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Anti-Diabetic Activity and Phytochemical Screening of Crude Extracts of PuerariaTuberosa DC. (FABACEAE) Grown in India on STZ -Induced Diabetic Rats

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ABSTRACT: The available drugs for diabetes, Insulin or Oral hypoglycemic agents have one or more side effects. Search for new antidiabetic drugs with minimal or no side effects from medicinal plants is a challenge according to WHO recommendations. In this aspect, the present work was undertaken to study indigenous plant Puerariatuberosa DC. (Fabaceae) is a perennial climber, commonly known as Vidarikand or Indian Kudzu. The tuber of P. tuberosa is used in indigenous system of Indian medicine as tonic, aphrodisiac, antirheumatic, diuretic and galactogogue. In the present study petroleum ether, chloroform, ethanol and aqueous extracts of P. tuberosa tubers were subjected to phytochemical investigation and evaluated for antidiabetic activity in Streptozotocin (STZ) induced diabetic rats. Dose selection was made on the basis of acute oral toxicity study (300-5000 mg/kg body weight) as per OECD guidelines. Oral glucose tolerance test was performed in experimental diabetic rats. Diabetes was induced by a single intraperitoneal injection of STZ (50 mg/kg b.w.). After STZ induction, the hyperglycemic rats were treated with all four extracts orally at the dose 500 mg/kg b.w. daily for 21 days. Glibenclamide (2.5 mg/kg b.w., p.o.) was used as reference drug. Fasting blood glucose level and changes in body weight were measured on days 0, 7, 14, and 21. Statistical analyses were performed using one-way ANOVA followed by Bonferronni's multiple comparison tests. Steroid, triterpenoid, glycoside, carbohydrate, alkaloids, flavanoid, tannin, protein and amino acid were found in the group tests. All the extracts at oral doses of 500 mg/kg b.w. significantly ($p<0.001$) exhibited antidiabetic activity in STZ-induced diabetic rats by reducing and normalizing the elevated fasting BGL as compared to those of STZ control group. The ethanol and aqueous extracts were most active. This study concludes that P. tuberosa tuber confirmed promising antidiabetic activity in STZ-induced diabetic rats.

Key words: PuerariaTuberosa, Streptozotocin, Glibenclamide, AntidiabeticActivity

ORIGINAL ARTICLE

INTRODUCTION

Diabetes is a chronic disease that occurs either when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin it produces. Insulin is a hormone that regulates blood sugar. Hyperglycaemia is a common effect of uncontrolled diabetes and over time leads to serious damage to many of the body's systems, especially the nerves and blood vessels. Long-term diabetes is associated with several multifactorial diseases, such as erectile dysfunction, blindness, poor wound healing, kidney failure, heart disease, etc. as a result of considerable damage, dysfunction, and failure of various organs that develop as the disease progresses.The incidence of diabetes worldwide is now estimated to be around 366 million, far beyond the 285 million projected by WHO for 2010 from global statistics gathered in 2008 (Danaei et al., 2011). This means that there may have been more than 4 million deaths or 6.8% of global mortality in 2010 that could be attributed directly or indirectly to diabetes (Roglietal., 2010). More than 80% of diabetes deaths occur in low- and middle-income countries (Mathers and Loncar, 2006). WHO projects that diabetes will be the 7th leading cause of death in 2030 (WHO, 2011). The two major forms of the syndrome result from either lack of the metabolism regulatory hormone, insulin (type 1 diabetes), or because body tissues fail to respond to the hormone (type 2 diabetes).Type 1 diabetes (previously known as insulin-dependent, juvenile or childhood-onset) is characterized by deficient insulin production and requires daily administration of insulin. The cause of type 1 diabetes is not known and it is not preventable with current knowledge. Symptoms include excessive excretion of urine (polyuria), thirst (polydipsia), constant hunger, weight loss, vision changes and fatigue. These symptoms may occur suddenly.Type 2 diabetes (formerly called non-

