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Hypoglycemic and Anti-Diabetic Profile of N-Hexane Extract of *Leptadenia Hastata* Leaves on Streptozotocin-Induced Diabetes in Albino Rats

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Abstract

Introduction: Diabetes mellitus is a metabolic disorder of the endocrine system. The disease occurs worldwide and its occurrence is increasing rapidly in most parts of the world to epidemic proportions. Management of diabetes is usually with prescribed medication and controlled lifestyle choices. Rural dwellers in Nigeria often resort to herbal remedy and dietary control in the treatment of diabetes mellitus because of availability and affordability of orthodox medication available for treatment of diabetes mellitus. This work was undertaken to provide the logic for the use of the leaves of *Leptadenia hastata* as a traditional anti-diabetic agent. Method: n-hexane extract of the leaves of *Leptadenia hastata* was prepared by soxhlet extraction using maceration method. The extract was evaluated for anti-diabetic effect in streptozotocin-induced diabetes in rats. The serum glucose levels were assayed as indices of diabetes. Results: Treatment of streptozotocin induced diabetes in rats with 100 and 200 mg/kg of n-hexane extract of L. hastata reduced ($p<0.001$) blood glucose level and prevented diabetic weight loss which was evident in the untreated diabetic rats. Conclusions: The results of the present study affirms the efficacy of *Leptadenia hastata* leaves in the treatment of diabetes mellitus.

Keywords: Leptadenia hastate; Diabetes; Hypoglycemic; Anti-Diabetic; N-Hexane extract.

1. Introduction

Diabetes is a heterogeneous group of metabolic disorders characterized physiologically by insulin deficiency or insulin activity and is clinically diagnosed by hyperglycemia or impaired glucose tolerance and other manifest able disorders [1]. It is also associated with hyper lipidemia and co-morbidities such as obesity and hypertension [2]. Lowering the serum glucose concentration is the best defense against the late complications and negative outcomes of diabetes mellitus such as blindness, renal failure and limb amputation [3]. Diabetes Mellitus is ranked seventh among the leading causes of death worldwide and is considered third when its fatal complications are taken into account [4]. It has been estimated that about 171 million people worldwide suffer from diabetes [5, 6].

Although insulin therapy is the primary treatment for lowering blood glucose, the first approach to diabetes mellitus treatment generally involves lifestyle changes which includes increasing physical activity, reducing weight and improving the diet [3]. Currently, conventional treatment of diabetes mainly involves a sustained reduction in hyperglycemia by the use of biguanides, thiazolidinediones, sulphonylureas, Diphenylalanine derivatives, meglitinides and α-glycosidase inhibitors in addition to insulin [3, 7]. However, due to unwanted side effects the effectiveness of these compounds are debatable and it has been shown that the use of orthodox drugs in the management of diabetes mellitus has not improved the situation [5]. There is therefore a demand for new substances useful in the treatment of diabetes [7] which have minimized side effects and is readily available and affordable.

Medicinal plants have been also used for a long period of time in treatment of ailments and are well known in traditional medicine for their hypoglycaemic activities including the prevention and control the complications associated with diabetes mellitus [3, 8]. Available literature indicates that there are more than 800 plants species showing hypoglycaemic activity [8].

With increasing interest in herbal medicine, more individuals are exploring the possibility of using natural medicines to complement conventional therapy, as is already the case in certain minority cultures [8-10]. *Leptadenia hastata* is one of the medicinal plants used locally in the treatment and management of diabetes mellitus especially in the northern parts of Nigeria. *Leptadenia hastata* (Pers.) Decne belongs to the family Asclepiadaceae, used as food by many African populations [9]. It is commonly used as a vegetable and is considered as a famine food due to its high content of valuable nutrients in Niger [10]. It has reportedly been used traditionally in the management of diabetes mellitus and treatment of stomach ache [11]. In the Northern part of Nigeria, traditional women use the leaf

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of this plant to cause anti-fertility or abortion on their rivals or mate [12]. In Nigeria, reports has supported the use of *Leptadenia hastata* as an antimicrobial agent [15], Also in Nigeria, local healers use the plant for hypertension, catarrh and skin diseases [13]. The sap, or the root in decoction is used for ophthalmia in Senegal [13, 14]. In association with other plants, it is used in Senegal for suckling babies with green diarrhea and for prostate and rheumatism complaints [14, 15]. In Burkina Faso, locally it is used for sexual potency (chewing leaves), trypanosomosis (decoction of leaves), skin diseases and wound-healing (application of latex) [14, 16]. The present research study seeks to investigate the anti-diabetic potential of the herb as an available and affordable alternative to orthodox medication used in the treatment of diabetes mellitus.

