Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.elsevier.com/locate/apjtb

Document heading doi: 10.1016/S2221-1691(13)60127-6

Histological changes and antidiabetic activities of *Icacina trichantha* tuber extract in beta-cells of alloxan induced diabetic rats

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PEER REVIEW

Peer reviewer

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Comments

This study is good and will be benefits to the scientific communities. Further work on this plant is like to be stimulated if the paper is published.
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ABSTRACT

Objective: To investigate the antidiabetic, hypolipidaemic activities and histopathological changes of *Icacina trichantha* (*I. trichantha*) tuber extract in alloxan induced diabetic rats. **Methods:** In the present study, 80% methanol extract of *I. trichantha* tuber was tested on alloxan induced diabetic rats. They were randomly grouped into control (distilled water and glibenclamide) and experimental (200, 400 and 600 mg/kg body weight). Diabetes was induced by a single intraperitoneal injection of 160 mg/kg body weight of alloxan. Blood glucose levels were measured using blood glucose test strips with AccuCheck Advantage II glucometer at 1, 3, 6, and 24 h on the first day and 1 h after treatment on Day 7, 14 and 21. Blood samples were collected and centrifuged to separate serum for estimation of lipid profile and other biochemical parameters. Histopathological changes in diabetic rats pancreas were also studied after extract treatment. **Results:** Daily oral administration of *I. trichantha* tuber extract (200, 400, and 600 mg/kg body weight) and glibenclamide (2 mg/kg) showed beneficial effects on blood glucose level ($P<0.01$) as well as improving liver, kidney functions and hyperlipidaemia due to diabetes. The extract had a favourable effect on the histopathological changes of the pancreas in alloxan induced diabetes. **Conclusions:** *I. trichantha* tuber extracts posses antidiabetic activities as well as improve liver and renal profile and total lipids levels. *I. trichantha* tuber extracts also have favourable effects to inhibit the histopathological changes of the pancreas in alloxan induced diabetes.

KEYWORDS

Icacina trichantha, Diabetes mellitus, Glibenclamide, Alloxan monohydrate, Rats

1. Introduction

Diabetes mellitus is a group of syndromes characterized by a persistent elevation of fasting blood glucose above 200 mg/dL, due to insufficient or complete cessation of insulin synthesis or secretion and/or peripheral resistance to insulin action[1]. The latest World Health Organization (WHO) publication (global burden of disease) estimates the prevalence of diabetes in adults to be around 173 million[2].

Despite considerable progress in the treatment of diabetes using oral hypoglycaemic agents, search for newer drugs continues because the existing synthetic drugs have several limitations. In recent times, there has been renewed interest in plant remedies for which WHO has recommended attention[3].

Icacina trichantha (Pflamzenfen) (*I. trichantha*), Icacinaceae, which is known as urumbia or eriagbo (meaning to induce vomiting when eaten) among the Ibo speaking tribes of eastern Nigeria, or gbegbe (meaning to carry away) by the Yoruba speaking tribes of western Nigeria[4,5], has played a significant role in traditional medicine among the natives of Nigeria. The tuber is the most widely used part of the plant; it is sometimes as large as yam and eaten during famine while the leaves are

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Foundation Project: Financially supported by the Institute of International Education (IIE) of the United States Department of State's Bureau for Educational and Cultural Affairs (Grant No. 15120356).

Article history:
Received 7 Jun 2013
Received in revised form 17 Jun, 2nd revised form 19 Jun, 3rd revised form 23 Jun 2013
Accepted 15 Jul 2013
Available online 28 Aug 2013

used for wrapping processed oil bean seeds[6]. *I. trichantha* is reportedly used as medicine in rural communities in Nigeria[7]. This is supported by the fact that it is regarded as a major handy household medicine for emergency and first aid treatment; hence, virtually all households have the macerated tuber in ethanol which is stored in corked bottles. Tubers of *I. trichantha* have been used by traditional herbalists to treat constipation, poisoning, malaria and induce emesis[5].

The antidiabetic potentials of methanolic leaf extracts of *I. trichantha* was previously reported[8], but our focus, considering the traditional use of this plant in diabetes treatment, is to investigate the antidiabetic, hypolipidaemic and histopathological effects of *I. trichantha* tuber extract in alloxan induced diabetic rats.

