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Research Article

Investigation of the Anti-Hyperglycaemic Effect of the Leaf Extracts of *Solanum Dulcamara* in Diabetic Rats

DC NWACHUKWU*[1], CN OKWUOSA[2], PU ACHUKWU[2], NKIRU AZUBIKE[2], GE EZE[2]

[1]Department of Physiology, College of Medicine, University of Nigeria, Enugu Campus

[2]Department of Medical Laboratory Sciences, Faculty of Health Sciences & Technology, College of Medicine, University of Nigeria, Enugu Campus

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ABSTRACT

The effect of the leaf extracts of *Solanum dulcamara* on blood sugar level of diabetic rats was investigated using sixty (60) albino wistar rats and twenty (20) albino mice. Diabetes mellitus was induced in experimental animals by the intra-peritoneal administration of alloxan monohydrate (8% w/v). Phytochemical tests revealed the presence of alkaloids, flavonoids, resins, glycosides, saponins, & carbohydrates. Results of acute toxicity tests (LD_{50}) indicated an intra-peritoneal LD_{50} of 154.91mg/kg and an oral LD_{50} of 2720mg/kg. Results showed that oral administration of 50mg/kg and 100mg/kg of the methanol extract of *Solanum dulcamara* (MESD) significantly reduced the blood glucose of diabetic rats at when compared with the untreated diabetic control and the 0 hour glucose value ($p \cdot 0.05$; $p<0.001$). The antihyperglycaemic action started 2 hours post-drug administration and was sustained throughout the duration of the experiment. The aqueous extract of *Solanum dulcamara* (AESD) at 100mg/kg reduced blood sugar significantly at 1 and 2 hours when compared with the 0 hour value ($p \cdot 0.05$; $p<0.001$).This reduction however, did not persist as the glycaemic control was lost after 4 hours. 50mg/kg AESD did not produce anti- hyperglycaemic effects on diabetic rats when compared with control and 0 hour value ($p>0.05$). The methanol and aqueous extracts of *Solanum dulcamara* possess significant anti-hyperglycaemic property.

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INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder of carbohydrate metabolism in which glucose is underutilized producing hyperglycaemia [1]. It is a syndrome of impaired carbohydrate, fat, and protein metabolism caused by lack of insulin secretion or decreased sensitivity of body tissues to insulin [2]. The incidence of Diabetes mellitus is on the increase and about 30 million people around the world are affected [3,4]. Treatment of DM based on nutrient interaction has been described by several workers. These workers inferred that different therapeutic regimes can inhibit certain metabolic processes leading to decrease in blood glucose. Examples are agents that limit the availability of fatty acids [5], agents that inhibit gluconeogenesis[6], and agents that enhance lipolysis while promoting fatty acid oxidation in a futile cycle that does not yield metabolic energy and stimulate peripheral glucose utilization[7]. Peroxisome proliferator-activated receptors (PPARs) are in the latter group and many of them have serious deleterious effects on the liver, especially the PPAR-γ agonist. Despite efforts in the management of this dreadful condition with allopathic medicinal agents, diabetes mellitus still ravages mankind at an alarming rate. Most of the drugs used in allopathic medical practice are not devoid of side effects [8]. Several medicinal plants have been shown to possess anti-hyperglycemic and hypoglycemic properties [9-16].

Solanum dulcamara belong to the family Solanaceae and genus Solanum. It has been traditionally used in many herbal remedies as stimulant, expectorant, diuretic, and detoxifier.

Author for Correspondence:
Email: danychukwu@yahoo.com

In the south eastern part of Nigeria it is claimed, in folklore, to be efficacious in the management of diabetes mellitus. However, this claim has not been validated scientifically to the best of our knowledge. In Nigeria, many plants are used traditionally for the management of diabetes mellitus. The anti-hyperglycemic effect of *Solanum dulcamara* is not well documented. Thus, the role of *Solanum dulcamara* in diabetes treatment shall be investigated with a view to ascertaining the validity or otherwise of its use as an anti-hyperglycaemic agent in folk medicine and also contribute to the on-going efforts all over the world towards combating the disease with drugs that cheap, affordable and are less deleterious to the body.

MATERIALS & METHODS

Animals

Sixty albino Wistar rats weighing 180-250g were recruited for this study. Twenty albino mice weighing 17-30g were used for the acute toxicity study. They were obtained from the Animal House of the College of Medicine, University of Nigeria, Enugu Campus. These animals were kept in clean gauzed cages and acclimatized for 2 weeks under standard conditions of temperature (25ºC± 3ºC) and a 12:12 hr light/dark cycle. They were fed with commercially available rat pellets (Guinea Feed®) and clean water *ad libitum*.

