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SCREENING OF ANTI DIABETIC ACTIVITY OF *STRYCHONOUS POTATORUM* LEAVES USING WISTAL ALBINO RATS

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ABSTRACT

In the present study, due to administration of alloxan the level of blood glucose was found to be increased by 330% on the fifteenth day of exposure period. In case of alloxanized rat due to adminstration of ethanol extract of *S. potatorum* the level of blood glucose was found to be increased by 123% and 128% on the fifteenth and thirtieth day of exposure respectively. Further, due to tolbutamide, the level of blood glucose was reduced in rat by 73% on the fifteenth day of exposure and the same was 90% on thirtieth day. The level of enzymes *viz* AST, ALT and ALP in the liver of rat maintained as control, alloxanised rat, the lone effect of ethanol extract of *S. potatorum*, antidiabetic drug, tolbutamide and alloxan in combination with ethanol extract of *S. potatorum* was assayed. In the present study, due to administration of alloxan the level of AST was found to be increased by 23.4% after fifteen days of exposure period. In alloxanized rat due to administration of ethanol extract of *S. potatorum* the level of AST was found to occur as 6.9 and 10.9 percentages on the fifteenth and thirtieth days of exposure respectively. Further, due to tolbudamide, the level of AST was decreased in rat by 2.1% on the 15th day of exposure.

INTRODUCTION

Diabetes mellitus is one of the most common disease affecting millions of people. Atleast 30 million people throughout the world suffer from diabetes mellitus. Diabetes not only kills, but it is a major cause of adult blindness, kidney failure, neuropathy, heart attack and strokes. Alloxan is rapidly reduced in the body to form dialuric acid and this undergoes auto-oxidant to yield detectable amount of H_2O_2, superoxide ion and hydroxyl free radicals. These radicals breakup DNA strands and activates poly ADP synthetase which utilizes NAD as a substrate causing depletion of pyrimidine nucleotide and it also concludes in cellular dysfunction and cell-death (Grover, 2002).

From ancient era plants have been recognized by their medicinal properties and some plant products form components of many popular drugs. Ethnobotanical information suggests that about 800 plants possess anti-diabetic potential, among them Momordica charantia, Pterocarpus marsupium, and Trigonella foenum greacum have been suggested for treatment of type 2 diabetes (Ponnusamy et al., 2011 and Patel et al., 2012). Wide arrays of plant derived active principles with different biological activities, like alkaloids, glycosides, galactomannan gun, polysaccharides, peptidoglycans, hypoglycans, guanidine, steroids, glycopeptides, terpenoids, amino acids and inorganic ions have demonstrated various medicinal effects including antidiabetic effect (Devi and Latha, 2013). Medicinal plants have been proposed as rich yet unexploited potential sources for antidiabetic drugs. Many of the antidiabetic synthetic drugs were discovered either directly or indirectly from the plant source (Kayarohanam and Kavimani, 2015).

The insulin secreting cells of pancreatic islets are unable to meet this demand in women predisposed to develop diabetes. Repeated pregnancy may increase the likelihood of developing irreversible diabetes, particularly in obeses women (Dunn *et.al.,* 2003). For women who do not have diabetes currently, pregnancy brings the risk of gestational diabetes. Pregnancy demands more insulin in the body than normal because of the increased production of hormone that can lead to insulin resistance. For women with diabetes, excellent blood glucose control before conception and then throughout pregnancy is vital to the health of the baby and the mother (Holman and Turner, 1991).

MATERIALS AND METHODS

Wister albino rats were used for the present investigation. The rats were obtained from Tetrox biosuppliers, Chennai-54 and reared in laboratory under standard conditions of light and darkness (12-12hours) and temperature (22± 2ºC). A total number of 30 rats were randomized into 5 groups of control and experimental animals. Lethal dose of plant drugs and alloxan were analyzed using Spraque (1963) method. From the lethal dose calculate one tenth of LD_{50} concentration value for treatment. **Group I :** Served as untreated control (normal) and did not receive any other treatment. **Group II:** Animals were treated with single intraperitonial (i.p) injection of alloxan monohydrate (100 mg / kg bw) after overnight fast for 12 hours. **Group III:** Animals were received ethanol extract of *S. potatorum* leaves (100 mg / kg bw) for 30 days after the diabetic state was assessed in alloxan induced diabetic rats.

