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Original Article

ANTIHYPERGLYCEMIC AND ANTIDYSLIPIDEMIC POTENTIAL OF *IPOMOEA BATATAS* LEAVES IN VALIDATED DIABETIC ANIMAL MODELS

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ABSTRACT

Objective: The present study was undertaken to investigate the antidiabetic potential of the leaves of *Ipomoea batatas*.

Methods: The crude powder, 95% ethanolic, 50% ethanolic and aqueous extracts of *Ipomoea batatas* leaves were administered to normoglycemic and streptozotocin (STZ)-induced diabetic rats in a single dose study. The chloroform, butanol and aqueous fractions of aqueous extract were investigated for their antihyperglycemic on STZ-induced diabetic rats. Multiple dose study of an aqueous fraction was also done in STZ and neonatal STZ-induced diabetic rats. Further, the aqueous fraction was measured against the alpha glucosidase and aldose reductase enzymes, and glucose uptake in L6 myotubes.

Results: The aqueous extract showed significant lowering of postprandial hyperglycemia of post sucrose loaded normal rats and significantly declined the blood glucose level of STZ-induced diabetic rats. The aqueous fraction at a single dose of 100 mg/kg b. w in comparison with chloroform and butanol fractions significantly lowered the blood glucose level of STZ-induced diabetic rats. The aqueous fraction in a multiple dose study were found to significantly improved the percent glycated hemoglobin (%HbA1c), fasting blood glucose, oral glucose tolerance (OGTT), serum insulin, lipid profile, liver and kidney parameters in STZ-induced diabetic rats. Marked improvement in OGTT and serum insulin levels was also found in neonatal STZ-induced diabetic rats. *In vitro* study, the aqueous fraction of *I. batatas* increased glucose uptake in L6 myotubes and inhibits the α-glucosidase and aldose reductase enzymes.

Conclusion: The present study demonstrated the significant antidiabetic activity of the *I. batatas* leaves by promoting insulin secretion, alpha glucosidase and aldose reductase enzyme inhibition.

Keywords: Antidiabetic activity, Antidyslipidemic activity, Glucose uptake, STZ-induced rats, Neonatal STZ induced diabetic rats, *Ipomoea batatas* leaves.

INTRODUCTION

Diabetes mellitus (DM) is a heterogeneous group of metabolic disorders characterized by chronic hyperglycemia i.e. persistent high blood glucose level. The chronic hyperglycemia in diabetes is associated with long-term damage, dysfunction, and failure of different organs, mainly the eyes, kidneys, heart and blood vessels [1]. Although currently available oral antidiabetic agents have a number of serious adverse effects; thus, managing diabetes without any side effects is still a challenge. Hence, the search for more safer, specific and valuable hypoglycemic agents has constantly been an area of important investigation. The plants exhibiting blood glucose lowering effect have been listed in the available literature; though partial mechanism (s) of their mode of action is available for only about one-fourth of them. However, in the traditional system the herbal drugs with antidiabetic effect have been acclaimed and accepted for their therapeutic purposes, still they are yet to get commercially formulated just like modern drugs. The easily available traditional plants provide prospective for finding antidiabetic drugs. Therefore, the present study was conducted to identify the antidiabetic potential of leaves of *Ipomoea batatas*.

Ipomoea batatas L. (Family: *Convolvulaceae*) is commonly known as sweet potato, it is the world's sixth largest food crop, which is widely grown in tropical, subtropical and warm temperate regions [2]. It is an important food crop in many countries, also cultivated for its use as animal feed and as a medicinal plant [3]. In the region of Kagawa, Japan, a variety of white sweet potato has been eaten raw to treating anemia, hypertension and diabetes [4]. A study in rats has shown that *I. batatas* leave are a good source of polyphenols, antioxidants and possess vascular relaxing properties [5]. The anti hyperglycemic and antidiabetic activities have been reported for *I. batatas* tuber roots in STZ-induced type 2 diabetes model rats and obese Zucker rats [6-9]. It has been shown by Li *et al.,* in 2009 that the flavonoids from *I. batatas* leaf exerts both antihyperglycemic and antidyslipidemic activities in alloxan-induced diabetic mice [10]. It has been also tested clinically that when patients with type 2 diabetes were given *I. batatas* treatment given for 3 months, resulted in lowering of plasma glucose and cholesterol levels in patients [4,11]. Therefore, the present study was focused on investigating the antidiabetic activity of crude extracts and role of an aqueous fraction of active aqueous extracts of *I. batatas* leaves in validated diabetic animal models.

MATERIALS AND METHODS

Chemicals

Streptozotocin, metformin, glybenclamide, Dulbeco Minimum Essential Medium (DMEM), fetal bovine serum, pencillin G, antibiotics, trypsin, insulin and other chemicals are from Sigma-Aldrich Chemical Company (St. Louis, MO (USA). All other reagents used were of the highest purity grade. 2-Deoxy-D-[3H]-glucose (2-DG) was procured from Perkin Elmer, USA. Sucrose, glucose, fructose, and cholesterol, scintillation cocktail were purchased from Sisco Research Laboratories, Mumbai (India). The serum triglycerides and total cholesterol were measured using Dialab diagnostic kits. The blood glucose level was measured using glucose strips which were obtained from Roche (India).

Animals

Male albino rats of Sprague Dawley (SD) Strain (140-180 g) of 8-10 weeks of age and male neonatal rats (weighing 7±2 gm) of 2 days were obtained from the animal colony of CSIR-Central Drug Research Institute, Lucknow. The animals were always housed in polypropylene cages in groups of 3 to 6. All the animals were kept in the animal room under standard conditions (23±2 °C, 50%-60% relative humidity, light and dark cycles of 12 h each). The animals were provided pellet diet and tap water *ad libitum* unless stated otherwise. As per the guidelines of the Committee for the Purpose of

Control and Supervision of Experiments on Animals (CPCSEA) formed in the year 1964 by the Indian government, suitable permission was acquired from CSIR-CDRI institutional animal ethics committee (Ethics Committee Approval Reference No. IAEC/2008/63/Renewal 04 dated 16.05.2012) for animal experiments.

Collection of plant material and preparation of extracts and fractions of *I. batatas* leaves

Leaves of *I. batatas* were purchased from the local market and its identity was authenticated in the laboratory of CDRI. The shade dried material was cut into fine pieces and powdered by a mechanical grinder, passed through 100 mesh sieve and the powder was stored in airtight containers until used. The powdered leaves (3.0 kg) of *I. batatas* were percolated in 10 volumes of 95 % ethyl alcohol five times successively. The combined extract was filtered and concentrated under high vacuum in a rotavapour and termed ethanolic extract. In the same manner the crude powder was extracted with ethanol: water (50:50) and distilled water, respectively.

The collective 50% ethanol and aqueous extracts were separated out and were further concentrated under reduced pressure at 45°C using rotavapour and termed as 50% ethanolic (ethanol: aqueous) and aqueous extracts. Ethanolic, 50% ethanolic, aqueous extracts were stored at – 20°C until used. The crude aqueous extract was macerated with n-hexane; the insoluble residue was macerated with chloroform to give chloroform soluble fraction and an insoluble residue. The chloroform insoluble residue was portioned between n-butanol and water to give n-butanol soluble fraction and water soluble fractions, these entire fractions were then concentrated under reduced pressure on rotatory evaporator at 50 °C and finally dried under vacuum to get final fractions for evaluation of antidiabetic activity.