insulin-dependent or adult-onset) results from the body's ineffective use of insulin. Type 2 diabetes comprises 90% of people with diabetes around the world (WHO, 1999), and is largely the result of excess body weight and physical inactivity. Symptoms may be similar to those of Type 1 diabetes, but are often less marked. As a result, the disease may be diagnosed several years after onset, once complications have already arisen. Until recently, this type of diabetes was seen only in adults but it is now also occurring in children.Gestational diabetes is hyperglycaemia with onset or first recognition during pregnancy. Symptoms of gestational diabetes are similar to Type 2 diabetes. Gestational diabetes is most often diagnosed through prenatal screening, rather than reported symptoms (WHO, 1999).There are different approaches to the treatment of diabetes, like insulin treatment in type 1 diabetes: Sulphonylureas, which release insulin from pancreas by blocking the ATP-sensitive potassium channels; Biguanides, which decrease the insulin resistance; Thizaolidinediones, which increase the insulin sensitivity; alphaglucosidase inhibitors like acarbose, which decrease glucose absorption from intestine, thereby decreasing postprandial hyperglycemia; metiglinides like repaglimide and nateglamide, which are insulin secretogogues (Tripathi, et al, 2012).These drugs are used as monotherapy or in combination to achieve better glycemic control. Each of the above oral antidiabetic agents is associated with a number of serious adverse effects (Moller, 2001). Hence antidiabetic drug discovery has shifted its focus to natural plant sources having minimal side effects (Aiman, 1970).Traditional antidiabetic plants might provide new oral anti-diabetic compounds, which can counter the high cost and poor availability of the current medicines for many rural populations in developing countries (Noor et al., 2008). Plant drugs are frequently considered to be less toxic and free from side effects than synthetic ones (Valiathan, 1998).

Concurrently, phytochemicals identified from traditional medicinal plants present an exciting opportunity for the development of new types of therapeutics. Phytochemicals can offer a new avenue to greatly impact the onset and progression of chronic diseases, oxidant stress and ageing. The phytoprotectants act as bioenhancers of several physical and biochemical processes (Krishnaswamy, 2008).In pursuit of this goal, the present work was undertaken to study indigenous plant Puerariatuberosa DC (Family: Fabaceae) is a reputed medicinal herb of Indian traditional system of medicines distributed throughout tropical parts of the India (Khare, 2008). It is a perennial woody climber which grows up to 6 cm tall. The leaves are compound, opposite, trifoliate, orate and coriaceous. Pods are flat, constricted between seeds (Gupta, 2003). The plant perinnates by producing large tubers (up to 10kg) at a depth of 1-2m and it has several vernacular names like in Sanskrit: Vidari, Bengali: shimia, English: Indian Kudju, Gujarati: vidarikand; Hindi: bilaikand; Kannada: gumadigida; Malayalam: mutukku; Marathi: badra; Tamil and Telugu: darigummadi. The tuberous roots are laxative, emetic, diuretic, cardiotonic, expectorant and are useful in arthiritis, burning sentation, constipation, and cardiac debility (Pullaiah, 2006). The tribles of Kesla block of Hoshangabad district, Madhya Pradesh have been using tubers of this plant for the treatment of diabetes (Jain, 2005). Earlier the plant has been studied for its, adaptogenic (Babu et al., 2006), antifertility (Gupta et al., 2004), antiinflammatory, wound healing (Kambhoja and Murthy, 2007), contraceptive (Prakash et al., 1985), antioxidant, antistress (Pramanik et al., 2010), hypoglycemic (Raghuwanshi, 2012), and nootropic (Venkata et al., 2008) activities.Considering the varied important activities reported in traditional system of medicine with this plant. It was planned to study the effects of tubers extracts of P. tuberosa DC for its antidiabetic activity. The present study reports the changes of blood glucose levels by administration to STZ- induced diabetic rats.

Figure 1: P. tuberosa tuber

MATERIALS AND METHODS

Experimental animals:

Wistar albino rats of 4 months, of both sexes, weighing between 100 to 200 gm. were used. They were provided from Sapience Bio-analytical Research Lab, Bhopal, and (M.P.). The experiment protocol was approved by the institutional ethics committee and as per CPCSEA guidelines (approval no. 1413/PO/a/11/CPCSEA).

Drugs and Chemicals:

Chemicals used in the study were of analytical grade and were procured from Merck specialties private limited, Mumbai, India. Streptozotocin was purchased from Himedia, India. Glibenclamide was provided as gift sample by Bioplus life sciences, Bangalore.