Plant Collection and Extraction

The leaves of *Solanum dulcamara* were obtained from Ogige market, Nsukka in Nsukka LGA of Enugu state, Nigeria. The leaves were identified by Dr. F. Onyishi of the Herbarium section, Department of Botany, University of Nigeria, Nsukka. A voucher specimen no UNH/56C was opened at the Herbarium for future reference. The leaves were air-dried under a shade and crushed into powder using a grinding machine.

Methanol Extraction

800g of powdered leaves of *S. dulcamara* was put in a container and 2.5 litres of 80% methanol was added to it and the container was well stoppered and shaken intermittently for a period of 48hrs. The filtrate was evaporated to dryness on a rotary evaporator (Model type 349/2 Corning Ltd) and the residue stored in the refrigerator (4± 2°C) until required. The methanol extract (ME) gave a yield of 14% W/W.

Aqueous Extraction

The method of extraction in Nsukka community, Enugu State, South East, Nigeria was used. 400g of fresh leaves were macerated in 200mls of distilled water and allowed to stand for 48 hours. The homogenate was strained through muslin. The extractive value of the aqueous extract (AE) was 100mg/ml.

Methanol and aqueous extractions were chosen to determine the lipid and water solubility respectively of the hypoglycaemic agent(s) present in the leaf of *solanum dulcamara.*

Pharmacological Studies

Acute Toxicity Test (LD_{50})

The intra-peritoneal and oral LD_{50} of the extract was determined in mice with the method of *Lorke* [17], 1983.

Phytochemical Analysis

This was carried out based on the procedures outlined by *Trease and Evans* [18], 1983.

Experimental Induction of Diabetes Mellitus

The rats were fasted for 24hrs but had access to portable water. They were then injected with alloxan monohydrate (freshly prepared as an 8% solution in saline) -120mg/kg intraperitoneally as described by Akah and Okafor [19]. After four days, animals with fasting blood sugar of 300mg/dl and above were selected for the study; thirty (30) rats were selected for this study. All animals were handled according to Institutional and International ethical guidelines for experiments involving the use of animals [20].

Experimental Design

The diabetic rats were divided into six groups (A – F) of 5 rats per group. On the 6th day post-alloxan administration, a 12hr fasting blood sugar (Fbs) was determined in all the rats. This was designated the 0 hour sample. Rats in groups A, B, & C received 50mg/kg body weight MESD,100mg/kg body weight MESD, 0 .07mg/kg b.w glibenclamide respectively while groups D and E received 50mg/kg b.w AESD and 100mg/kg b.w AESD respectively. Group F animals received only Vehicle (3% dimethylsulphoxide -5ml/kg) and served as negative control. All administration was by oral route (p.o). Blood glucose levels were determined for all the animals in each group at fixed interval of 1, 2, 4, 8, and 24 hours via the tail vein. Blood glucose was measured using the One Touch-ultra blood glucose monitoring system (LifeScan, California, USA).

Statistical Analysis

Results were expressed where appropriate as Mean ± SEM. Differences between means was determined using the univariate ANOVA with repeated measures followed by Tukey's multiple comparison. Results were considered significant at $P< 0.001$ and $P< 0.05$.

RESULTS

Preliminary phytochemical analyses revealed the presence of abundant amount of alkaloids, moderate amounts of carbohydrates, glycosides, and resins, and small amounts of flavonoids and saponins. Terpenoids, steroids, and tannins were absent.

Acute toxicity tests showed that *Solanum dulcamara* leaf extracts had an intraperitoneal LD_{50} of 154.91mg/kg and an oral LD_{50} of 2720mg/kg body weight in mice.

Administration of two cumulative doses (50 and 100mg/kg) of the methanol extracts (MESD) to diabetic rats resulted in significant reduction in blood glucose level when compared with the 0 hour values and the untreated diabetic control respectively ($P< 0.001$). This reduction started at 2 hrs for the 100mg/kg dose and persisted for 24hrs. Diabetic rats treated with the aqueous extracts (100mg/kg) showed significant reduction in blood glucose concentration when compared with the 0 hour value and the untreated diabetic control repectively ($P< 0.001$). The reduction was observed at 2 and 4 hours. However, glycaemic control was lost at 8 hours (Table 1). The 50mg/kg AESD did not produce glucose lowering effect in the acute study. Glibenclamide (0.07mg/kg body weight) did not cause significant reduction in blood glucose in the rats ($p>0.05$).

