10.2*</td>
<td>116.5±4.2*</td>
<td>122.6±6.4*</td>
<td>123.3±8.3*</td>
<td>126.0±7.5*</td>
<td>126.6±6.5d*</td>
</tr>
<tr>
<td>B (Negative)</td>
<td>0.25ml</td>
<td>364.3±72.4</td>
<td>387.6±61.2c*</td>
<td>420.4±15.7c*</td>
<td>455.7±25.8c*</td>
<td>487.2±29.1d*</td>
<td>506.7±8.6c*</td>
</tr>
<tr>
<td>C (Positive)</td>
<td>0.1mg/kg</td>
<td>399.6±56.7</td>
<td>189.5±45.5</td>
<td>123.6±6.3*</td>
<td>101.2±2.4c*</td>
<td>089.3±6.4</td>
<td>075.6±6.3c*</td>
</tr>
<tr>
<td>D (Extracts)</td>
<td>100</td>
<td>322.4±16.7</td>
<td>316.5±32.3*</td>
<td>290.7±11.4c*</td>
<td>212.7±8.7c*</td>
<td>175.6±12.8</td>
<td>138.7±29.6d</td>
</tr>
<tr>
<td>E (Extracts)</td>
<td>200</td>
<td>354.2±17.9c</td>
<td>324.7±17.9c</td>
<td>302.9±36.2c*</td>
<td>245.3±33.4</td>
<td>163.3±34.6</td>
<td>121.3±7.6d</td>
</tr>
<tr>
<td>F (Extracts)</td>
<td>300</td>
<td>377.5±32.4</td>
<td>282.3±31.9c*</td>
<td>225.7±11.6c*</td>
<td>209.7±17.8c</td>
<td>145.8±19.7</td>
<td>098.4±19.5*</td>
</tr>
<tr>
<td>G (Extracts)</td>
<td>400</td>
<td>392.4±16.8</td>
<td>265.9±21.3</td>
<td>178.8±45.3</td>
<td>125.3±19.3c*</td>
<td>102.4±11.2</td>
<td>087.8±11.6*</td>
</tr>
<tr>
<td>H (Extracts)</td>
<td>500</td>
<td>432.3±11.7</td>
<td>232.6±23.8</td>
<td>129.7±11.9</td>
<td>105.3±12.4c*</td>
<td>093.4±33.5*</td>
<td>064.4±23.7*</td>
</tr>
</tbody>
</table>

Value with superscripts * with a group along the row is significantly (P<0.05) higher than zero hours’ blood glucose value with superscript d within the group along the row is significantly (P<0.05) lower than zero hours’ blood glucose value. While value with superscript * between groups along the column is significantly (P<0.05) lower than blood glucose value in the diabetic control group.

Table 2: Effect of Leptadenia hastata Stem-bark extract on Mean blood glucose Level of diabetic rats

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Dose mg/kg</th>
<th>0</th>
<th>3</th>
<th>7</th>
<th>14</th>
<th>21</th>
<th>28</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (Normal)</td>
<td>0.25ml</td>
<td>103.7±11.2</td>
<td>105.3±6.4</td>
<td>111.6±2.6</td>
<td>113.5±4.3</td>
<td>119.0±9.4</td>
<td>120.2±2.3</td>
</tr>
<tr>
<td>B (Negative)</td>
<td>0.25ml</td>
<td>344.4±12.3</td>
<td>368.6±4.12</td>
<td>418.4±13.7c</td>
<td>449.7±65.3c</td>
<td>468.6±17.3c</td>
<td>501.2±11.3c</td>
</tr>
<tr>
<td>C (Positive)</td>
<td>0.1mg/kg</td>
<td>388.4±16.5</td>
<td>178.5±47.6e</td>
<td>118.6±5.3g</td>
<td>098.7±1.6h*</td>
<td>076.3±7.1h*</td>
<td>055.6±9.2h*</td>
</tr>
<tr>
<td>D (Extracts)</td>
<td>100</td>
<td>332.4±18.3</td>
<td>314.3±12.3</td>
<td>288.7±23.4</td>
<td>202.9±7.3</td>
<td>164.6±11.5</td>
<td>128.9±45.5c</td>
</tr>
<tr>
<td>E (Extracts)</td>
<td>200</td>
<td>358.7±56.7</td>
<td>331.5±28.2*</td>
<td>235.6±55.4</td>
<td>180.3±24.8</td>
<td>130.9±9.3c</td>
<td></td>
</tr>
<tr>
<td>F (Extracts)</td>
<td>300</td>
<td>370.5±14.4</td>
<td>294.3±11.7d</td>
<td>209.8±45.6f</td>
<td>189.6±15.8g</td>
<td>133.9±38.4</td>
<td>094.4±17.8*</td>
</tr>
<tr>
<td>G (Extracts)</td>
<td>400</td>
<td>399.7±35.8g</td>
<td>255.7±51.5</td>
<td>150.7±15.7j</td>
<td>105.7±34.2</td>
<td>098.4±71.4*</td>
<td>073.3±66.4*</td>
</tr>
<tr>
<td>H (Extracts)</td>
<td>500</td>
<td>444.7±66.7</td>
<td>242.5±43.6</td>
<td>139.6±32.4</td>
<td>115.3±72.4</td>
<td>083.7±22.7*</td>
<td>044.5±63.5*</td>
</tr>
</tbody>
</table>