Plant Materials:

The tubers of P. tuberosa (Fabaceae) were purchased from local area of Jabalpur, (M.P.) India. Parts of plant were inspected to be healthy and botanically

identified and authenticated by Dr D.K. Pahalwan, Senior Scientist & PI-IVLP Directorate of Extension J.N.K.V.V., Jabalpur (M.P.). All voucher specimens were deposited in Department of Crop & Herbal Physiology, J.N.K.V.V., Jabalpur, (M.P.) for future reference.

Extraction of plant material and preparation of test dose: About 400 gm of coarse dried powder of tubers of P. tuberosa was taken in the soxhlet apparatus and extracted successively using different solvents according to their increasing order of polarity, for the present investigation (i.e. petroleum ether→ chloroform→ ethanol). The extraction for each solvent was carried out for 18 to 24 hours. Finally crude drugs were macerated with water. The extract was collected by evaporating the solvents by slow heat treatment. Percentage yield of obtained extracts were calculated (Harborne, 1998).

Proximate analysis:

Ash values: Quantitative estimation of ash value total, acid insoluble ash, and water- soluble ash may serve as useful indices for identification of the powdered drug was performed as per the reported methods (Khandelwal, 2002).

Extractive values: Extracts were prepared with various solvents. Percentages of the extractive values were calculated with reference to air-dried drug (Kokate, 1994).

Fluorescence analysis: Many drugs fluorescence when their powder is exposed to ultraviolet radiation. It is important to observe all materials on reaction with different chemical reagents under U.V. light. The fluorescence characteristics of powdered drug were studied under U.V. light after treating with different chemical reagents is reported (Khandelwal, 2002).

PH value: The pH value of the solutions was determined by means of standard glass electrode, a reference electrode and a digital pH meter. First the apparatus was calibrated using buffer of 4, 9 and 7 pH. 5 g powdered drug were taken and dissolved in 100 ml demineralized water (5%w/v solution) and 10 g powdered drug were taken and dissolved in 100 ml demineralized water (10 % w/v solution). The electrodes were immersed in the solution and the pH was measured.

Phytochemical Screening: The extracts were subjected to preliminary Phytochemical analysis in order to detect the presence of various groups of phytoconstituents by carrying out the following chemical analysis such as sterols and triterpenoids (Salkowski; Libermann-Burchard and Libermann'stest), Carbohydrates (Molisch's; Fehling's solution; Benedict's and Barfoed's test) Tannins and phenol compounds (Ferric chloride; Lead acetate and Gelatin solution test), glycosides (Keller-Killiani and Baljet's test), alkaloids (Mayer's; Dragendorff's; Wagner's and 1% picric acid reagents), Saponin (frothing and haemolysis tests), Proteins (Biuret; Million's and Xanthoprotein test), Amino Acids (Ninhydrin test) and Flavonoids (Shinoda test) are identified using various reagents (Tripathi and Kohli, 2012).

Acute oral toxicity: Acute toxicity studies were performed according to the guidelines set by Organization for Economic Co-operation and Development (OECD), received draft guidelines 423, received from Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India. Administration of the stepwise doses of all four extracts of P. tuberosa tubers from 300 mg/kg b.w. up to a dose of 5000 mg/kg b. wt. caused no considerable signs of toxicity in the tested animals. One -tenth of the upper limit dose was selected as the level for examination of antidiabetic activity (OECD, 2000).

Experimental models:

Oral glucose tolerance test (OGTT): OGTT for non-diabetic rats were performed according to the standard method (Du Vigneaud and Karr, 1925). In short, Group I to Group VII was selected for OGT test after starving at water for Diagnostics Private Limited, Delhi). Group I stands for normal control group. Group II served as diabetic control received glucose (2g/kg b.w.) in the form of solution. Group III is treated with Glibenclamide (2.5 mg/kg b.w.). Groups IV-VII received the different fractions of P. tuberosa at a dose of 500 mg/kg b.w. as a fine 0.5 % CMC suspension. After 30 min of extract administration, the rats of all groups were orally treated with 2 g/kg of glucose. Serum glucose of blood sample from tail vein was estimated by using glucometer at 0, 30, 60 and 90 min.