DISCUSSION

The aqueous and methanol extracts of *Solanum dulcamara* is used in folklore medicine for the treatment of diabetes mellitus and as a stimulant, detoxifier, expectorant, and diuretic. There is evidence that the mechanism of action of alloxan induced diabetes mellitus involves the degeneration of the islet cells of the pancreas. This degeneration is due to the accumulation of cytotoxic free radicals [21]. Alloxan is concentrated in the liver and in the islet cells after administration from where it is reduced to dialaric acid which undergoes oxidation back to alloxan followed by the generation of superoxide (0_2^-), hydrogen peroxide (H_20_2), and hydroxyl radicals by fenton type reaction. The action of these free radicals is reversed in the liver due to the action of antioxidant enzymes like superoxide dismutase, catalase, and glutathione peroxidase. These enzymes are deficient in the islet cells of the pancreas leading to their destruction by alloxan administration [21].

In the present study, the aqueous and methanol extracts of *Solanum dulcamara* effectively decreased the blood glucose in alloxan-induced diabetic rats. The action of *Solanum dulcamara* extracts was dose dependent. This reduction was superior to that of glibenclamide- a sulphonylurea anti-hyperglycemic agent. The precise mechanism of action of this plant was not elucidated in this work. However, preliminary phytochemical analysis revealed the presence of abundant amount of alkaloids, moderate amounts of carbohydrates, glycosides, resins, and small amount of saponins and flavonoids. Most plants that contain glycosides, alkaloids, terpenoids, flavonoids, carotenoids, etc., are frequently implicated as having antidiabetic effect [22]. Many secondary metabolites participate in a variety of anti-diabetic functions *in vivo* [23]. Polysaccharides, coumarins, flavonoids, terpenoids and a host of other secondary plant metabolites, including arginine and glutamic acid, possess hypoglycaemic effects in various experimental animal models [24-27]. Effect of the flavonoids- quercetin and ferulic acid on pancreatic cells leading to their proliferation and secretion of more insulin have been proposed [28, 29] as the mechanism by which they reduced hyperglycaemia caused by Streptozotocin in diabetic rats. The presence of flavonoids, carbohydrates, alkaloids, and glycosides in the crude extracts of *Solanum dulcamara* could also act in a similar manner thereby decreasing the high blood glucose levels of alloxan-diabetic rats [30]. Hypoglycaemic polysaccharides lower blood glucose level by impeding glucose absorption from the gastrointestinal tract and thus reducing postprandial hyperglycaemia. This may explain the conclusion that the majority of plants with blood glucose lowering activity contain polysaccharides[25]. The extract also contains moderate amounts of flavonoids. Flavonoids have been found to be inhibitors of glucose -6-phosphatase system [31].

Table 1: Effect of Solanum dulcamara leaf extracts on blood glucose levels in alloxan-induced diabetic rats (mg dL-1)

Group	Dose (mg/kg)	Fbs (0hr)	1hr	2hrs	4hrs	8hrs	24hrs
A (MESD)	50	428.12 ±44.82	398.24 ±48.78	289.52 ±26.64[a]	213.56 ±20.70[b]	105.76 ±30.60[b]	196.68 ±36.36[b]
B (MESD)	100	343.80 ±19.26	264.24 ±20.70	199.08 ±27.00[a]	165.60 ±11.88[b]	112.68 ±15.48[b]	183.68 ±39.24[a]
C (glibenclamide)	0.07	443.52 ±21.24	483.12 ±24.12	466.56 ±22.86	439.20 ±18.36	477.00 ±20.88	458.64 ±24.66
D (AESD)	50	418.32 ±19.44	589.32 ±27.54	572.04 ±24.66	558.88 ±23.40	584.44 ±18.90	567.72 ±15.30
E (AESD)	100	410.76 ±52.20	298.44 ±21.42[a]	221.22 ±20.16[b]	361.96 ±21.42	403.20 ±24.30	378.02 ±27.18
F (negative diabetic control)	5ml/kg	385.92 ±25.56	456.12 ±32.04	505.44 ±24.30	545.04 ±27.00	528.12 ±6.48	396.00 ±72.36

Values are mean concentration of blood glucose ± S.EM. (n=5).
[a]Significantly decreased values compared with 0 h data ($p<0.05$).
[b]Significantly decreased values compared with 0 h data ($p<0.001$).