Induction of diabetes

To the 16 h overnight fasted animals, streptozotocin freshly prepared in citrate buffer (0.1 M, pH 4.5) at a dose of 60 mg/kg was orally administered intraperitoneally (i. p.) for induction of diabetes. Diabetes rats were characterized by identification of features specific for diabetes, such as polydipsia, polyuria and increased fasting blood glucose levels 72 h observed in STZ treated rats. Rats with blood glucose levels above 280-450 mg/dl after were selected for experiments.

Single dose effect

Oral glucose tolerance test of normal rats

Male albino rats of Sprague Dawley (SD) (140-180 g) were selected and kept on an overnight starvation. Next morning the blood glucose level (0 min) of each animal was measured by glucometer using glucostrips. Animals showing their fasting blood glucose levels between 60 to 80 mg/dl (3.33-4.44 mM) were finally selected and divided into groups, consisting six animals in each group. Each rat of experimental groups was given suspension of the test substances made in 1.0% gum acacia at desired dose levels, i.e. 250 mg/kg b.w. in the case of crude/extracts and 25 mg/kg in the case of standard antidiabetic drug i.e. glybenclamide. An equal amount of 1.0% gum acacia (vehicle) was given to each rat of control group animals, and termed as sham treated control. An oral sucrose load (10 gm/kg b. w) was given to each animal exactly 30 min post administration of the test sample/standard drug/vehicle and the blood glucose level was measured at 0, 30, 60, 90 and 120 min post sucrose load. During the course of the experimentation food was withheld, but water was provided to the animals in the cages. Comparing the AUC of the experimental vs. control group determined the % lowering of blood glucose [12].

Effect of crude powder and extracts/fractions of leaves of *I. batatas* on blood glucose of streptozotocin-induced diabetic rats

Streptozotocin induced diabetic rats with marked elevation in fasting blood glucose levels above 280-450 mg/dl were used for this study. The selected diabetic rats were randomly divided into groups consisting of six animals in each group. The plant crude powder and its extracts, fractions and standard antidiabetic drug, metformin were prepared as suspensions made in 1.0% gum acacia and were orally administered at a pre selected dose of 250 mg/kg for crude powder/extracts and 100 mg/kg b.w. dose for fractions and metformin. At post administration of the test sample/ vehicle/standard drug the blood glucose level of each animal was measured by glucostrips at 0, 30, 60, 90, 120, 180, 240, 300, and at 1440 min [13]. During 0 to 300 min of experimentation food was withheld, but water was provided to the animals in the cages. The percentage lowering of blood glucose levels by test samples or standard drug was determined by plotting the mean blood glucose levels against time and calculating the area under the curve (AUC) between 0-300 min and 0-1440 min.

Multiple dose effect

Effect of aqueous fraction of aqueous extract of *I. batatas* leaves on streptozotocin-induced diabetic rat model

Streptozotocin induced diabetic rats with marked elevation in fasting blood glucose levels above 280-450 mg/dl were used for this study. These STZ-induced diabetic rats were left untreated for a period of 10 weeks to develop secondary complications in association with the chronic diabetes. The diabetic animals which showed percent glycated haemoglobin more than 8 were selected for this study and grouped into three with six animals in each group. The group, which served as a diabetic control was administered with 1.0 % gum acacia as a vehicle, whereas, the second and third groups were given an aqueous fraction of aqueous extract of *I. batatas* leaves and metformin at the desire dose levels of (100 mg/kg) for a period of 30 days. The fasting blood glucose level and OGTT post glucose loads of each rat were examined at the time of start and on day 7, 14, 21, and 28.

Effect of aqueous fraction of aqueous extract of *I. batatas* leaves on Neonatally-streptozotocin treated diabetic rats

Two-day-old pups of Sprague Dawley strain (weighing 7±2 gm), were injected 90 mg/kg STZ prepared in citrate buffer 0.1 M, *p*H 4.5 and left along with their mothers for 4 weeks. The rats separated from the mothers were further kept for 3 months in polypropylene cages and given a pellet diet and water *ad libitum*. These animals showed signs of polydypsia, polyurea and abnormal OGTT at the end of the period. These rats were randomly divided into three groups of five animals. Group I (diabetic control group) received 1% gum acacia**,** Group II (experimental group) received test sample i.e. aqueous fraction of leaves of *I. batatas* at 100 mg/kg body weight dose and Group III (standard group) received standard drug, metformin at 100 mg/kg body weight dose. Fasting blood glucose, serum insulin and OGTT post glucose loads of these animals were followed at weekly intervals for 30 days [14].

Oral glucose tolerance test

For the oral glucose tolerance test, 3.0 gm/kg post glucose load glucose was administered to rats that had been fasted for 16 h. Blood samples were collected from a tail vein and blood glucose was measured at 0, 30, 60, 90 and 120 min. Effect on oral glucose tolerance was obtained by calculating the area under the curve for the values of blood glucose between 0-120 min.

Blood biochemical parameters

On day 10, 20, and 30, the blood was withdrawn from the retro-orbital plexus of each rat for the estimation of the triglycerides, cholesterol, LDL-C and HDL-C, ALT, AST, urea, uric acid, creatinine and %HbA1c levels were measured on semi-auto-analyzer (Dialab) using the assay kits and instructions from the manufacturer. The serum insulin content was determined using the rat Insulin enzyme-linked immunosorbent assay (ELISA) kit (Mercodia, Uppsala, Sweden).

Cell culture

L6 rat skeletal muscle cell lines, procured from the National Center of Cell Sciences (NCCS), Pune, India were cultured in DMEM supplemented with 10% FBS, penicillin (100 units/ml), streptomycin (200 µg/ml) and gentamycin (50 µg/ml) in a humidified atmosphere of 5% CO2 and 95% air at 37 °C 26, 27. Differentiation was induced by switching confluent cells to medium

supplemented with 2% FBS. Experiments were performed in differentiated myotubes 4-6 days after seeding. The cells were maintained for another five to seven days and media were changed every 48h prior to use in experiments.

Glucose uptake assay

Glucose uptake was performed as previously described by Srivastava *et al.,* 2014 [13]. In brief, differentiated L6 myotubes grown in 24-well plate (6×10^4 cells/well) were incubated with different concentrations of the aqueous fraction of leaves of *I. batatas* or standard drug, metformin at a 500µM concentration for 16 h with final 3 h in serum-deprived medium and a sub-set of cells were given stimulation with insulin at 100 nM concentration for 20 min. Glucose uptake was assessed for 5 min in HEPES-buffered saline (pH 7.4)] containing 10 µM 2-DG (0.5 µCi/ml 2-[^{3}H] DG) at room temperature. After the uptake period, radioactive solution was rapidly aspired, and the cell mono layers were rinsed with an ice-cold HEPES buffered containing 0.9% NaCl and 25 mM D-glucose. The radioactivity associated with cell was quantified, by cell lysis and lysates were counted with scintillation fluid in β-counter (Beckman Coulter, USA). The assays were performed in triplicates and normalized to total protein, was expressed as fold change with respect to control.

***In-vitro* enzyme inhibition assays**

α-Glucosidase assay

α-Glucosidase enzyme inhibition assay was performed according to a slight modification of the procedure reported by Pistia-Brueggeman and Hollingsworth, 2001 [15]. 100 µl of the purified µ-glucosidase (0.1 mg/ml) was added to the assay system containing 500 µl of 67 mM phosphate buffer (pH 6.8), 100 µl of glutathione (1.0 mg/ml) and the desired concentrations of the test sample or standard inhibitor acarbose and the final volume was made to 1000 µl. The reaction was started by the addition of 100 µl *p*-nitrophenyl-α-D-glucopyranoside (1.0 M) and terminated by the addition of 500 µl of 0.1 M sodium carbonate. The IC_{50} value was defined as the concentration of α-glucosidase inhibitor to inhibit 50% of its activity under the assay conditions. The absorbance of colored p-nitrophenol liberated was read on 410 nm. One unit of enzyme activity is 1.0 µmol p-nitrophenol formed per min per mg protein.