2. Materials and Methods

2.1. Chemicals and Reagents

Streptozotocin (Bristol Scientific Company, Missouri, United States), soluble insulin injection (Novo Nordsick, Denmark) were commercially purchased. Sodium citrate granules (BDH chemicals, Poole, England) and citric acid granules (BDH chemicals, Poole, England) were dissolved in distilled water to make citrate buffer in which streptozotocin powder was dissolved in, Combostik11 Urinalysis strips (DFI Co, Ltd, Iran) was procured from a pharmacy and also from the University of Maiduguri Teaching Hospital, Maiduguri.

3. Plant Materials

3.1. Preparation of Plant Materials

3.1.1. Collection, Identification and Storage of Plant Material

Leptadenia hastata was collected from a garden in the University of Maiduguri, Borno State, authenticated by a plant taxonomist, from the Department of Biological Sciences, Faculty of Science, University of Maiduguri. The leaves were harvested, washed and shade dried for a period of two weeks and then ground to powder using a mortar and pestle. The powder was sieved to obtain the fine powder, it was then labeled and stored for use.

3.2. Extraction of Plant Material

Maceration technique as described by Azwanida [17] was used for extraction in the current study. The leaf powder weighing 500g was dissolved in 3 liters of n-hexane in a 5 liter stoppered container. Maceration involved soaking the plant which is allowed to stand at room temperature for a period of 3 days at the minimum with periodic agitation. The process softened and broke the plant's cell wall to release the soluble phytochemicals. After 3 days, the mixture was filtered using Whatman's filter paper. The resulting n-hexane filtrate was concentrated to dryness in-vacuo using an evaporator and the resulting powder was kept in an air–tight container and refrigerated.

3.3. Animals

Thirty adult albino rats of both gender (120-200g) were obtained from the Animal House of the University of Maiduguri, Nigeria. The animals were maintained in a well-ventilated room in the animal house standard conditions of under 12 h light: 12 h dark cycle and were acclimatized for a period of 3 weeks before the start of the experiment. Animals were allowed to freely feed on their standard pellet diet (ECWA feeds, Jos) and water ad libitum. All the experimental procedures utilized were performed in accordance with the approval of the Institutional Animal Ethics Committee of the institution.

3.4. Experimental Induction of Diabetes in Rats

Hyperglycemia was induced in overnight fasted albino Wistar rats by a single intra-peritoneal injection of 50mg/kg streptozotocin (Bristol-Sigma, Bristol Scientific Company, Missouri, United States of America) dissolved in 0.1M ice-cold sodium citrate buffer, (pH = 4.5), immediately before use in a volume of 1ml/kg body weight as described by Etuk [18]. Hyperglycemia was confirmed by the elevated plasma glucose levels determined in tail blood sample using a glucometer (Roche, Germany). Rats whose fasting blood glucose levels exceeded 250mg/dl (13mmol/dl) after one week were considered as diabetic and used for the study. Urinalysis was also carried out to confirm diabetes in all groups according to a method adopted by Houcine, *et al.* [19].

3.5. Experimental Design

A total of thirty rats (20 diabetic and 10 normal) of both sexes were randomly divided into 6 groups of 5 animals each and treated as presented below. This treatment commenced after diabetes was confirmed and continued for a period of 28 days.