Streptozotocin induced diabetic model: The animals were allowed to fast for 24 hr and a freshly prepared solution of Streptozotocin (50 mg/kg, i.p.) in 0.1M citrate buffer, pH 4.5, was injected intra-peritoneally in the animals (single injection) in a volume of 1ml/kg body wt. After the animals were left aside for 4 hrs and then 10 % glucose solution was placed in the cages for 24hrs. The diabetes was confirmed by estimation of blood glucose level (BGL) on the third day. Rats having BGL > 140mg/dl were used for study and during the experiment the animals were divided into seven groups of six animals in each group.Group I served as a control which received vehicle alone. Group II kept as negative control, i.e., treated with STZ (50 mg/kg, i.p). Group III received Glibenclamide (2.5 mg/kg) after 3rd day of the treatment with STZ (50 mg/kg, i.p). Group IV treated orally with of 500 mg/kg b.w. P. tuberosa petroleum ether extract (PEPT) after 3rd day of the treatment with STZ (50 mg/kg, i.p). Group V treated orally with of 500 mg/kg b.w. P. tuberosa chloroform extract (CEPT) after 3rd day of the treatment with STZ (50 mg/kg, i.p). Group VI treated

orally with of 500 mg/kg b.w. P. tuberosa aqueous extract (AEPT) after 3rd day of the treatment with STZ (50 mg/kg, i.p) and Group VII treated orally with of 500 mg/kg b.w. P. tuberosa ethanol extract (EEPT) after 3rd day of the treatment with STZ (50 mg/kg, i.p) for 21 days. The blood glucose concentrations and body weight of the animals were measured using glucometer at the beginning of the study and measurements were repeated on 7th day, 14th day and 21st day of the experiment. The blood glucose levels were expressed in mg/dl. The data was represented as mean blood glucose level and standard error of mean (SEM) (Pinakini et al., 2005).

Statistical Analysis:

The data are expressed as mean ± SEM. The results were analyzed statistically by software (Graph pad instat 3) using ANOVA followed by Bonferronni's multiple comparison tests. The minimum level of significance was fixed at $p<0.05$.

RESULTS

Organoleptic evaluation of plant material is shown (Table 1). The proximate analysis revealed that moisture content was 4.12 %, total ash 9.6 %, acid insoluble ash 3.2%, water soluble ash 2.8 %, alcohol soluble extractives 12 %, water soluble extractives 25%, chloroform soluble extractives 3.6 % and petroleum ether soluble extractives 1.8% (Table 2). The fluorescence analysis of powdered material was subjected to analysis under Long Ultra Violet light after treatment with various chemical and organic reagents is shown (Table 3).

Table 1: Organoleptic Characters

S No.	Name of plants	Part used	Description		
			Color	Odor	Taste
1	*P. tuberosa*	Tuber	Light brown	No characteristic	Sweet

Table 2: Physico-chemical parameters of P. tuberosa tubers

S. No.	Parameters studied	Values obtained on dry weight basis (w/w)
1	Loss on drying	4.12 % w/w
2	Total Ash	9.6 % w/w
3	Acid insoluble Ash	3.2 % w/w
4	Water soluble Ash	2.8 % w/w
5	Alcohol soluble extractives	12 % w/w
6	Watersoluble extractives	25% w/w
7	Chloroformsoluble extractives	3.6 % w/w
8	Pet-Ethersoluble extractives	1.8 % w/w

Table 3: Florescence analysis of crude drug (P. tuberosa tubers)

S No.	Solvent used	Ordinary light	UV light 254 nm	UV light 366 nm
1	Powder as such	Light brown	Buff	Light brown
2	Powder + dilHcl	Reddish brown	Dark brown	Light brown
3	Powder + dil HNo_3	Yellow	Light brown	Yellow
4	Powder + dil H_2So_4	Reddish black	Black	Brownish black
5	Powder + glacial acetic acid	Yellow	Light brown	Brown
6	Powder + 5% NaOH	Light green	Light green	Brown
7	Powder + 5%KOH	Brownish black	Light green	Brown
8	Powder + 5%$Fecl_3$	Yellowish brown	Brown	Yellow
9	Powder + ammonia	Buff	Yellowish brown	Dark brown
10	Powder + Pet Ether	Light brown	Light green	Light brown
11	Powder + benzene	Reddish brown	Light green	Light brown
12	Powder + acetone	Light brown	Light green	Brown
13	Powder + ethyl acetate	Buff	Light green	Light brown
14	Powder + ethanol (95%v/v)	Light brown	Light green	Light brown
15	Powder + water	Light brown	Dark brown	Brown