Aldose reductase (AR) assay

The lens homogenate was prepared according to the protocol as previously described [16]. The lenses from both the normal as well as STZ-induced diabetic rats, after cervical dislocation, were immediately enucleated and washed with saline, and their fresh weight was recorded. 40 eye lenses of each were pooled and 10% lens homogenate was prepared in 0.1 M phosphate buffer saline (pH 7.4) by using Potter Elvejhem glass homogenizer with Teflon pestle, the prepared homogenate was separated out by muslin cloth and the filtrate was centrifuged at 5000×*g* for 15 min at 4 °C, further the supernatant was collected and used as an enzyme source in applications, and kept at-70 °C for storage until used. Protein content in the supernatant of the lens homogenate was estimated according to Lowry *et al.*, (1951) method [17].

The method of Hayman and Kinoshita (1965) was used to measure AR activity of Lens. In brief, the decrease in the absorption of NADPH at 340 nm over a 3-min period was measured by using DL±Glyceraldehyde as a substrate [18]. The aldose reductase inhibition was determined as follows: 0.7 ml of 67 mM sodium phosphate buffer of pH 6.2, 0.1 ml of NADPH (25×10^{-5} M), 0.1 ml of DL±Glyceraldehyde (substrate, 1×10^{-3} M) and 0.1 ml of lens supernatant to a final volume of 1 ml were mixed in the sample cuvette and read against a reference cuvette containing all components except the substrate, DL±Glyceraldehyde. The pH of the reaction mixture was finally adjusted at pH-6.2.

The enzymatic reaction starts with the addition of the substrate into the solution mixture and the absorbance in optical density (O. D) was recorded at 340 nm for 3 min at 30s intervals. The aldose reductase inhibitory activity was calculated and expressed as ΔO. D./min/mg protein. The percentage inhibition (%) was calculated as [(ΔO. D. Sample/min)/(ΔO. D. Control/min) X100-100], where ΔO. D. Sample/min depicts the decrease in absorbance for 3 min with test sample and ΔO. D. Control/min represents the same, but with 1% DMSO instead of samples.

Statistical analysis

Each parameter was expressed as mean±S. E. Statistical comparisons between groups were made by Dunnett's test. The results were considered significant if *p* values are 0.5 or less.

RESULTS

Effect of crude powder and extracts of *I. batatas* leaves on post-sucrose loaded normal rats

Table 1 and fig. 1A represent the effect of crude powder, ethanolic, ethanolic: aqueous and aqueous extracts and standard drug, glybenclamide on percent improvement of glucose tolerance on post-sucrose loaded normal. It is evident from the results that the crude powder of *I. batatas* leaves showed average percent improvement of glucose tolerance around 14.0%, while among all the extracts, the aqueous extract of *I. batatas* leaves showed the most significant improvement (18.4%, $p<0.01$) on oral glucose tolerance of post sucrose loaded normal rats. The standard drug, glybenclamide caused significant improvement around by 30.4% ($p<0.01$) of inhibition on the rise of post-prandial hyperglycemia of sucrose loaded normal rats at 25 mg/kg dose.

Table 1: Effect of crude powder and ethanolic, ethanolic: aqueous and aqueous extracts of *I. batatas* leaves and standard antidiabetic drugs on the improvement of glucose tolerance post sucrose load in normal rats and blood glucose lowering on STZ-induced diabetic rats

Treatment	Dose (mg/kg)	Blood glucose level (AUC±SEM)		
		Sucrose loaded normal rats (OGTT)	STZ-induced diabetic rats	
		(0-120 min)	0-300 min	0-1440 min
Sham treated Control (1.0% Gum acacia)	-	15160±257.3	118000±2940	610000±16080
Crude powder	250	12950±279.9 (14.0%)^{ns}	87300±706 (26.0%)**	473000±23240 (22.6%)**
Ethanolic extract	250	12700±122.3 (16.2%)**	101000±2060 (14.4%)*	544400±15950 (10.8%)^{ns}
Ethanolic: aqueous extract	250	13070±355.8 (13.8%)^{ns}	107000±4000 (9.32%)^{ns}	539200±14230 (11.6%)^{ns}
Aqueous extract	250	12280±90.63 (18.4%)**	93400±848 (20.8%)**	504200±10450 (17.3%)*
Glybenclamide	25	10550±77.8 (30.4%)**	-	-
Metformin	100	-	83900±1220 (28.9%)**	426000±8845 (30.2%)**

Values are mean±SE of six rats, p*<0.05, p**<0.01,ns: not significant

Effect of crude powder and extracts of *I. batatas* leaves on blood glucose levels of STZ-induced diabetic rats

Table 1 presents the blood glucose profile of the STZ-induced diabetic rats treated with crude powder, ethanolic, ethanolic: aqueous, aqueous extracts of *I. batatas* leaves and the standard antidiabetic drug, metformin. Of these, crude powder and its aqueous extract showed significant decline in fasting blood glucose level at the selected doses of 250 mg/kg b. w on STZ-induced diabetic rats. The average antihyperglycemic activity of the crude

powder and its aqueous extract was calculated to be around 26.0% and 20.8 % during the period 0-300 min, respectively, and 22.6% and 17.3 % during the period 0-1440 min, respectively. Whereas, the standard drug, metformin caused blood glucose lowering effect on STZ-treated diabetic rats at 100 mg/kg b. w dose. The average antihyperglycemic activity of metformin was estimated to be around 28.9% and 30.2 % during the period 0-300 min and 0-1440 min, respectively (fig. 1B and 1C).

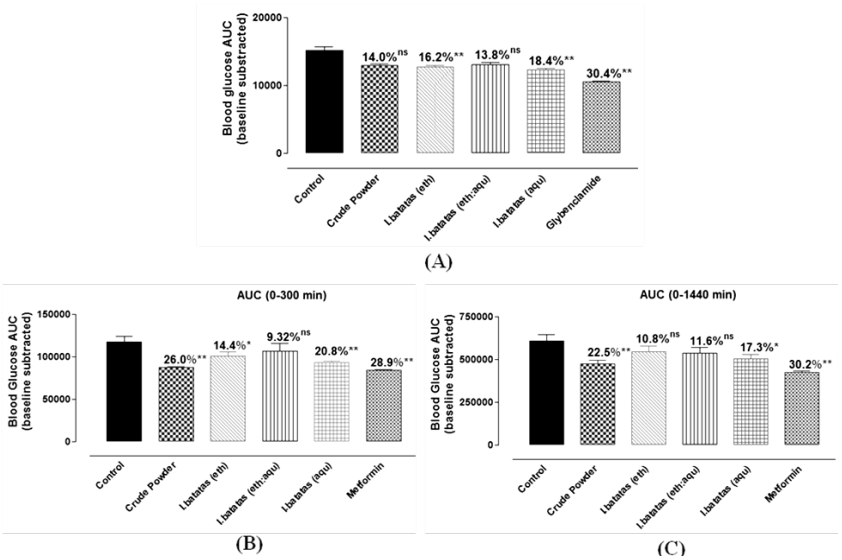

Fig. 1: Effect of crude powder and ethanolic, ethanolic: aqueous and aqueous extracts of *I. batatas* leaves and standard antidiabetic drugs (A) on post sucrose loaded normal, during 0-120 min and on STZ-induced diabetic rats (B) during 0-300 min (C) during 0-1440 min

Effect of fractions of aqueous extract of *I. batatas* leaves on blood glucose levels of STZ induced diabetic rats

Table 2 represents the antihyperglycemic effect of the fractions i.e. aqueous, butanol and chloroform of aqueous extract of *I. batatas* leaves and the standard antidiabetic drug, metformin, on fasting blood glucose level of STZ-induced diabetic rats. The corresponding blood glucose lowering were calculated to be around 17.6%, 1.43% and 12.5%, during 0-300 min and around 15.0%, 8.93% and 14.7%, during 0–1440 min, at post treatment, respectively for each aqueous, butanol and chloroform fractions. The standard antidiabetic drug metformin caused lowering in fasting blood glucose levels of STZ-induced diabetic to the tune of 31.2% (p<0.01) and 24.8% (p<0.01) during 0-300 min and 0–1440 min, respectively.