Value with superscripts * with a group along the row is significantly (P<0.05) higher than zero hours’ blood glucose value with superscript h within the group along the row are significantly (P<0.05) lower than zero hours’ blood glucose value. While value with superscript * between groups along the column is significantly (P<0.05) lower than blood glucose value in the diabetic control group.

Table 3: Effect of Leptadenia hastata Roots extract on Mean blood glucose Level of diabetic rats

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Dose mg/kg</th>
<th>0</th>
<th>3</th>
<th>7</th>
<th>14</th>
<th>21</th>
<th>28</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (Normal)</td>
<td>0.25ml</td>
<td>115.3±23.2a*</td>
<td>119.5±4.2a*</td>
<td>126.8±4.4a*</td>
<td>123.3±7.5a*</td>
<td>128.0±5.5a*</td>
<td>129.6±3.2a*</td>
</tr>
<tr>
<td>B (Negative)</td>
<td>0.25ml</td>
<td>394.3±72.4</td>
<td>387.6±61.2</td>
<td>420.4±15.7</td>
<td>455.7±25.8c</td>
<td>487.2±29.1c</td>
<td>506.7±8.6c</td>
</tr>
<tr>
<td>C (Positive)</td>
<td>0.1mg/kg</td>
<td>399.6±56.7</td>
<td>189.5±45.5d*</td>
<td>212.7±8.7d*</td>
<td>175.6±12.8d*</td>
<td>138.7±29.6d*</td>
<td></td>
</tr>
<tr>
<td>D (Extracts)</td>
<td>100</td>
<td>322.4±16.2</td>
<td>316.5±32.3</td>
<td>298.7±51.4</td>
<td>212.7±15.3</td>
<td>175.6±12.8d*</td>
<td>138.7±29.6d*</td>
</tr>
<tr>
<td>E (Extracts)</td>
<td>200</td>
<td>354.2±17.9a*</td>
<td>324.7±17.9a*</td>
<td>302.9±36.2d*</td>
<td>245.3±33.4d*</td>
<td>163.3±34.6d*</td>
<td>121.3±7.6d</td>
</tr>
<tr>
<td>F (Extracts)</td>
<td>300</td>
<td>377.5±32.4</td>
<td>282.3±31.9</td>
<td>225.7±11.6d*</td>
<td>209.7±17.8d*</td>
<td>145.8±19.7d*</td>
<td>108.4±19.5d*</td>
</tr>
<tr>
<td>G (Extracts)</td>
<td>400</td>
<td>392.4±16.8</td>
<td>265.9±21.3</td>
<td>178.8±45.3</td>
<td>125.3±19.3d*</td>
<td>102.4±11.2d*</td>
<td>087.8±11.6d</td>
</tr>
<tr>
<td>H (Extracts)</td>
<td>500</td>
<td>442.3±22.7</td>
<td>221.6±13.8d*</td>
<td>125.7±45.9d*</td>
<td>109.3±18.7d*</td>
<td>091.4±11.4*</td>
<td>061.1±13.9*</td>
</tr>
</tbody>
</table>

Value with superscripts * with a group along the row is significantly (P<0.05) higher than zero hours’ blood glucose value with superscript d within the group along the row are significantly (P<0.05) lower than zero hours’ blood glucose value. While value with superscript * between groups along the column is significantly (P<0.05) lower than blood glucose value in the diabetic control group.
4. RESULTS AND DISCUSSION