2. Materials and methods

2.1. Plant material collection and extract preparation

Fresh tubers of *I. trichantha* were collected in the month of June, 2011 from Orba village in Nsukka local government area of Enugu state, Nigeria. The tubers were identified by Mr. Ozioko A., a botanist with BDCP laboratories, Nsukka; voucher specimen (UNN/FVM 456) was preserved in the pharmacology laboratory for reference purposes. The tubers were washed, sliced into small pieces and dried under mild sunlight. The dried tuber slices were pulverized to a coarse powder. A bulk extraction was carried out using about 1.5 kg of the pulverized material. This was done by soaking the plant material in 80% methanol for 48 h. They were shaken at regular intervals of 2 h. The extracts were filtered using No. 1 Whatman filter paper and concentrated *in vacuo* to dryness using a rotary evaporator and kept at 4 °C until use.

2.2. Experimental animals

Male wistar albino rats (250-300 g) procured from the laboratory animal unit of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka, were used for the experiment. The rats were kept under standard environmental conditions of temperature (25 °C), relative humidity (45%-55%), dark/light cycle (12 h), and were fed with standard feed pellets (Feed Masters®) and tap water *ad libitum*. The rats were fasted for 12 h before the experiment, but were allowed free access to water; all the studies were conducted in accordance with the Animal Ethical Committee Guidelines of the University.

2.3. Experiment and design

Hyperglycemia was induced by a single intraperitoneal injection of 160 mg/kg body weight alloxan monohydrate (Sigma Chem. Co., St Louis, USA), freshly dissolved in distilled water immediately before use to overnight feed-fasted albino rats. After 10 d, rats with fasting blood glucose of 6.0 mmol/dL or more were considered diabetic and were used for the study. Rats were divided into six groups of six rats each. Group I served as negative control (non diabetic) receiving distilled water (10 mL/kg, *per os*); Group II served as positive control (diabetic but not treated) and also received distilled water at 10 mL/kg orally; Group III received glibenclamide at 2 mg/kg body weight; while Groups IV, V and VI received *I. trichantha* tuber extract at 200, 400, and 600 mg/kg *per os* respectively. The animals were treated once daily and fasting blood glucose level measured. Blood samples were collected by a snip-cut at the tip of the tail under mild anesthesia and blood glucose level was measured using an auto-analyzer - AccuCheck Advantage II glucose kit at 1, 3, 6, and 24 h on the first day, and 1 h after treatment on Day 7, 14 and 21. Blood samples were collected and centrifuged to separate serum for estimation of lipid profile and other biochemical parameters[9].

Total cholesterol, HDL, LDL, triglycerides were analyzed from serum. Total cholesterol was estimated according to Liebermann Burchard Reaction Method as reported[10]. LDL was estimated indirectly by Friedwald's method[11]. Triglycerides were determined using Hantzsch condensation method[12]. Alkaline phosphatase (ALP), serum alanine transaminase (ALT) and serum aspartate transaminase (AST) were measured by autoanalyser (Erba Chem 7, Mannheim, Germany) using Erba diagnostic kits[13,14].

2.4. Phytochemical analysis

Phytochemical screening was carried out according to the methods described by Trease and Evans[15].

2.5. Acute toxicity studies

The acute toxicity of the extract was conducted by the method Lorke as modified by Deora *et al*[16]. Rats fasted for 12 h were randomly divided into drug treated 'test' groups and vehicle treated 'control' group, total making up six groups of six rats per cage. *I. trichantha* tuber extract 200, 400, 800, 1 000 and 1 500 mg/kg body weight was separately administered orally to the rats in each of the test groups. The rats were observed for behavioral changes over a period of 48 h and the number of mortality caused by the extract within this period was also noted.

2.6. Histopathology of pancreas

The whole pancreas from each animal was removed after sacrificing the animal and washed on ice cold saline immediately. A portion of pancreatic tissue was fixed in 10% neutral formalin fixative solution for histological studies. After fixation tissues were embedded in paraffin, solid sections were cut at 5 μm and the sections were stained with haematoxylin and eosin[17].

2.7. Statistical analysis

All the data were presented as mean±SEM. The differences between groups were evaluated by one-way analysis of variance (ANOVA) followed by the Dunnette multiple comparisons test. $P<0.01$ was considered to be significant.

3. Results

3.1. Phytochemical test

Phytochemical studies indicate that the tuber extracts of *I. trichantha* contained alkaloids, terpenes, flavonoids, glycosides, steroids, saponins and tannins (Table 1).