It is evident from the results that aqueous fraction showed most significant decline in fasting blood glucose level of STZ-induced diabetic rats at the dosage of 100 mg/kg of body weight. The effect was started from 60 min which persisted till the end of the experiment i.e. 1440 min as shown in fig. 2.

Table 2: Effect of fractions of aqueous extract of *I. batatas* leaves and standard antidiabetic drug, metformin on blood glucose levels of STZ induced diabetic rats

Treatment	Dose (mg/kg)	Blood glucose lowering (STZ rats) (AUC±SEM)	
		0-300 min	0-1440 min
Sham treated control (1.0% Gum acacia)	-	125900±3354	632600±16440
Chloroform fraction	100	110200±4517 (12.5%)^{ns}	539500±19180 (14.7%)*
Butanol fraction	100	124100±8891 (1.43%)^{ns}	576100±11420 (8.93%)^{ns}
Aqueous fraction	100	103700±3148 (17.6%)**	537600±34780 (15.0%)**
Metformin treated	100	86620±2282 (31.2%)**	475800±19160 (24.8%)**

Values are mean±S. E. of six rats. p*<0.05, p**<0.01, ns: not significant

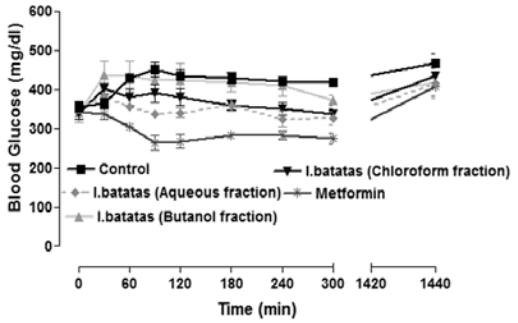

Fig. 2: Effect of fractions *I. batatas* leaves and standard antidiabetic drug, metformin on blood glucose levels of STZ induced diabetic rats

Effect of aqueous fraction of aqueous extract of *I. batatas* leaves on fasting blood glucose and glucose tolerance (OGTT) of STZ-induced diabetic rats

Table 3 depicts the effect of an aqueous fraction of aqueous extract of *I. batatas* leaves and metformin on fasting blood glucose and oral glucose tolerance (OGTT) of STZ-induced diabetic rats. It is evident from the table 3 that an aqueous fraction of *I. batatas* leaves when given to STZ-induced diabetic rats at 100 mg/kg p. o. for 30 consecutive days declined their fasting blood glucose level and improved OGTT as compared to the sham treated control group. The decline in the fasting blood glucose was calculated to be around 17.8 % (p<0.01) and 28.0% (p<0.01), whereas an improvement on OGTT, was around 13.5% (p<0.05), and 24.7% (p<0.01 on day 14 and 28, respectively in aqueous fraction of *I. batatas* leaves treated group. While the metformin treated group showed a decline in fasting blood glucose and an improvement on

OGTT were calculated to be around 20.6 % (p<0.01) and 29.7% (p<0.01) and 17.2% (*p*<0.01) and 30.6% (*p*<0.01) on day 14 and 28, respectively at the dose of 100 mg/kg p. o. when compared to the sham treated control group (fig. 3A and fig. 3B).

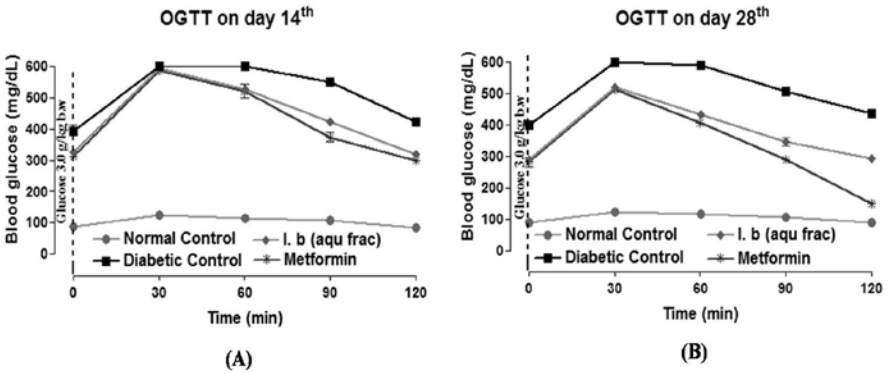

Fig. 3: Effect of aqueous (aqu) fraction of I. batatas (I. b) Leaves on glucose tolerance (OGTT) of streptozotocin induced diabetic rats (A) on day 14, and (B) on day 28

Table 3: Effect of an aqueous fraction of *I. batatas* leaves and standard drug metformin on fasting blood glucose and improvement in OGTT of STZ-induced diabetic rats

Group	Dose (mg/kg)	Fasting blood glucose(mg/dl)					Oral Glucose tolerance (0-120 min) (AUC±SEM)				
		Day 0	Day 7	Day 14	Day 21	Day 28	Day 0	Day 7	Day 14	Day 21	Day 28
Normal Control (1.0% Gum acacia)	-	74.0±2.73	88.6±2.62	89.3±2.66	89.8±2.55	90.7±2.92	12580±207.8	12710±14 4.7	12950±12 6.7	12860±12 6.6	13080±92 .10
Sham Control (1.0 % gum acacia)	-	384.2±7.09	389.2±7.13	394.0±7.57	399.7±6.73	401.8±4.92	64710±446.8	64940±13 8.3	64740±204.7	63880±951.9	63460±518.4
Aqueous fraction of aqueous extract of *I. batatas* leaves treated	100	386.0±25.3	349.0±13.8 (10.3)*	324.2±6.20 (17.8)**	306.8±4.16 (23.2)**	289.2±7.70 (28.0)**	64710±757.6	59120±92 3.3 (8.76)ns	55970±35 7.5 (13.5)*	51430±11 52 (19.5)**	47760±60 7.2 (24.7)**
Metformin treated	100	398.0±13.3	335.0±10.3 (13.9)*	313.2±3.22 (20.6)**	301.2±6.35 (24.6)**	282.7±14.9 (29.7)**	66370±459.7	57040±45 7.2 (12.2)*	53600±12 63 (17.2)**	48140±13 81 (24.7)**	42770±45 76 (30.6)**

Values are mean±SE. of six rats. *p**<0.05, *p***<0.01, ns: not significant

Effect of aqueous fraction of aqueous extract of *I. batatas* leaves and standard drug metformin on body weight and %HbA1c of STZ-induced diabetic rats

Table 4 depicts the effect of an aqueous fraction of *I. batatas* leaves and standard drug, metformin, on body weight and percentage (%) HbA1c of STZ induced diabetic rats after 30 days of treatmentat the doses of 100 mg/kg, respectively. The aqueous fraction of *I. batatas* leaves treatment when given at 100 mg/kg post oral (p. o.) for 30 consecutive days to STZ induced diabetic rats showed no significant improvement in body weight, while metformin treated group showed significant (19.2%, *p*<0.01) restoration of the body weight on day 30 at the dose of 100 mg/kg (fig. 4A).