Group 1 - Non-diabetic control group were administered olive oil as vehicle

Group 2 - Non-diabetic group that was administered 200mg/kg of n-hexane extract of *Leptadenia hastata*

Group 3 – Induced with 50mg/kg of Streptozotocin and received olive oil as vehicle

Group 4 - Induced with 50mg/kg of Streptozotocin and treated with 100mg/kg of n-hexane extract of *Leptadenia hastata*

Group 5 - Induced with 50mg/kg of Streptozotocin and treated with 200mg/kg of n-hexane extract of *Leptadenia hastata*

Group 6 – Induced with 50mg/kg of Streptozotocin and treated with insulin (6IU/kg)

3.6. Determination of Serum Glucose Levels

Serum glucose levels were determined weekly by testing the blood obtained from the tail of rats in all groups using Acucheck glucometer strips. At the end of 28 days, the serum glucose levels were determined using Labkit protocol (Barcelona, Spain) kits using the glucose oxidase method as described by Kanagasabapathy and Kumari [20], Trinder [21].

The mass spectrometer was adjusted to zero with blank or reagent. The sample was pipetted into a curvette along with the following solutions: In the first test tube, 1.0ml of the reagent was added, in the second test tube, 1.0ml of reagent and 10µL of calibrator was added and in the third test tube, 1.0ml of reagent and 10µL was added. These solutions were mixed and incubated for 5 minutes at 37^{o}C or 10 minutes at room temperature (15-25^{o}C). The absorbance (A) of the samples and calibrator were observed against the blank and calculated if the colour was observed to be stable for at least 30 minutes.

Calculations: Glucose (mg/dl) = $\frac{\text{(A) Sample X 100 (Calibrator conc)}}{\text{(A) Calibrator}}$

Conversion factor: mg/dL X 0.555 = mmol/L

3.7. Measurement of Weight

The rats were measured weekly using a digital weighing balance. The weight in each of the groups was recorded.

3.8. Statistical Analysis

Data was statistically analyzed using GraphPad InStat software (version 3.75) by using One-way Analysis of Variance (ANOVA) and expressed as mean ± SEM and percentage followed by Bonferroni Multiple Comparisons Test. $p<0.05$ was considered to be statistically significant.

Table-1. Effect of n-hexane extract of Leptadenia hastata on fasting blood glucose in experimental rats

Groups	Treatment	Initial	Fasting Blood Glucose levels (mg/dl)				
			Day 1	Day 7	Day 14	Day 21	Day 28
I	-	105.2 ± 4.3^{a}	100.2 ± 3.3^{a}	96.5 ± 7.4^{a}	99.9 ± 6.9^{a}	92.3 ± 3.5^{a}	110.6 ± 12.6^{a}
II	Extract (200mg/kg)	108.3 ± 6.6^{a}	112.3 ± 4.8^{a}	114.2 ± 5.5^{a}	108.8 ± 8.1^{a}	119.2 ± 7.3^{a}	105.0 ± 9.3^{a}
III	-	120.2 ± 4.1^{a}	420.2 ± 5.2^{b}	444.8 ± 8.7^{b}	547.0 ± 4.2^{c}	567.8 ± 2.4^{c}	539.0 ± 8.5^{c}
IV	Extract (100mg/kg)	108.0 ± 8.8^{a}	450.0 ± 6.9^{b}	494.0 ± 3.6^{b}	414.2 ± 3.3^{b}	124.6 ± 12.9^{a}	180.7 ± 8.7^{b}
V	Extract (200mg/kg)	116.8 ± 5.4^{a}	440.8 ± 5.6^{b}	446.0 ± 4.7^{b}	391.4 ± 4.7^{b}	214.4 ± 6.3^{b}	93.4 ± 8.9^{a}
VI	Insulin	113.4 ± 3.7^{a}	462.4 ± 6.8^{b}	441.0 ± 9.3^{b}	374.4 ± 3.3^{b}	291.7 ± 6.0^{b}	222.4 ± 3.0^{b}

The values are expressed as mean ± SEM (n=5). Values in the same column with different superscript are significantly different at $p<0.05$. Values in the same column with the same superscript are not significant