Table 1
Phytochemical composition of methanol extract of *I. trichantha* tuber.

Component	Availability
Alkaloids	strong response
Flavonoids	strong response
Tannins	weak response
Saponins	strong response
Carbohydrates	strong response
Sterols and terpenes	weak response
Glucose	strong response

3.2. Acute toxicity

Acute toxicity studies revealed the non-toxic nature of the *I. trichantha* tuber extract as the treated rats appeared normal and did not display any significant changes in behavioral or neurological responses up to 1 500 mg/kg body weight of the extract. There was no mortality or toxicity reaction at any of the doses until the end of the study.

3.3. Antihyperglycemic and antihyperlipidemic activity

Administration of alloxan (160 mg/kg, intra peritoneal) led to elevation of fasting blood glucose levels, which was maintained over a period of 3 weeks, and daily treatment with various concentration of *I. trichantha* tuber extract led to a decrease in blood glucose levels. The serum glucose level of the *I. trichantha* tuber extract treated groups were significantly ($P<0.01$) reduced especially as from the 6 h onward when compared to the diabetic control group, but 600 mg/kg body weight of extract having the highest depletion power from (14.95±1.40) to (2.00±0.10) mmol/L on Day 21 (Table 2). Table 3 shows the data for total bilirubin, total protein, cholesterol, triglycerides, HDL, and LDL respectively. The total bilirubin level of the 200 mg/kg tuber extract treated group [(0.98±0.03) mg/dL] showed statistically significant ($P<0.01$) compared to diabetic control group [(3.70±0.09) mg/dL] on Day 21 (Table 4). Similar results were noticed in the case of total protein, HDL and LDL on Day 21.

Table 2
Effects of *I. trichantha* extract on blood glucose level in alloxan induced diabetic rats.

Treatment (mg/kg)	Blood glucose level (mmol/L)						
	1 h	3 h	6 h	24 h	Day 7	Day 14	Day 21
Normal control	3.35±0.32^{a}	3.30±0.32^{a}	3.60±0.10^{a}	2.80±0.18^{a}	3.20±0.30^{a}	2.70±0.21^{a}	2.80±0.21^{a}
Diabetic control	14.95±1.40^{bc}	14.50±1.46^{b}	14.40±1.33^{b}	14.50±1.60^{b}	15.40±1.23^{c}	15.70±1.23^{b}	16.10±1.42^{b}
Glibenclamide 2	11.84±0.99^{b}	7.60±0.72^{c}	3.60±0.35^{a}	3.70±0.35^{a}	3.20±0.24^{a}	2.60±0.23^{a}	2.60±0.34^{a}
extract 200	18.30±1.85^{c}	14.90±1.42^{b}	12.50±1.19^{b}	11.60±1.17^{c}	7.80±0.80^{d}	6.10±0.72^{c}	5.20±0.46^{c}
extract 400	15.63±1.43^{bc}	11.40±0.89^{d}	9.20±1.08^{c}	8.40±0.89^{d}	5.80±0.38^{b*}	3.70±0.29^{a*}	2.70±0.55^{a*}
extract 600	13.98±0.56^{b}	9.90±0.26^{cd}	5.00±0.38^{a}	5.00±0.24^{a}	3.90±0.14^{ab*}	2.60±0.56^{a*}	2.00±0.10^{a*}

Values are mean±SEM, $n=6$. $^{*}P<0.01$.

Table 3
Effects of *I. trichantha* extract on serum biochemical parameters in alloxan induced diabetic rats.

Treatment (mg/kg)	Serum biochemical parameters				
	Creatinine (mg/dL)	Urea (mmol/L)	AST (IU/L)	ALT (IU/L)	ALP (μmol/L)
Normal control	0.47±0.01^{a}	22.34±0.15^{a}	40.29±3.62^{a}	49.39±1.23^{a}	48.04±0.61^{a}
Diabetic control	0.92±0.02^{b}	32.15±0.37^{b}	128.38±0.98^{b}	153.99±1.24^{b}	125.33±0.94^{b}
Glibenclamide 2	0.68±0.01^{c}	14.20±0.15^{c}	64.02±0.58^{c}	83.98±0.63^{c}	61.24±2.72^{c}
extract 200	0.85±0.01^{d}	17.63±0.46^{d}	97.07±2.53^{d}	107.07±2.11^{d}	103.05±2.35^{d}
extract 400	0.78±0.04^{e}	16.30±0.13^{e*}	71.00±0.46^{e*}	96.92±0.65^{e*}	84.60±1.19^{e*}
extract 600	0.64±0.01^{c*}	20.95±0.39^{f*}	63.41±0.23^{c*}	82.44±0.24^{c*}	61.53±0.53^{c*}

Values are mean±SEM, $n=6$. $^{*}P<0.01$.