The % HbA1c level of aqueous fraction of *I. batatas* leaves treated group on day 30 at the dose of 100 mg/kg was suppressed 40.2% (*p*<0.01), as compared to diabetic control group and in metformin treated group % HbA1c level observed was around 52.1% (*p*<0.01) (fig. 4B).

Effect of aqueous fraction of aqueous extract of *I. batatas* leaves on serum insulin level and lipid profile of STZ-induced diabetic rats

Table 5 presents effect of aqueous fraction of aqueous extract of *I. batatas* leaves and metformin treatment at the doses of 100 mg/kg b. w on the serum insulin level and lipid profile of STZ-induced diabetic rats. The aqueous fraction of leaves of *I. batatas* and metformin treated groups showed significant increased in serum insulin level compared to sham treated control group on day 10 and 30, respectively. Table 5 also shows the effect of an aqueous fraction of *I. batatas* on the serum lipid profile of STZ-induced diabetic rats. The aqueous fraction of leaves of *I. batatas* caused 10.8% and 29.9% decline in serum triglycerides, around 9.42% and 34.3% decline in serum cholesterol level and 11.7 and 33.5% decline in serum LDL-cholesterol levels of STZ rats on day 10 and 30, respectively. The aqueous fraction of leaves of *I. batatas* caused a rise in HDL-cholesterol level to around 6.52% and 12.6% on day 10 and 30, respectively. The standard drug, metformin, when given at the

dosage of 100 mg/kg body weight (b. w) for 30 successive days did not significantly either lowers the serum triglycerides, cholesterol and LDL-cholesterol levels nor raised the level of serum HDL-cholesterol of streptozotocin-induced diabetic rats.

Table 4: Effect of aqueous fraction of *I. batatas* leaves and standard drug metformin on body weight and %HbA1c of STZ-induced diabetic rats

Group	Dose (mg/kg)	Body Weight (gm)		% HbA1c	
		Initial (0 day)	Final (30 day)	Initial (0 day)	Final (30 day)
Normal Control (1.0% Gum acacia)	-	182.7±2.67	196.0±3.99	4.87±0.599	4.90±0.33
Sham treated control (1.0% Gum acacia)	-	152.6±3.46	139.8±5.03	11.9±0.84	12.4±0.92
Aqueous fraction treated	100	152.0±3.28	156.9±4.92 (12.2%)^{ns}	11.4±0.90	7.41±0.60 (40.2%)**
Metformin treated	100	150.3±3.90	166.7±3.32 (19.2%)**	11.9±0.98	5.94±0.29 (52.1%)***

Values are mean±SE of six rats. p*<0.05, p**<0.01, ns: not significant

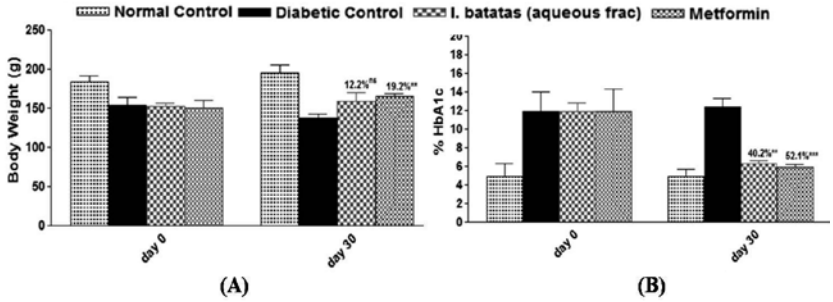

Fig. 4: Effect of aqueous fraction (frac) of leaves of *I. batatas* and standard drug, metformin, on (A) Body weight and (B) %HbA1c of STZ-induced diabetic rats. Values are mean±SEM of six rats. Statistical significance *p<.01, **p<0.001 compared to sham control

Table 5: Effect of aqueous fraction of *I. batatas* leaves and standard drug metformin on serum insulin level and lipid profile of STZ-induced diabetic rats

Group	Day	Insulin ((µg/l)	Biochemical profiles (Serum)			
			TG (mg/dl)	TC (mg/dl)	LDL-C mg/dl)	HDL-C (mg/dl)
Sham treated Normal Control (1.0% Gum cacia)	0	0.174±0.006	62.3±13.0	84.3±2.41	30.0±2.93	39.8±2.64
	10	0.167±0.007	64.0±3.06	86.7±2.46	31.5±0.76	39.7±3.93
	20	0.163±0.009	64.5±5.24	89.0±1.90	32.2±0.60	39.3±0.76
	30	0.164±0.018	65.8±2.41	89.2±1.85	33.3±0.49	40.3±2.90
Sham treated Diabetic Control (1.0% Gum cacia)	0	0.084±0.001	134.7±3.12	116.3±2.35	65.7±5.09	23.7±1.66
	10	0.082±0.001	136.0±2.58	117.8±2.00	65.6±0.80	23.0±0.80
	20	0.082±0.003	137.5±1.61	120.8±0.99	67.2±1.01	22.8±1.60
	30	0.081±0.003	139.0±1.44	122.3±1.33	68.0±2.17	22.5±1.86
Aqueous fraction of aqueous extract of *I. batatas* leaves treated (100 mg/kg p. o)	0	0.087±0.001	135.2±3.50	114.8±2.18	66.2±7.18	22.5±1.77
	10	0.095±0.004 (15.9)*	121.3±1.45 (10.8)^{ns}	106.7±2.83 (9.42)^{ns}	57.9±2.18 (11.7)^{ns}	24.5±1.11 (+6.52)^{ns}
	20	0.099±0.005 (20.7)**	112.7±2.43 (18.0)**	99.2±3.48 (17.6)**	49.8±2.16 (25.9)**	25.0±1.34 (+9.50)^{ns}
	30	0.112±0.002 (38.3)**	97.5±4.78 (29.9)**	80.3±4.72 (34.3)**	45.2±1.63 (33.5)**	24.3±1.43 (+12.6)^{ns}
Metformin treated (100 mg/kg p. o)	0	0.085±0.001	134.7±4.02	117.8±3.61	69.2±8.46	25.7±2.39
	10	0.092±0.001 (12.2)^{ns}	125.5±2.40 (7.72)^{ns}	111.7±2.06 (5.18)^{ns}	62.2±1.23 (5.27)^{ns}	23.5±1.11 (+2.17)^{ns}
	20	0.097±0.006 (18.3)*	124.3±1.46 (9.60)^{ns}	109.8±3.49 (8.73)^{ns}	61.7±3.06 (8.19)^{ns}	24.5±1.52 (+7.31)^{ns}
	30	0.099±0.005 (22.2)**	121.2±2.09 (12.8)^{ns}	107.0±3.20 (12.3)^{ns}	60.9±2.06 (10.4)^{ns}	25.0±1.51 (+11.1)^{ns}

Values are mean±SE. of six rats. p*<0.05, p**<0.01, ns: not significant

Effect of aqueous fraction of aqueous extract of *I. batatas* leaves on liver and kidney function parameters of STZ-induced diabetic rats

Table 6 depicts the effects of aqueous fraction of aqueous extract of *I. batatas* leaves and metformin on liver and kidney function markers of STZ-induced diabetic rats. The aqueous fraction of leaves of *I. batatas* caused 8.75 and 35.4 % decline in serum ALT activity and around 7.67 and 21.3 % decline in serum AST activity on day10 and 30, respectively. In comparison metformin caused 11.4 % and 33.8% decline in ALT activity, 8.99 and 23.7% decline in AST activity on day 10 and 30, respectively. The kidney functions were also improved in the aqueous fraction of *I. batatas* treated group as nearly 5.99 and 18.9 % decline in serum urea levels on day10 and 30, respectively, 13.5 and 50.1% decline in serum uric acid levels and 8.79 and 21.9% decline in serum creatinine levels were observed on day10 and 30, respectively in the aqueous fraction of *I. batatas* treated group. Metformin treatment lowered serum urea level by 7.36 and 21.4%, uric acid level by 14.4 and 40.7% and serum creatinine level by 9.90 and 31.1% on day10 and 30, respectively.