The pH of powdered drug solution at 5% and 10% w/v concentration was found to be 5.45 and 6.14 respectively as shown (Table 4). Successive solvent extraction values in various organic solvent were observed as petroleum ether 2.30 %, chloroform 1.50%, and ethanol 10 % and aqueous solvent 15% as shown (Table 5). The aqueous extract of tubers of P. tuberosa results maximum yield value than that of petroleum ether extract, chloroform extract and ethanol extract. All extracts subjected to chemical evaluation and results are shown (table 6). In petroleum ether extract, steroid, triterpenoid and alkaloid were prominently seen. The chloroform extract showed presence of steroid, glycoside, alkaloid and flavanoid. Whereas ethanol extract was found to contain steroid, triterpenoid, glycoside, tannin, protein and amino acid. The aqueous extract was observed with steroid, triterpenoid, glycoside, carbohydrate, flavanoid, tannin, protein and amino acid respectively.

The data in the table is the mean ± SEM (n = 6 wistar rats per groups), p<0.05, ** p<0.01, ***p<0.001 compared with multiple groups using Bonferronni's multiple comparison test followed by one way ANOVA. a - Significance difference as compare to Control (Group-I) , b- Significant inhibition as compare to Negative control (Group-II), c- Significance difference as compare to Standard (Group-III).

The data in the table is the mean ± SEM (n = 6 wistar rats per groups), * p<0.05,** p<0.01,***p<0.001 compared with multiple groups using Bonferronni's multiple comparison test followed by one way ANOVA., a- Significance difference as compare to Control (Group-I), b -Significant inhibition as compare to Negative control (Group-II) c -Significance difference as compare to Standard (Group-III).

Table 4: pH of crude drugs

S. No.	Solution of different concentration	pH values (*P. tuberosa* tubers)
1	pH of 5% solution	5.45
2	pH of 10% solution	6.14

Table 5: Percentage yield of various extracts of P. tuberosa tubers

S. No.	Solvent	Color	% Yield (w/w)
1	Pet-ether (60-80°C)	Yellowish brown	2.30
2	Chloroform	Dark brown	1.50
4	Ethanol (95% v/v)	Dark brown	10
5	Water	Cream coloured	15

Table 6: Phytochemical screening of different extracts of P. tuberosa tubers

S. No.	Phytoconstituents	Pet-ether (60-80°c)	Chloroform	Ethanol	Water
1	Steroids	+	+	+	+
2	Triterpenoids	+	-	+	+
3	Saponins	-	-	-	-
4	Glycosides	-	+	+	+
5	Carbohydrates	-	-	-	+
6	Alkaloids	+	+	-	-
7	Flavonoids	-	+	-	+
8	Tannins & Phenolic	-	-	+	+
9	Proteins	-	-	+	+
10	Amino acid	-	-	+	+

+ Present; - Absent

Table 7: Effect of different extracts on blood glucose levels on glucose loaded normal rats (OGTT Study)

Groups	Treatment	Blood Glucose (mg/dl)		
		0 min	30 min	90 min
I	Normal Control (NC)	79.12±2.34	82.45±1.24	80.13±2.7
II	Negative control	89.45±2.34	110.57±2.67a**	135.12±1.45 a***
III	Std(Glibenclamide 2.5 g/kgb.w)	76.23±2.56	80.37±1.65 b**	91.34±2.55 a**,b***
IV	Test (PEPT 500 mg/kg)	78.23±2.25	97.57±1.23 a**,b***,c***	104.24±2.78 a**,b**,c**
V	Test (CEPT 500 mg/kg)	84.47±2.78	98.87±1.76 a**,b**,c**	119.29±2.38 a***,b**,c**
VI	Test (AEPT 500 mg/kg)	73.37±2.45	95.45±2.78 a**,b***,c**	104.72±1.89 a**,b**,c**
VII	Test (EEPT 500 mg/kg)	78.27±3.02	89.56±2.99 a*,b**,c**	109.19±1.17 a**,b**,c**