4.1 Results

The effect of the extracts of Leptadenia hastata Leaves, Roots and Stem bark extract on the diabetic albino rats fasted for 28days with a dose of 100-500 mg/kg body weight, is shown in Tables 1, 2 and 3. The mean blood glucose levels of rats treated with 100,200,300,400 and 500mg/kg bwt of the extract at 0 days’ table 1, were 322.4±16.2, 354.2±16.7, 377.5±32.4, 392.4±16.8 and 432.3±11.7; table 2, was 332.4±18.3, 358.7±56.5, 370.5±14.4, 399.7±35.8 and 444.7±66.7 while table three was reported to be 322.4±16.2, 354.2±16.7, 377.5±32.4, 392.4±16.8 and 442.3±22.7 respectively. After three days the blood glucose level of the rats treated with 100mg/kg bwt showed statistically significant increase compared to zero-day blood glucose value, the value significantly (P<0.05) increased to 316.5±32.3, 324.7±17.9, 282.3±31.9, 265.9±21.3 and 232.6±23.8 for leaves extract; 314.3±12.3, 333.5±28.2, 294.3±11.7, 255.7±51.5 and 242.5±43.6 for stem bark and 316.5±32.3, 324.7±17.9, 377.5±32.4, 265.9±21.3, 221.6±13.8 for the roots extract respectively. However, the group treated with 200-500mg/kg bwt in all the extracts in tables 1, 2 and 3 at days 28 had their blood glucose significantly (P<0.05) values decreased to 138.7±29.6, 121.3±7.6, 098.4±19.5, 087.8±11.6, 064.4±23.7 (Leaves); 128.9±45.5, 130.7±9.3, 094.4±17.8, 073.3±66.4, 044.5±63.5 (Stem bark) and the roots 138.7±29.6, 121.3±7.6, 108.4±19.5, 087.8±11.6, 061.1±13.9; thus the diabetic group treated with insulin (Reference drug) had their blood glucose levels significantly (P<0.05) reduced from zero days’ value for all the extract at days 3,7,14,21 and 28days. While the diabetic untreated groups had their blood glucose levels increased significantly (P<0.05) at zero -28days experiment. But the normal groups had their blood glucose level maintained between 112.3±10.2 and 126.6±6.5 for leaves extract, 103.3±11.2 and 120.2±2.3 for Stem bark extract and roots at 115.3±23.2 and 129.6±3.2 respectively.

In comparing blood glucose values between the tested groups (insulin, 100, 200, 300, 400,500mg/kg) with the diabetic control groups, all the groups showed no difference between their values with that of the diabetic control at day zero. Similarly results obtained for all the test groups at days 3,7,14,21 and 28 were significant (P:0.050 than the the diabetic control group respectively in all the plant extract. However, the blood glucose in the non-diabetic normal group was significantly (P<0.05) when compared with the extracts of the plants at 100, 200and 300mg/kg bwt in some cases, but higher when compared to the leaves, stem-bark and roots extracts at 400-500 mg/kg bwt at days 28. This result showed that there the blood glucose values between the test group (100,200, 300, 400, and 500mg/kg bwt) and that of the control group is significant (P<0.05) thus reduced the level of blood glucose with increase in days of administration with increase in extract dose.

4.2 Discussion

Glucose is the simplest metabolic end-product of carbohydrate metabolism which is mostly readily absorbed into the porta blood from the gastro-intestinal tract following its oral ingestion [12]. Therefore, several in vivo acute and chronic drug-induced hyperglycaemic models have been developed and used to investigate the hypoglycaemic effects of medicinal plants with antidiabetic potentials. Most of the models include oral glucose loading- and nicotine-induced hyperglycaemia [13]. This plant Leptadenia hastata has been popularly used locally in the treatment of diabetes in Nigeria and west Africa at large [14]. The research work on the leaves, roots and stem bark of Leptadenia hastata on diabetic induced rats showed that extracts at dose of 300-500mg/kg bwt is capable to reduce the diabetic blood glucose level. At 500mg/kg leaves extract the extract reduced the diabetic glucose level (444.7±66.7) to aa significantly non-diabetic Level (064.4±23.7) which also applies to all the ariel parts of the plant as shown in table 2 and3. Since Alloxan is known to destroy pancreatic beta cells, these research findings suggest that the extracts may have extra pancreatic anti-hyperglycaemia mechanism of the secondary to their insulin secretion[15]. Thus studies on the phytochemical should be carried out for the exploration of the bioactive potential of the plant parts as an agent for antidiabetic.

5. CONCLUSION

The present study has shown that the Hexane extract of Leptadenia hastata leaves, stem-bark and roots caused significant reductions in diabetic drug-induced hyperglycaemia in albino rats. Elucidation and Characterization of the plant extracts are currently on-going Natural product research laboratory Universiti Malaysia Sarawak. Also, results obtained in this preliminary study will form a template for subsequent studies on the mechanisms involved.

6. CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

7. ACKNOWLEDGMENT

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REFERENCE