Table 6: Effect of aqueous fraction of aqueous extract of *I. batatas* leaves and standard drug, metformin, on liver and kidney function markers of STZ-induced diabetic rats

Group	Day	Biochemical Parameters (Serum)				
		Liver Function Markers		Renal Function Markers		
		ALT (U/l)	AST (U/l)	Serum-Urea (mg/dl)	Serum-Uric acid (mg/dl)	Serum-Creatinine (mg/dl)
Normal Control (1.0% Gum acacia)	0	11.4±1.08	13.6±1.19	21.0±1.69	2.04±0.18	0.37±0.02
	10	12.8±0.61	13.9±1.13	23.2±1.14	2.05±0.17	0.39±0.05
	20	12.8±0.60	14.1±1.08	24.2±1.01	2.05±0.28	0.39±0.02
	30	13.7±0.76	14.5±1.13	24.5±0.88	2.08±0.19	0.40±0.2
Sham treated Control (1.0% Gum acacia)	0	82.0±4.07	88.3±3.20	85.5±2.41	8.42±0.72	0.83±0.04
	10	81.8±2.54	89.0±2.82	86.2±1.96	8.55±0.73	0.83±0.04
	20	84.0±2.49	89.2±2.73	88.0±2.29	8.59±0.67	0.86±0.05
	30	86.7±1.86	89.2±2.73	88.2±2.12	8.66±0.68	0.86±0.03
Aqueous fraction of aqueous extract of *I. batatas* leaves treated (100 mg/kg p. o)	0	88.0±6.32	85.5±3.58	84.2±3.11	8.66±0.54	0.79±0.04
	10	74.7±2.29 (8.75)^{ns}	82.2±3.45 (7.67)^{ns}	81±2.45 (5.99)^{ns}	7.40±0.59 (13.5)^{ns}	0.75±0.01 (8.79)^{ns}
	20	64.3±3.72 (23.4)**	78.0±1.29 (12.5)^{ns}	79.5±2.20 (9.66)^{ns}	5.71±0.38 (33.6)**	0.72±0.02 (15.9)**
	30	56.0±2.39 (35.4)**	70.2±4.62 (21.3)**	71.5±0.99 (18.9)**	4.32±0.32 (50.1)**	0.67±0.02 (21.9)**
Metformin treated (100 mg/kg p. o)	0	87.5±4.89	89.7±2.65	89.2±3.35	8.80±0.57	0.86±0.07
	10	72.5±2.99 (11.4)^{ns}	81.0±4.48 (8.99)^{ns}	79.8±2.60 (7.36)^{ns}	7.32±0.54 (14.4)*	0.75±0.05 (9.90)^{ns}
	20	61.5±2.62 (26.8)**	77.8±3.93 (12.7)*	77.0±5.02 (12.5)*	6.29±0.33 (26.7)**	0.74±0.03 (13.5)*
	30	57.3±2.39 (33.8)**	68.0±2.82 (23.7)**	69.3±1.72 (21.4)**	5.13±0.60 (40.7)**	0.59±0.05 (31.1)**

Values are mean±SE. of six rats. p*<0.05, p**<0.01, ns: not significant

Effect of aqueous fraction of aqueous extract of *I. batatas* leaves and metformin on fasting blood glucose and OGTT of neonatally STZ induced diabetic rats

Table 7 depicts the effect of an aqueous fraction of aqueous extract of *I. batatas* leaves and metformin on fasting blood glucose and oral glucose tolerance of post glucose loaded neonatally STZ-induced diabetic rats. Mentally STZ-induced diabetic rats showed abnormal glucose tolerance after 12 weeks compared to sham treated control (table 7). The aqueous fraction of aqueous extract of *I. batatas* leaves caused a decline in fasting blood glucose level and improved oral glucose tolerance of neonatally STZ-induced diabetic rats start from 7 days, which continued up to the termination of experiment i.e. on day 28 as shown in table 7. The treatment of aqueous fraction of *I. batatas* leaves and metformin at the doses of 100 mg/kg b.w. to these diabetic rats caused significant improvement on their glucose intolerance.

The aqueous fraction of *I. batatas* leaves treatment improved their OGTT around 22.6% (p<0.01) and 51.8% (p<0.01) on 14 and 28 days, respectively. Whereas, metformin improved the OGTT around 30.5% (p<0.01) and 59.2% (p<0.01) on day 14 and 28, respectively.

Table 7: Effect of aqueous fraction of aqueous extract of *I. batatas* leaves and standard drug, metformin on fasting blood glucose, improvement in OGTT of neonatally STZ treated diabetic rats

Group	Fasting blood glucose (mg/dl)					Oral Glucose tolerance (0-120 min) (AUC±SEM)				
	Day 0	Day 7	Day 14	Day 21	Day 28	Day 0	Day 7	Day 14	Day 21	Day 28
Normal Control (1.0% Gum acacia)	76.7±3.57	80.5±2.54	80.9±2.87	88.7±2.62	89.1±3.04	12441±147.2	12569±154.3	12850±132.2	12910±28.3	12810±189.3
Sham Control (1.0 % gum acacia)	191.7±3.39	190.8±3.59	191.3±3.34	192.7±2.78	193.7±2.26	46070±642.5	47090±563.9	47580±959.1	47340±1519.0	51920±1590.0
Aqueous fraction of aqueous extract of *I. batatas* leaves treated (100 mg/kg p. o)	191.8±3.10	161.3±4.22 (15.5)*	144.0±3.39 (24.7)**	111.5±2.01 (42.1)**	106.8±1.76(44.8)**	46230±117.7	39710±565.0 (15.7)*	36850±719.6 (22.6)**	23940±970.2 (49.4)**	25030±752.6 (51.8)**
Metfor	191.7±1	164.0±2.	129.5±3.	104.7±2.	107.2±1.81	46830±84	36900±60	33080±19	23280±665	21200±768

min treated (100 mg/kg p. o)	5.6	38 (14.0)**	03 (32.3)**	24 (45.6)**	(44.7)**	5.1	27 (21.6)**	5.9 (30.5)**	.6 (50.8)**	.7 (59.2)**

Values are mean±SE. of six rats. p*<0.05, p**<0.01, ns: not significant

Effect of aqueous fraction of aqueous extract of *I. batatas* leaves and metformin on serum insulin level of neonatally STZ treated diabetic rats

Fig. 5 represents the effect of an aqueous fraction of aqueous extract of *I. batatas* leaves and metformin on serum insulin levels of neonatally STZ-induced diabetic rats at the doses of 100 mg/kg b.w. In the aqueous fraction of *I. batatas* leaves and metformin treated groups, the serum insulin levels were increased by 47.4% and 29.2%, respectively on day 30 post treatment.