*=p<0.05,**=p<0.01,***=P< 0.001

Table 8: Effect of different extracts of P. tuberosa on blood glucose level of STZ induced diabetic rats after prolonged treatment (after 21 days)

Groups	Treatment	Blood Glucose (mg/dl)			
		Initial value	Day 7	Day 14	Day 21
I	Normal Control (NC) (vehicle only)	85.23±2.45	82.83±4.63	83.17±3.08	87.18 ±4.62
II	Diabetic Control(DC), (STZ)	182.56±3.67	236.16±5.76 a***	256.46±3.85 a***	268.46±6.38 a***
III	Stand. (STZ +Glibenclamide)	171.53±5.51	130.57±3.65 a**,b***	111.34±5.55 a***,b***	106.33±6.16 a**,b***
IV	Test.(STZ+ PEPT)	176.23±5.85	167.07±5.83 a**,b***,c**	154.34±6.08 a**,b***,c**	148.83±3.45 a**,b***,c**
V	Test.(STZ+ CEPT)	188.47±4.78	187±1.76 a**,b**,c**	179.29±2.38 a**,b***,c**	174.47±2.78 a**,b***,c**
VI	Test.(STZ+ AEPT)	176.37±5.32	165.75±6.78 a**,b***,c**	134.75±8.80 a**,b***,c**	133.37±5.40 a**,b***,c**
VII	Test.(STZ+ EEPT)	178.27±6.82	159.56±2.99 a**,b***,c**	149.19±1.17 a**,b***,c**	128.27±3.02 a**,b***,c**

*=p<0.05,**=p<0.01,***=P< 0.001

Table 9: Effect of various extracts on body weight of STZ induced diabetic rats after prolonged treatment (after 21 days)

Groups	Treatment	Dose (mg/kg)b.w.	Average Body Weight (g)±SEM.			
			Initial	Day 7	Day 14	Day 21
I	Normal Control (NC) (vehicle only)	-	113.32±3.37	114.83±2.27	113.92±2.07	114.86±3.26
II	Diabetic Control(DC), (STZ)	50	120.63±2.26	118.42±3.91	110.93±2.26	108.62±3.45
III	Stand. (STZ +Glibenclamide)	2.5	121.21±2.75	114.34±2.54	111.21±2.45	110.38±1.54
IV	Test.(STZ+ PEPT)	500	177.11±1.66	172.69±2.93	170.21±3.60	172.09±2.98
V	Test.(STZ+ CEPT)	500	197.06±1.28	197.27±3.02	192.86±3.08	190.28±3.72
VI	Test.(STZ+ AEPT)	500	115.72±2.92	118.41±3.68	117.32±3.82	115.32±2.18
VII	Test.(STZ+ EEPT)	500	131.28±2.63	133.03±3.19	131.36±3.62	130.38±3.13

The data in the table is the mean ± SEM (n = 6 wistar rats per groups)

DISCUSSION

In acute toxicity study, none of the studied extracts of P. tuberosa tubers showed any significant toxicity sign when observed for the parameters during the first 3 hrs and followed by daily observations for 7 days and mortality was also not observed, the drug was found to be safe at the tested dose level of 5000 mg/kg b.w. One-tenth of this dose level was taken as effective dose. All the extracts were experimented at the same dose of 500 mg/kg b.w. In order to ascertain a scientific base for the usefulness of this plant in the treatment of diabetes, it was decided to evaluate experimental design of antidiabetic activity by following glucose tolerance test and STZ - induced model.The effect of different extracts on glucose tolerance test in normal rats is shown (Table 7). Result indicates that all extract showed significant tolerance. The blood glucose concentration increased rapidly from its initial value in normal control group 30 min after starting the glucose tolerance test, but the extract treated groups with 500 mg/kg b.w. of PEPT at 30 min and 90 min (97.57±1.23 a**, b***,c*** and 104.24±2.78 a**,b**,c**) and 500 mg/kg b.w. of AEPT at 30 min and 90 min (95.45±2.78 a**,b***,c** and 104.72±1.89 a**,b**,c**) prevented glucose- induced hyperglycemia significantly as compared to that of negative control at 30 min and 90 min (110.57±2.67a** and 135.12±1.45a***). Glibenclamide treated group (2.5 mg/kg) also prevented glucose induced hyperglycemia significantly at 30 min and 90 min (80.37±1.65 b** and 91.34±2.55 a**, b***) as compared to negative control at 30 min and 90 min (110.57±2.67a** and 135.12±1.45a***). Maximum glucose tolerance was observed in pet ether extract (104.24±2.78 a**, b***, c**) and minimum glucose tolerance was observed in chloroform extract of (119.29±2.38 a***, b**, c**) Puerariatuberosa in 90 minutes compared with the negative control (135.12±1.45a***). The pet ether (PEPT) and aqueous (AEPT) extracts of the tubers of P. tuberosa exhibited remarkable blood glucose lowering effect as compared to other extract at 90 min.Streptozotocin is a glucosamine-nitrosourea compound. As with other alkylating agents in the nitrosourea class, it is toxic to cells by causing damage to the DNA. During the decomposition of STZ, highly reactive carbonium ions are formed .which cause alkylation of DNA bases and also STZ may damage to β cell membrane and break the DNA strand which leads to the activation of poly (ADP- ribose) synthetase which