The ability of the methanol extracts to significantly reduce blood glucose level in the absence of functional pancreatic β- islet tissue could be by reducing gluconeogenesis and glucose absorption from the gastrointestinal tract similar to the action of biguanides [32]. Furthermore, due to the ability of the extracts to reduce blood glucose in the fed state, it is likely that they could act by inhibiting brush border enzymes (α-glucosidase) in the same manner as acarbose and meglitol[33]. Another plausible mechanism of action is that the extract might have stimulated residual pancreatic beta-cell function or produced the hypoglycaemia through an extra-pancreatic mechanism [32]. Administration of glibenclamide (0.07mg/kg) to diabetic rats did not lower the blood glucose level of treated rats. Glibenclamide act by binding to and blocking ATP-sensitive K^+ - channels in the β-islet cells of the pancreas leading to reduced potassium conductance with consequent membrane depolarization and influx of calcium ions through voltage sensitive Ca^{2+} channels finally culminating in insulin secretion [33]. Therefore, the inability of glibenclamide to reduce blood sugar in alloxan induced diabetic rats could be due to complete destruction of the pancreatic β-islet cells by alloxan. This further shows that the extracts may likely have acted through extra- pancreatic mechanisms. The skeletal muscles also play significant role in removal of glucose from the blood by its conversion to glycogen. It is possible that AESD and MESD acted by increasing the sensitivity of peripheral tissues to insulin action or the extracts may possess insulin-like activity. They may also act by stimulating key enzymes involved in glycogenesis. The extracts may improve the insulin mediated upregulation of glucose transporters (GLUT-4) which enhance the uptake of glucose by the skeletal muscle.

This study justifies the use of *Solanum dulcamara* leaf extracts in folklore for the management of diabetes mellitus. Further work should be done with different fractions of the crude extract in order to characterize the substance (s) responsible for the observed effect. Their effects on GIT motility and transit time should also be investigated with a view to investigating their action on glucose absorption.

Chronic research studies on their long term control of blood glucose are also recommended.

REFERENCES

[1] David B. and Sack S. Diabetes In: Tietz Fundamentals of Clinical Chemistry. 5th edition, WB Saunders, Philadelphia. 2001: P 433-435.

[2] Guyton CA and Hall JE. Textbook of Physiology. 10th edition, WB Saunders Company, Philadelphia. 2000: P 894.

[3] World Health Organization. Expert Committee on Diabetes Mellitus Second report: World Health organization Technical Report Series. Geneva. 1980: 646: 61.

[4] Wallace JL. Management of diabetes in the elderly. Clinical Diabetes. 1999 (17): 1-6.

[5] Poley JE. Rationale and Application of Fatty acid oxidation inhibitors in the treatment of diabetes mellitus. Diabetes Care. 1992; 15: 773-84.

[6] Moneva AA and Dagogo-Jack S. Multiple targets in the management of type 2 diabetes mellitus. Current Drug Targets. 2002; 3: 203-21.

[7] Bebernitz, G.R and Schuster, HF. The impact of fatty acid oxidation on energy utilization: targets and therapy. Current Pharmaceutical Design. 2002; 8: 1199-227.

[8] Nolte MS and Karam, JH. Pancreatic hormones and anti-diabetic drugs. In: Basic and Clinical Pharmacology. Katzung, B.G (editor), 9th edition, McGraw Hill, USA.2004: 693-715.

[9] Chattopadhyay RR. Possible mechanism of antihyperglycemic effect of Azadirachta indica leaf extract. Part IV. Gen Pharmacol 1996; 27: 431-4.

[10] Das AV, Padayatti PS, Paulose CS. Effect of leaf extract of Aegle marmelose (L.) Correa ex Roxb. on histological and ultrastructural changes in tissues of streptozotocin induced diabetic rats. Indian J Exp Biol. 1996; 34: 341-5.

[11] Yadav P, Sarkar S and Bhatnagar D. Action of Capparis deciduas against alloxan-induced oxidative stress and diabetes in rat tissues. Pharmacol Res 1997; 36: 221-8.

[12] Rai V, Iyer U and Mani UV. Effect of Tulasi (Ocimum sanctum) leaf powder supplementation on blood sugar levels, serum lipids and tissue lipids in diabetic rats. Plant Foods Hum Nutr. 1997; 50: 9-16.

[13] Shapiro K and Gong WC. Natural product used for diabetes. Journal of American Pharmacological Association. 2002; 42: 217-26.