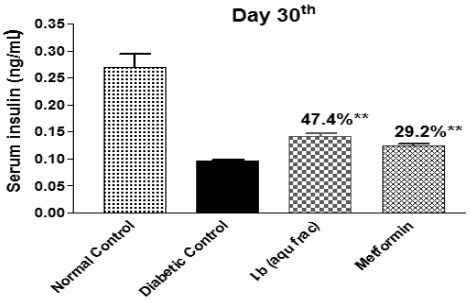

Fig. 5: Effect of aqueous fraction (aqu frac) of aqueous extract of *I. batatas* (I. b) leaves and standard drug, metformin on serum insulin profile of neonatally STZ induced diabetic rats. Statistical significance p<0.01 compared to sham control**

Effect of aqueous fraction of aqueous extract of *I. batatas* leaves on the 2-DG uptake

Fig.6 represents the effect of an aqueous fraction of aqueous extract of *I. batatas* leaves on glucose uptake in L6 cells. Aqueous fraction of *I. batatas* leaves were evaluated for glucose uptake assay using 2-deoxyglucose (2-DOG) in L6 cells, increased basal glucose uptake in a concentration dependent manner in L6 cells.

Aqueous fraction of *I. batatas* leaves increased basal glucose uptake in L6 myotubes to a significant level at a minimum concentration of 10 µg/ml (1.37-fold, p<0.05). Maximum stimulation was observed at 20 µg/ml concentration (1.61-fold, p<0.05). Pre-incubation of myotubes with metformin for 16 h at a 500 µM concentration increases the glucose uptake 1.78-fold (p<0.01).

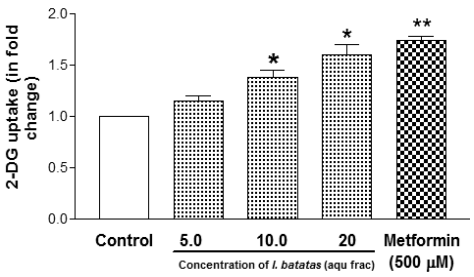

Fig. 6: Effect of aqueous fraction of *I. batatas* leaves and standard drug, metformin at 500 µM concentration on 2-deoxyglucose uptake by differentiated myotubes (L-6). Results are expressed as fold change over control. Values are mean±S. E. of three independent experiments; p values **<0.01, *<0.001 relative to control**

Effect of aqueous fraction of aqueous extract of *I. batatas* leaves on α-glucosidase enzyme

Fig. 7 shows the inhibition on α-glucosidase by an aqueous fraction of *I. batatas* leaves and acarbose. The aqueous fraction of *I. batatas* leaves exerted an inhibitory effect on a α-glucosidase by showing around 62.9% inhibition at the concentration of 100 µg/ml compared the acarbose which showed around 66.2% inhibition on α-glucosidase at 100 µg/ml. The aqueous fraction of *I. batatas* leaves caused 50% inhibition (IC_{50}=53.0 µg/ml). Whereas, the standard drug, an acarbose showed 50% inhibition at (IC_{50}=43.6 µg/ml) (fig.7).

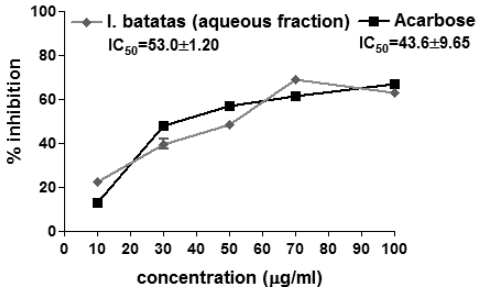

Fig. 7: *In vitro* inhibition of α-glucosidase enzyme by the aqueous fraction of *I. batatas* leaves. IC_{50}=Values are means±SE of three replicates

Effect of aqueous fraction of aqueous extract of *I. batatas* leaves, on aldose reductase (AR) activity from the eye lenses of both normal rats and STZ-induced diabetic rats

The specific activities of AR in normal and streptozotocin-induced diabetic rats were calculated to be around 0.0823 and 0.1493 µmol/min/mg protein, respectively. Quercetin, a standard inhibitor, showed 50% inhibition (IC_{50}) on aldose reductase at 6.04µg/ml (20µM) in normal rat eye lens and at 0.69 µg/ml (2.1 µM) in diabetic rat eye lens. The aqueous fraction of *I. batatas leaves* showed significant 50% inhibition on aldose reductase from the dose response curve at 3.31 µg/ml in normoglycemic rats and IC_{50} for aldose reductase activity from eye lens of diabetic rats at 5.81 µg/ml as shown in fig. 8A & 8B.

DISCUSSION

Ipomoea batatas L. (Sweet Potato), has been recognized as a folk medicine for the treatments of diabetes and other metabolic diseases [19, 20]. Lako *et al.*, [21] results, showed that sweet potato leaves have high contents of total phenolics and flavonoids that could have profound benefits for combating chronic degenerative disorders [22, 23]. White-skinned sweet potato has been known to exhibit antihyperglycemic activity in normal rats and diabetic rats [8, 9, 20]. It has been shown that the active ingredients of white-skinned sweet potato, presumably an acidic glycoprotein [9], prevents and improves the symptoms of diabetes and hypoglycemia as well as, stimulate the immune response [24]. Liao *et al.*, [25] study demonstrated that the water extract of sweet potato leaves has strong antioxidant activity.

In the present study, ethanolic, ethanolic: aqueous and aqueous extracts of *I. batatas* leaves were evaluated for their antihyperglycemic effect on sucrose loaded normal rats and STZ-induced diabetic rats. Of all these extracts, the aqueous extract of *I. batatas* leaves showed most significant improvement in the blood

glucose profile of post sucrose loaded normal rats and STZ-induced diabetic rats compared to that of standard antidiabetic drugs, glybenclamide and metformin, respectively, indicating the insulin mimetic, insulin secretaceous or insulin sensitizing effect of aqueous extract of leaves of *I. batatas* for glucose utilization by peripheral tissues on postprandial rise hyperglycemia in sucrose loaded normal and STZ-induced diabetic rats or attributed to inhibition of the intestinal α-glucosidase enzymatic action of the breakdown of polysaccharide sugars in its monomeric form and thus inhibiting the postprandial increase of plasma blood glucose.

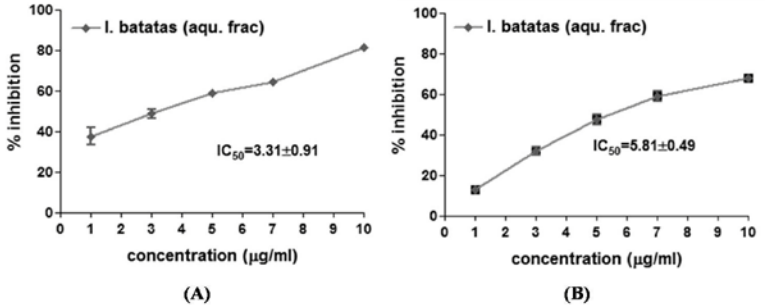

Fig. 8: *In vitro* inhibition of aldose reductase by the aqueous fraction of *I. batatas* leaves (A) Normal eye lens (B) Diabetic eye lens. IC_{50}=Values are means±SE of three replicates

Further, the isolated fractions, from the aqueous extract of *I. batatas* leaves were evaluated for their antihyperglycemic effect on STZ-induced diabetic rats. Of these fractions, the aqueous fraction of the leaves of *I. batatas* showed most significant decline in fasting blood glucose level of STZ-induced diabetic rats. However, antidiabetic efficacy and hypoglycemic mechanisms of aqueous fraction of aqueous extract of *I. batatas* leaves are not fully defined in different diabetic models. Therefore, in the present study, antihyperglycemic and anti-dyslipidemic activities of the aqueous fraction of *I. batatas* leaves were identified in validated animal models of type 2 diabetes mellitus i.e. STZ-induced diabetic rats; and 2-days neonates (n) 2-STZ-induced diabetic rats.