Table 4
Effects of *I. trichantha* extract on lipid profile of alloxan induced diabetic rats.

Treatment (mg/kg)	Lipid parameters (mg/dL)					
	HDL	LDL	Tbilirubin	Tprotein	Cholesterol	Triglyceride
Normal control	42.83±1.50^{a}	52.92±0.90^{a}	1.19±0.05^{a}	7.30±0.07^{a}	81.52±0.22^{a}	88.32±0.32^{a}
Diabetic control	20.02±0.42^{b}	177.69±1.16^{b}	3.70±0.09^{b}	3.71±0.46^{b}	127.68±0.50^{b}	130.80±0.39^{b}
Glibenclamide 2	34.05±0.66^{c}	55.48±0.59^{c}	1.15±0.08^{ac}	6.30±0.12^{c}	81.79±0.60^{a}	86.78±0.36^{c}
extract 200	25.92±0.18^{d}	74.35±0.91^{d}	0.98±0.03^{c}	4.50±0.13^{d}	121.39±0.34^{c}	121.44±0.52^{d}
extract 400	33.75±0.71^{e*}	44.14±0.89^{e*}	2.42±0.03^{d}	5.30±0.03^{e}	91.68±0.76^{d}	97.91±0.40^{e*}
extract 600	41.95±0.38^{a*}	55.48±0.32^{c*}	2.11±0.05^{e}	6.50±0.11^{c*}	82.01±0.35^{a*}	90.71±0.37^{f*}

Values are mean±SEM, $n=6$. $^{*}P<0.01$.

3.4. Histopathology of pancreas

The islets of langerhans in normal group were unevenly scattered in the pancreatic tissue and they were often quite abundantly distributed and were of varying sizes in the sane lobule of pancreas (Figure 1). The acinar cells which stained strongly were arranged in lobules with prominent nuclei. The islets cells were seen embedded within the acinar cells and surrounded by fine capsule. Pancreatic islets of diabetic control rats revealed significant reduction in size and number of the acinar cells around the islets though seemed to be in normal proportion does not look classical (Figure 2).

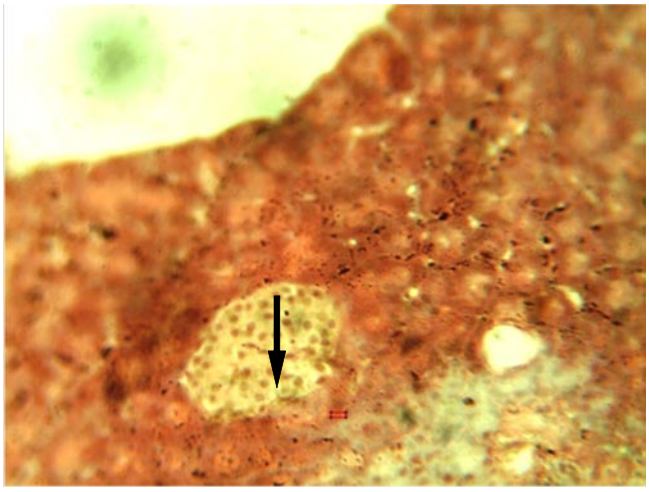

Figure 1. Photo micrograph of rat with normal pancreas showing normal pancreatic islet cells (arrow) (H&E ×400).

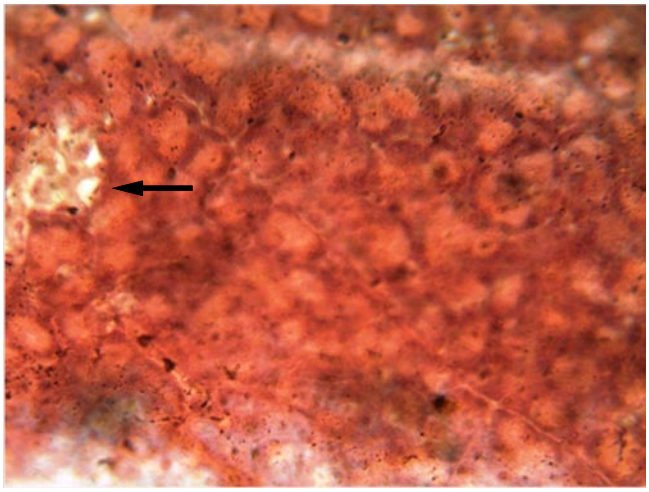

Figure 2 Photomicrograph of pancreas of alloxan induced diabetic rats showing reduced size and number of islets cells (arrow) (H&E ×400).