ultimately leads to cell death. Diabetes induced by STZ that produces a selective toxic effect on β cells and induces diabetes in most laboratory animals (Koteswara et al. 2006).

As expected in the diabetic control, there was severe hyperglycemia as compared to the normal animals. Compared to the diabetic control, all the four extracts (PEPT, CEPT, AEPT and EEPT) lowered the elevated blood glucose levels in sub-acute treatment. It was observed that the standard drug glibenclamide lowered the blood glucose level significantly, bringing it nearly back to normal, whereas AEPT and EEPT extract significantly ($P< 0.001$) decreased fasting blood serum glucose in the diabetic rats on 7th, 14th, and 21st days as compared to initial blood serum glucose levels (Table 8 & Fig. 2). When AEPT and EEPT extracts of P. tuberosa were compared for their antidiabetic activity in comparison to active control, particularly Glibenclamide, the results showed that their potential was lesser but significant ($P< 0.001$) than the standard drug at subacute level. Statistical analyses were performed by one way analysis of variance followed by Bonferronni's multiple comparison tests. From this data, mean change in blood glucose level and SEM were calculated and tabulated. The change in the body weight of animals in different groups during the study period is shown in (Table 9). Normal vehicle control animals were found to have gain in their body weight but diabetic control rats showed significant reduction in the body weight, which is reversed by extract treated groups during 21 days study. The treatment with aqueous, ethanolic, chloroform and petroleum ether extracts of P. tuberosa (500 mg/kg b.w.) showed improvements in the body weight compared with the diabetic control (118.42 ±3.91, 110.93 ±2.26 and 108.62 ±3.45) in 7 , 14 and 21 day of study. Glibenclamide treated (110.38 ±1.54) group also prevented this reduction in body weight. The phytochemical screening test result showed that ethanol and aqueous fraction of P. tuberosa contains steroid, triterpenoid, glycoside, alkaloid, flavonoid and tannin compounds, which are known to be hypoglycemic. From the phytochemistry of the plant it is indicated that different active secondary metabolites are present in its extracts and perhaps some of these compounds may function in a synergistic manner.

Diabetes mellitus, a common heterogeneous metabolic syndrome, is prevalent throughout the world and has been projected to become one of the world's main disablers and killers within the next 25 years. Blood glucose level and body weight have been commonly measured to monitor the glycemic control mechanism. In the present study, diabetic rats had lower body weight, high blood levels as compared to normal rats. However, orally administered AEPT and EEPT extract significantly increased the body weight and decreased the blood glucose level. This could be due to potentiation of the insulin effect of plasma by increasing the pancreatic secretion of insulin from existing β-cells of islets of Langerhans or its release from bound insulin. The significant and consistent antidiabetic effect of AEPT and EEPT in STZ diabetic rats may also be due to enhanced glucose utilization by peripheral tissues. Therefore, it can be concluded that the extracts of P. tuberosa tuber possessed remarkably effective antidiabetic potential against STZ- induced diabetes in rats. In the light of ourpharmacological studies we can assume that further experiment should be carried out for isolating the possible hypoglycemic compounds and then explain the actual mechanism of hypoglycemic actions of the plant fractions. The present study has given some preliminary idea of the hypoglycemic compounds present in the reported plant fractions.

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