STZ has been used to induce diabetes in animals in the present study as, STZ injection selectively destroys pancreatic β-cells and leads to hyperglycemia, markedly declining the level of serum insulin and its biosynthesis and produces diabetes in STZ treated rats [26]. The persistent hyperglycemia is responsible for the development of diabetic complications like dysfunction, and failure of different organs, especially the eyes, renal and blood vessels. The STZ induced model is the confirmatory model for the screening of antidiabetic agents for diabetes mellitus. The glycosylated haemoglobin increase in the state of persistent hyperglycemia, positively correlate with the concentration of glucose in the blood [27, 28].

In the present study, STZ induced diabetic rats when treated with an aqueous fraction of aqueous extract of *I. batatas* leaves and metformin showed significant reduction in the HbA1c level on day 30post treatment, however, significantly no gain in body weight was observed. In accordance with the results of Rasineni *et al.*, [29] similar diabetic features such as polyphagia, polydipsia, and polyuria, loss of body weight, hypo insulinemia, hyperglycemia and hyperlipidemia were observed in the STZ-induced diabetic rats in the present study. Despite of high food intake, a reduction in the body weight of diabetic rats was reasonable because of excessive catabolism of fats and protein [28, 29]. It has been shown that both alloxan and STZ-induced diabetic rats have insulin deficiency, responsible for the accumulation of lipids [30].

It is evident from the present study that the treatment with the aqueous fraction of *I. batatas* leaves significantly improved the oral glucose tolerance, increases the insulin production and decreases the serum level of TG, TC and LDL-C, while increases the serum level of HDL-C in STZ-induced diabetic rats, an insulin increase might lead to activation of the enzyme lipoprotein lipase which further lowers the serum triglyceride level. These results are in accordance with previous studies on the white skinned tuber roots of sweet potato (*I. batatas*), declined the blood glucose level in STZ-induced type 2 diabetic rats by inducing pancreatic β-cells regeneration, increasing insulin expression, secretion and re-granulation of pancreatic islet beta cells, improvement in β-cells mass [6, 7, 9, 20].

The chronic hyperglycemia also induces the elevation of serum levels of of ALT, AST, and urea, uric acid and creatinine, which are considered as the significant markers of liver and renal dysfunctions [31-33]. The oral administration of the aqueous fraction of *I. batatas* leaves to STZ-induced diabetic rats for 30 days improved the liver and renal functions as evidenced in the present study by significant (p<0.01) decreased in the serum level of ALT, AST, and urea, uric acid, and creatinine, thus indicating the hepatoprotective and renoprotective activity exhibited by the aqueous fraction of *I. batatas* leaves.

The neonatal-STZ treated rats are suitable model of type 2 diabetes as it has the potential advantage over others by exhibiting the various stages of type 2 diabetes mellitus such as impaired glucose tolerance, mild, moderate and severe hyperglycemia with alteration of dose and days of STZ injection. It has been shown in the previous studies that neonatally STZ-induced diabetic rats exhibits lowered plasma insulin level, increased glucose levels and reduction in pancreatic insulin content [34]. The neonatally STZ-induced diabetic rats are considered to be better tools for the elucidation of the mechanisms associated with regeneration of the beta cells, the functional exhaustion of the beta cells and the emergence of defects in insulin action [35, 36]. A single injection of STZ at the dose range of 80-100 mg/kg of STZ to one or two or five days old Sprague-Dawley neonatal rats has been reported to produce type 2 diabetic conditions [35, 37]. The results of the present study indicate that neonatally STZ induced diabetic rats develop moderate type 2 diabetes when compared with normal rats, however, the animals showed gradual improvement in oral glucose tolerance and serum insulin level on treatment with the aqueous fraction of *I. batatas* leaves in comparison to the control group as shown in table 7 and fig. 5.

The α-glucosidase are found on the surface of brush border of small intestinal cells, which catalyzes carbohydrates into oligosaccharide units and α-glucosidases inhibitors inhibit maltase, sucrose and other disaccharide hydrolases (i.e., represses the degradation of disaccharides, oligosaccharides, and polysaccharides into monosaccharides) in the brush border membrane of the small intestine [38, 39]. In the present study, the aqueous fraction of aqueous extract of *I. batatas* leaves showed marked *in vitro* inhibition of α-glucosidase enzyme, it indicates the potential enzyme inhibiting constituents present in the aqueous fraction of *I. batatas* leaves. Drugs that diminish the postprandial hyperglycemia by repressing the absorption of starches are shown to be efficient for prevention and management of type 2 diabetes [39]. Several natural existing flavonoids possess hypoglycemic potential and have been depicted as α-glucosidase inhibitors; they have been considered as a promising source for drug development and have also demonstrated their marked biological effects in both *in vitro* and *in vivo* system [40]. AR is the first enzyme in the polyol pathway, which catalyzes

the reduction of glucose to the corresponding sugar alcohol, sorbitol utilizing NADPH as a cofactor, which is subsequently metabolized to fructose by sorbitol dehydrogenase [41, 42]. Sorbitol accumulation leads to osmotic swelling, membrane permeability changes and oxidative stress, which finally cause cellular injury [42, 43]. Many studies with experimental animals suggest that inhibition of aldose reductase could be effective in the prevention of some diabetic complications, including cataract, retinopathy, nephropathy and neuropathy [44]. In the present study the aqueous fraction of *I. batatas* leaves also showed *in vitro* inhibition of aldose reductase (AR) activity in a dose-dependent manner. It is evident from the results shown in fig. 7 and fig. 8 that the aqueous fractions of aqueous extract of *I. batatas* leaves possess both an α-glucosidase, and aldose reductase inhibitory activities along with antidiabetic properties, is a good finding in the field of treatment of diabetes mellitus.

L6 muscle cell lines have been considered as a suitable *in vitro* model to study the glucose transport activity [45], since skeletal muscles are the major site for primary glucose disposal and glucose utilization [46, 47]. It was found in the present study that the aqueous fraction of *I. batatas* leaves significantly enhances the basal glucose uptake in a concentration dependent manner, with most significant stimulation in glucose uptake at the concentration of 20 µg/ml showed in L6 skeletal muscle cells. Thus, the *in vitro* glucose uptake activity displayed in L6 cells indicate the antihyperglycemic effect exhibited by the aqueous fraction of *I. batatas* leaves.

In the present study the antihyperglycemic and antidyslipidemic effects have been shown to be exhibited by the aqueous fraction of the leaves of *Ipomoea batatas*. The studies in the literature, are available which have demonstrated that the leaf extract of *Ipomoea batatas* contains phytochemicals like alkaloids and flavonoids like anthocyanins, saponins which are known to posses' potent antidiabetic and hypolipidemic properties [48, 49]. Anthocyanins are natural water-soluble pigments and impart colors in fruits, leaves, and flowers of plants. Hence, the antidiabetic effect of the aqueous fraction of the leaf extract of *Ipomoea batatas* might be due to the high level of occurrence of anthocyanins, which mediate the lowering of blood glucose level and improvement in TG cholesterol in serum in type 2 diabetic models used in the study.

In conclusion, the aqueous fraction of *I. batatas* leaves have definite antihyperglycemic, antidyslipidemic, α-glucosidase, and aldose reductase inhibiting activities; it also demonstrates improvement in insulin secretion and increase the sensitivity of the insulin dependent tissues, i.e. the liver, and muscle tissues resulting in glucose utilization. Thus, the present study advocates the permissibility of isolation, identifying the active compounds and synthesis of plant based antidiabetic agents.

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CONFLICT OF INTERESTS

Declared None

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