This showed that islets were damaged, shrunken in size and infiltration of lymphocytes was observed. The islets cells of the glibenclamide treated group seen to be normal in position, but few in numbers comparable to normal group (Figure 3). The size of cell was to be back in normal position after 21-day treatment of glibenclamide. The islet cells were compactly arranged, with negligible intercellular space. In the treated group of 200 mg/kg body weight, the islets cells were seen in fewer numbers (Figure 4).

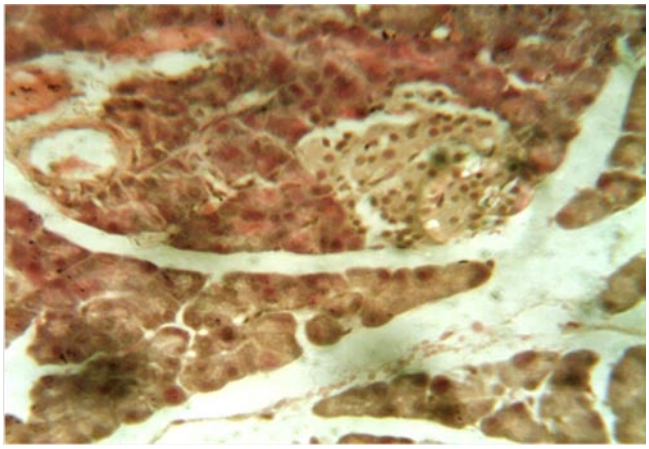

Figure 3. Photomicrograph of alloxan induced diabetic rats treated with glibenclamide showing full pancreatic islet cells (H&E ×400).

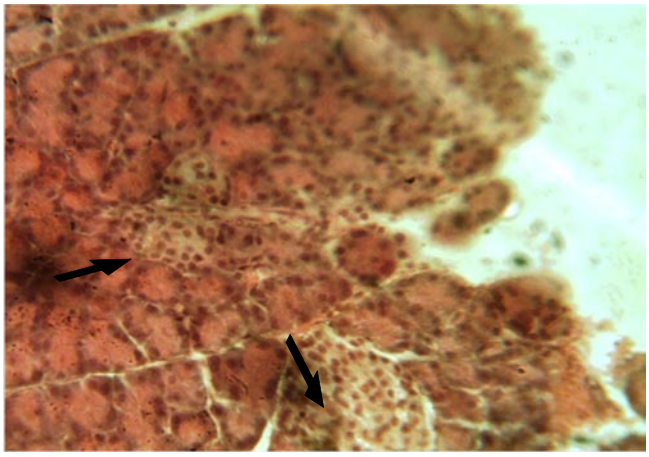

Figure 4. Photomicrograph of alloxan induced diabetic rats treated with 200 mg/kg of extract showing healthy pancreatic islet cells (arrows) (H&E ×400).

The size of the cell was shrunken with architectural disarray and hydrolysis compared to diabetic control group. The islets cells of 400 mg/kg body weight extract in treated group were shown to be in normal position (Figure 5).

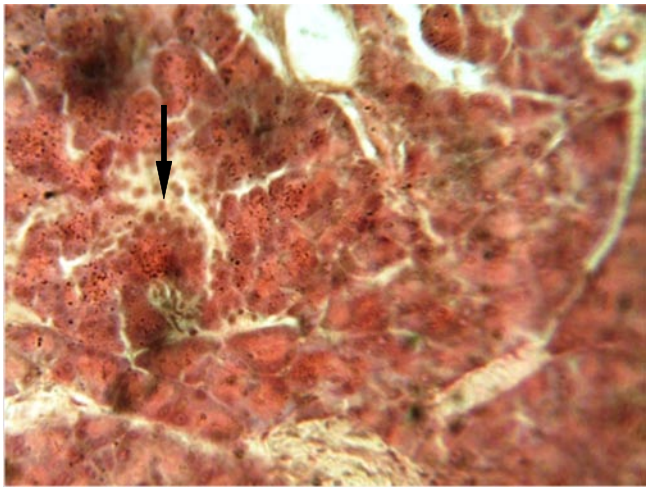

Figure 5. Photomicrograph of alloxan induced diabetic rats treated with 400 mg/kg of extract. Note the normal islet cells (arrow) (H&E × 400).