[14] Olusola L, Ike CO and Mariam S. Hypoglycemic properties of aqueous bark extract of Ceiba pentandra in streptozotocin-induced diabetic rats. Journal of Ethnopharmacology. 2003; 84: 139-42.

[15] Jain AK, Mehta SC, and Shrivastava, NM. Synthesis, Structure elucidation, and antimicrobial activity substituted α-benzamido-β-(3-methoxy-4- chloro-p-toluene sulphonyloxy)- cinnamamides. Asian Journal of Chemotherapy. 2004; 16: 357-64.

[16] Claudia ENM, Julius EO, Dogbert T. and Etienne, D. Antidiabetic and hypolipidemic effects of Laportea ovalifolia (Urticaceae) in alloxan induced diabetic rats. Afr J Complement. Alternat. Med. 2006; 3: 36-43.

[17] Lorke D. A new approach to practical acute toxicity testing.Arch Toxicology. 1983(54): 275-287. 28. Mahesh Ta and Menon PV. Quercetin alleviates oxidative stress in streptozotocin induced diabetic rats. Phytother. Res. 2004; 18: 123-7.

[18] Trease GE and Evans WC. Textbook of Pharmacognosy. 12th edition, Balliere Tindall and Company Publisher, London. 1983:Pp. 343- 383.

[19] Akah PA, Okafor CL. Blood Sugar lowering effect of Vernonia amygdalina (Del) in an experimental rabbit model. Phytotherapy Research. 1992; 6: 171-3.

[20] American Physiological Society. Guiding principles for research involving animals and human beings. Am J Physiol Regul Integr Comp Physiol 2002; 283: R281-3.

[21] Halliwell B and Gutteridge JM. A Nitro oxide releasing derivative of ursodeoxycholic acid selectively delivers nitric oxide to the liver and protects against development of portal hypertension. Radicals in Biology and Medicine. 1989 2nd Edition

[22] Loew, D. and M. Kaszkin. Approaching the problem of bioequivalence of herbal

medicinal products. Phytother. Res. 2002; 16: 705-11.

[23] Kako M, Miura T, Nishiyama Y, Ichimaru M, Moriyasu M,and Kato A. Hypoglycemic Activity of Some Triterpenoid Glycosides. J Nat. Prod. 1997; 60: 604-5.

[24] Akah PA, Gamaniel KS, Wambebe CN, Shittu A, Kapu SD, and Kunle, OO. Studies on the gastrointestinal properties of Ficus exasperate. Fitoterapia. 1997; 68: 17-20.

[25] Marles RJ and Farnsworth NR. Anti-Diabetic Plants and their Active Constituents. Phytomedicine. 1995; 2(2): 137-89.

[26] Ross IA. Medicinal Plants of the World – Chemical Constituents, Traditional and Modern Medicinal Uses. Humana Press Inc. New Jersey, USA. 2001: pp. 30-45.

[27] Ojewole JAO. Hypoglycaemic effect of Clausena anisata (Wild) Hook methanolic root extract in rats. J. Ethnopharmacol. 2002; 81: 231-7.

[28] Sri-Balasubashini M, Rukkumani R, Viswanathan P and Menon PV. Ferulic acid alleviates lipid peroxidation in diabetic rats. Phytother. Res. 2004; 18: 310-14.

[29] Oliver B. Oral Hypoglycaemic Plants in West Africa. J. Ethnopharm, 1980; 2: 119-27.

[30] Estrada, O. "Evaluation of Flavonoids from Bauhinia megalandra Leaves as inhibitors of Glucose-6-Phosphatase System." Phytother. Res. 2005; 19(10): 859-63.

[31] Stumvoll M, Nurjham N, Perriello G, Darley G, and Gerich JE. Metabolic effects of metformin in non-insulin dependent diabetes mellitus. New England Journal of Medicine. 1995(333): 550-554.

[32] Kameswaararao B., Kesavulu M M., and Apparao C. Evaluation of antidiabetic effect of Momordica cymbalaria fruit in alloxan-diabetic rats. Fitoterapia, 2003; 74:7-13.

[33] Anandharajan R, Pathmanathan K, Shankernarayanan NP, Vishwakarma RA, Balakrishnan A. Upregulation of Glut-4 and PPAR gamma by an isoflavone from Pterocarpus marsupium on L6 myotubes: a possible mechanism of action. J Ethnopharmacol. 2005; 97(2):253-60.