Table-2. The Effect of n-hexane Extract of *Leptadenia hastata* on Body Weight

Group	Treatment	Body weight (g)					Difference between Day 28 -Day 1 (%)
		Day 1	Day 7	Day 14	Day 21	Day 28	
I	-	160.5 ± 2.9^{a}	160.3 ± 2.9^{a}	186.8 ± 3.6^{a}	187.1 ± 3.1^{a}	182.4 ± 3.0^{a}	13.7
II	Extract (200mg/kg)	150.0 ± 2.2^{a}	171.54 ± 1.4^{a}	197.2 ± 1.7^{a}	180.1 ± 2.6^{a}	177.1 ± 2.6^{a}	18.0
III	-	190.2 ± 1.4^{b}	133.8 ± 2.4^{b}	110.8 ± 2.4^{b}	123.6 ± 2.0^{b}	123.7 ± 1.9^{b}	-35.0
IV	Extract (100mg/kg)	143.5 ± 2.1^{b}	140.6 ± 2.0^{b}	140.6 ± 1.6^{b}	131.8 ± 1.2^{b}	129.0 ± 1.2^{b}	-10.1
V	Extract (200mg/kg)	135.6 ± 1.8^{b}	135.6 ± 1.8^{b}	130.9 ± 1.3^{b}	129.8 ± 1.2^{b}	131.5 ± 1.2^{b}	-3.0
VI	Insulin	138.2 ± 4.2^{b}	138.1 ± 3.4^{b}	136.5 ± 3.3^{b}	152.0± 3.0^{a}	153.8 ± 3.0^{b}	10.7

The values are expressed as mean ± SEM (n=5). The values are expressed as mean ± SEM. Values in the same column with different superscript are significantly different at $p<0.05$. Values in the same column with the same superscript are not significant.

4. Results

4.1. Effect on Fasting Blood Glucose

The anti-diabetic activity of the extract was evaluated by demonstrating the effect of the extract on fasting blood glucose and the results presented in Table 1. The extract demonstrated and produced a progressive reduction in fasting blood glucose level in diabetic rats which became significant on days 21 and 28 for rats that received 100mg/kg and 200mg/kg of extract respectively. This was comparable to the reduction produced by insulin (days 14, 21 and 28). However, no significant reduction was observed in the fasting blood glucose in normal rats.

4.2. Effect of n-Hexane Extract of Leptadenia Hastata on Body Weight

Table 2 presents the body weight changes in the diabetic and non-diabetic rats treated with either olive oil, extract or insulin. There was progressive increase in body weight of the non-diabetic rats (groups I and II). In contrast, diabetic non-treated rats (group III) had a progressive weight loss that amounted to a reduction of 35% of the initial weight on day 28. However, the reduction was significantly lower in diabetic rats treated with 100mg/kg (10.1%) and 200mg/kg (3.0%) of the extract by day 28.

5. Discussion

Weight loss is one of the clinical features of diabetes mellitus and may be due to the degeneration of the adipocytes and muscle tissues to make up for the energy lost as a result of frequent urination and over conversion of glycogen to glucose. Weight loss is a very serious issue in the management of diabetes mellitus [22]. The body weight changes serve as a sensitive indication of general health status of animals during the course of the experimental study [22, 23]. Treatment with n-hexane extract of *Leptadenia hastata* with 100mg/kg and 200mg/kg doses appeared to protect the diabetic rats from drastic body weight loss observed in diabetic untreated rats which may be due to protein sparing effect. This was also observed in a study carried out by [24], [25] who observed that plant extracts also reversed the weight loss in diabetic rats. The improvement in body weight gain in diabetic rats that ingested the extract may highlight the blood glucose homeostasis which in turn promotes the body weight gain of glucose by the liver, and decreased utilization of glucose in peripheral tissues [11, 26-30].

The elevated blood glucose level observed in diabetic rats was significantly reduced in the extract treated groups suggesting insulin stimulatory effects *of Leptadenia hastata*. The reduced glucose levels also suggests that the extract might exert insulin-like effect on peripheral tissues by either promoting glucose uptake, metabolism or by inhibiting hepatic gluconeogenesis [31] or by interfering with the absorption of glucose into the muscle and adipose tissue or by stimulating the regeneration of pancreatic beta cells [25].

The hypoglycemic activity could be due to the presence of phytochemicals, e.g. terpenoids which have been reported to have anti-diabetic properties as reported by Aliero and Wara [32], Bello, *et al.* [11], Dambatta and Aliyu [13], Garba, *et al.* [12].

6. Conclusion

The current study confirmed the use of *Leptadenia hastata* in the traditional system to treat diabetes. Further comprehensive and pharmalogical research investigations are required on this plant to determine the exact mechanism of the extract.

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