The islets of 600 mg/kg body weight extract in treated group were present with a large proportion of islets cells, though

with a smaller volume compared to normal group (Figure 6). The treated group of 600 mg/kg body weight extract showed better restoration of beta cells in comparison with 200 mg/kg and 400 mg/kg body weight tuber extract of *I. trichantha*.

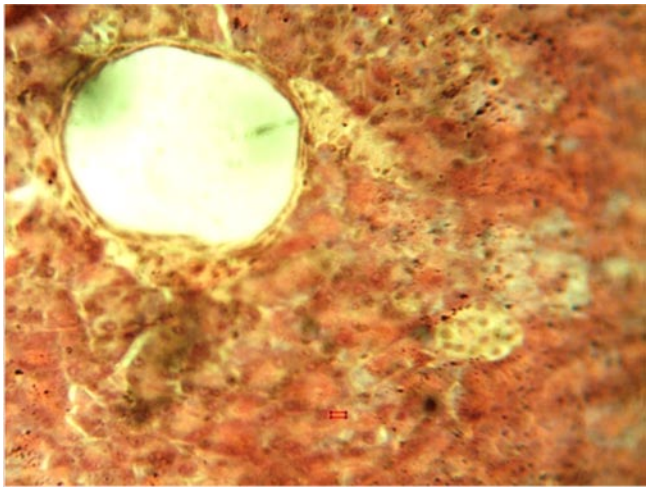

Figure 6. Photomicrograph of alloxan induced diabetic rats treated with 600 mg/kg of extract with no visible pathologic lesion (H&E ×400).

4. Discussion

The major characteristics of diabetes mellitus are polydipsia, polyuria, polyphagia, weight loss, muscle weakness and hyperglycemia[18]. Alloxan, a beta cytotoxin, destroys beta-cells of islets in the langerhans of pancreas resulting in a decreased endogenous insulin secretion leading to a decreased utilization of glucose by body tissues[19]. It results in the elevation of blood glucose level, decreases protein content, and increases levels of cholesterol and triglycerides[20].

Studies in the last several decades have shown that plant and plant based therapies have a potential to control and treat diabetes and its complications[21,22]. They are better than allopathic drugs, which have a lot of adverse side effects[23]. For testing antidiabetic potential of plants, alloxan and streptozotocin induced hyperglycemia in rats is considered to be a good preliminary screening model and is widely used[24]. Alloxan is well known for its selective pancreatic islet cell toxicity and has been extensively used to induce diabetes mellitus in animals.

The plant *I. trichantha* used in this study is widely used in both ayurveda and homeopathic medicine. *I. trichantha* is commonly used by many traditional healers in most of the herbal preparations for diabetes, antiprotozoan and as aphrodisiac[5]. Therefore, the present study was to demonstrated the efficacy of *I. trichantha* in reducing blood glucose level as well as determining the restoration of beta cells of the pancreas. This study also evaluated biochemical parameters such as serum total bilirubin, total protein, triglycerides, cholesterol, LDL, HDL and liver and renal enzymes in experimental diabetes caused by alloxan in rats. This study indicated that *I. trichantha* tuber extracts have good antidiabetic activity. Among different tuber extract concentrations, 400 mg/kg and 600 mg/kg were found to cause a greater decline ($P<0.01$) on Day 7, 14 and 21 which showed statistically high significance. Three weeks of daily treatment with various extract concentration of *I. trichantha* led to a dose-dependent decline in blood sugar levels. They can also improve the conditions of diabetes mellitus as indicated by parameters like triglycerides, serum LDL and HDL. The 400 mg/kg extract and 600 mg/kg extract showed some improvement in serum triglycerides levels on Day 21 when compared to diabetic control group which were found to be statistically significant ($P<0.01$).

Liver enzyme (AST, ALT and ALP) levels were elevated in diabetic rats; the elevated serum levels of these enzymes were significantly reduced by *I. trichantha* treatment. Some diabetic complications such as increased gluconeogenesis and ketogenesis may be attributed to elevation of levels of these enzymes[25]. *I. trichantha* also improved renal function in diabetic rats by reducing serum urea and creatinine; this implies generally that *I. trichantha* normalizes the function of vital organs of rats.

The possible mechanism by which *I. trichantha* brings about its hypoglycaemic action may be due to polyphenols and flavonoids present in this plant which stimulate the receptor on the cytoplasm side of the membrane, a protein phosphokinase of the tyrosine-specific type. It phosphorylates itself with the aid of ATP, undergoes a conformational change, and activates via a G-proteins, which liberates several second messenger further activates protein P-kinases which open a Ca^{2+} influx gives insulin like effect[26]. Another possible mechanism may be due to alkaloids causing inhibition of mitochondrial function that increases the AMP/ATP ratio, which could explain the activation pathway in the treatment of diabetes[27]. The third most important probable mechanism of action may be by potentiating the insulin effects of plasma by increasing either the pancreatic secretion of insulin from the existing beta cells or by its release from the bound form. Though the exact mechanism is unknown, we are assuming that various active constituents of this plant help to improve treatment of diabetes.

In conclusion, the results of this study shows that oral administration of the methanolic tuber extract of *I. trichantha* reduces blood glucose and serum lipids which could be due to improvement in insulin secretion by recovery of pancreatic beta-cells. Alkaloids and flavonoids have also been found to be beneficial in controlling diabetes and many other diseases as evident from earlier studies. It is therefore concluded from the data that *I. trichantha* tuber extract possess antidiabetic activity and it may prove to be effective for the treatment of type 1 diabetes. This also validates the traditional use of this plant tuber for the management of diabetes mellitus.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

The authors hereby acknowledge the Institute of International Education (IIE) of the United States Department of State's Bureau for Educational and Cultural Affairs for providing funding for this research through its Fulbright Program (Grant No. 15120356).

Comments

Background

Plant bioactive agent for a global health condition such as diabetes is of particular interest in Africa where the incidence of the diabetes mellitus is increasing due to medical awareness. The acceptance of traditional medicine by WHO is the more reason for investigation into medicinal plant of health significance. On this premises, this study was carried to screen for antidiabetic effects of a medicinal herb, *I. trichantha*.

Research frontiers

Biological activity of ethno botanicals is the beginning of drug discovery, and this paper reports clinical parameters associated with diabetes to justify the traditional claim of *I. trichantha* as an antidiabetic herb. This is innovative.

Related reports

There are related reports but not on the tuber of this plant. The only closely related work is on the leaf of this plant and that was on mice by Ezeigbo *et al*. This is the first time report on antidiabetic effect of the tuber of this plant.

Innovations and breakthroughs

For the first time this paper reports the antidiabetic effect of *I. trichantha* and histological changes. This is something new.

Applications

This work has given supportive reasons for the use of this plant material in the treatment of diabetes by the traditional medicine practitioners. It will also open a new area of research focus for antidiabetic drug discovery.

Peer review

This study is good and will be benefits to the scientific communities. Further work on this plant is like to be stimulated if the paper is published.

References

[1] Ortiz-Andrade, RR, Rodríguez-López V, Garduño-Ramírez ML, Castillo-España P, Estrada-Soto S. Anti-diabetic effects on alloxanized and normoglycemic rats and some pharmacological evaluations of *Tournefortia hartwegiana*. *J Ethnopharmacol* 2005; **101**(1-3): 37-42.

[2] Wild S, Roglic G, Sicree GA, King H, editors. Global burden of diabetes mellitus in the year 2000[C]. Geneva: WHO, 2003.

[3] World Health Organization. Expert committee on diabetes mellitus: second report[R]. Geneva: WHO, 1980: 1-80.

[4] Dalziel JM. *Icacina trichantha Oliv. family Icacinaceae: The useful plants of west tropical Africa*. London: Crown Agents Press; 1937, p. 291.

[5] Asuzu IU, Abubakar II.- The effects of *Icacina trichantha* tuber extract on the nervous system. *Phytother Res* 1995; **9**(1): 21-25.

[6] Ude NE, Nwaehujor CO. Antioxidant and hepatoprotective activities of ethylacetate leaf extract of *Icacina trichantha* on paracetamol-induced liver damage in rats. *Cont J AnimVet Res* 2011; **3**: 11-15.

[7] Timothty O, Idu M. Preliminary phytochemistry and *in vitro* antimicrobial properties of aqueous and methanol extracts of *Icacina trichantha* Oliv. leaf. *Int J Med Arom Plants* 2011; **1**: 1-5.

[8] Ezeigbo II. Antidiabetic potential of methanolic leaf extracts of *Icacina trichantha* in alloxan induced diabetic mice. *Int J Diabetes Dev C* 2010; **30**: 150-152.

[9] Harold V, Alan HG, Janet RM, Donald MM. *Varley's practical clinical biochemistry*. 6th ed. New Delhi: Heinemann Medical Books; 2006.

[10] Sangameswaran B, Ramdas P. Antihyperglycaemic and antihyperlipidaemic activities of *Amaranthus spinosus* Linn extraction on alloxan induced diabetic rats. *Malays J Pharm Sci* 2010; **8**(1): 13-22.

[11] Friedwald WT, Levy RI, Freidrickson DS. Estimation of LDL-Cholesterol in plasma without preparation of ultra centrifuge. *Clin Chem* 1972; **18**: 449-502.

[12] MacDonald RP. *Standard methods of clinical chemistry*. New York: Academic Press; 1970, p. 215-222.

[13] Donga S, Shukla VJ, Ravishankar B, Ashok BK, Mishtry IU. Chronic toxicity study of *Butea monosperma* (Linn) kuntze seeds in albino rats. *Ayu* 2011; **32**(1): 120-125.

[14] Kumar S, Malhotra R, Kumar D. Antidiabetic and free radicals scavenging potential of *Euphorbia hirta* flower extract. *Indian J Pharm Sci* 2010; **72**(4): 533-537.

[15] Trease GE, Evans MC. *Textbook of pharmacognosy*. 16th ed. Philadelphia: W. B. Saunders Co.; 2009, p. 194-353.

[16] Deora PS, Mishra CK, Mavani P, Ashar R, Shrivastava B, Rajesh KN. Effective alternative methods of LD_{50} help to save number of experimental animals. *J Chem Pharm Res* 2010; **2**(6): 450-453.

[17] Strate T. Micro circulatory function and tissue damage is improved after the repeated injection of bovine haemoglobin in server acute rodent pancreatitis. *Pancreas* 2005; **30**(3): 254-259.

[18] Schnell O, Standl E. Impaired glucose tolerance, diabetes, and cardiovascular disease. *Endocr Pract* 2006; **12**(Suppl 1): 16-19.

[19] Szkudelski T. The mechanism of alloxan and streptozotocin action in B cell of the rat pancreas. *Physiol Res* 2001; **50**: 536-546.

[20] Dhanabal SP, Raja MK, Ramanathan M, Suresh B. Hypoglycemic activity of *Nymphaca stellata* leaves ethnolic extract in alloxan induced diabetic rats. *Fitoterapia* 2007; **78**: 288-291.

[21] Marles RJ, Farnsworth NR. Antidiabetic plants and their active constituents. *Phytomedicine* 1995; **2**(2): 137-189.

[22] Grover JK, Vats V, Rathi SS, Dawar R. Traditional Indian anti-diabetic plants attenuate renal hypertrophy, urine volume and albuminuria in streptozotocin induced diabetic mice. *J Ethnopharmacol* 2001; **76**: 233-238.

[23] Grover JK, Yadav S, Vats V. Medicinal plants of India with anti-diabetic potential. *J Ethnopharmacol* 2002; **8**: 81-100.

[24] Etuk EU. Animal models for studying diabetes mellitus. *Agric Biol J N Am* 2010; **1**: 130-134.

[25] Ghosh S, Suryawansi SA. Effect of *Vinca rosea* extracts in treatment of alloxan diabetes in male albino rats. *Indian J Exp Biol* 2001; **39**: 748-759.

[26] Song J, Kwon O, Chen S, Daruwala R, Eck P, Levine M. Flavonoid inhibition of sodium-dependent vitamin C transporter 1 (SVCT 1) and glucose transporter isoform 2 (GLUT 2), intestinal transporters for vitamin C and glucose. *J Biol Chem* 2002; **18**: 15252-15260.

[27] Sharma B, Balo MC, Roy SN. Antidiabetic potential of ulhabid rich fraction from *Cappais decidus* on diabetic mice. *J Ethanopharmacol* 2010; **127**: 457-462.