Group IV: Animals were received ethanol extract of *S. potatorum* leaves (100 mg / kg) for 30 days. **Group V:** Animals were received tolbutamide (standard antidiabetic drug) (100 mg/ kg) after inducing diabetes.

Biochemical Estimations: Biochemical estimations were carried out in blood and liver homogenate samples of control and experimental animals in each group. Biochemical estimations include following parameters like glucose, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP).

Preparation of blood and liver homogenate samples: Blood samples were collected from normal and treated animals into heparinized tubes using cardiac puncture bleeding techniques. Plasma was separated from the blood sample by centrifugation at 1000g for 15minutes.

The normal and treated animals were sacrificed and aseptically removed liver from the animal and stored in clean sterile bottle at room temperature for further analysis. The liver tissue samples were washed with ice cold saline and dried in filter paper. The liver tissue samples were weighed and homogenized with required buffers for biochemical estimations using glass homogenizer with Teflon pestle. The homogenate was centrifuged at 1000g for 5 minutes and the supernatant was used for biochemical estimations.

Glucose estimation: The glucose level was qualitatively tested in urine samples using Benedict's method. Five ml of Benedict's solution was boiled, and then added to 0.5 ml (8 drops) of urine samples collected from normal and treated animals. The colour change was indicated presence of glucose in urine sample. It was mentioned as follows: green colour denoted as single plus (+), yellowish green colour denoted as double plus (++) and reddish brown colour denoted as three plus (+++). If glucose obtained in samples, then the blood and liver tissue homogenate samples were taken and estimated the level of glucose in the samples.

Glucose level was estimated by the method of O-toluidine using the modified reagent of Sasaki *et.al.* [1972]. Separately 0.1 ml of freshly drawn blood and 0.1 mg of liver tissue homogenate samples were immediately mixed with 1.9 ml of 10% TCA to precipitate the proteins and then centrifuged. One ml of the supernatant was mixed with 4.0 ml of O-toluidine reagent and was kept in boiling water bath for 15 minutes. When green color developed, it was read colorimetrically at 620 nm. The level of glucose was calculated using standard graph of known concentration of glucose level (20-100mg). The concentration glucose level was expressed as mg/d1 of blood.

Alanine aminotransferase (ALT) estimation: **(**Reitman and Frankel, 1957): Transamination is the process in which an amino group is transferred from one α- amino acid to another α- keto acid. The amount of pyruvate formed was measured by means of 2, 4 trinitrophenyl hydrazine of pyruvicacid. The colour of which is read at 520nm. Two test tubes were taken and marked as test and blank. To both the tubes, 1ml of substrate was added and kept for a few minutes at 37˚C to attain the room temperature. To the tube marked as test; 0.2ml of serum was added. Both the tubes were incubated at 37˚C for 30 minutes 0.2 ml serum was added to blank and then 0.1ml of phenyl hydrazine was added and allowed to stand for 20 minutes followed by the addition of 10ml of 0.4N NaOH and colour developed was read at 540nm. The activity of ALT was expressed as IU/dl.

Aspartate aminotransferase (AST) estimation: **(**Reitman and Frankel, 1957): Transamination is the process in which an amino group is transferred from an α-amino acid to a keto acid. AST catalyses the reaction that oxaloactetate is converted to pyruvate by decarboxylation and the pyruvate is estimated colorimetrically by the addition of 2,4 dinitrophenyl hydrazine. Two test tubes were taken and marked as test and blank. To both of the tubes 1ml of substrate solution was added and incubated for a few minutes at 37˚C to attain the temperature. To the tube marked test 0.2ml serum was added. After incubation at 37˚C added 1ml of 2, 4 dinitrophenyl hydrazine and allowed addition to stand for 20 minutes. Followed by the addition 10ml of 0.4N NaOH, the color developed was read at 540nm using a colorimeter.

Simultaneously a set of standards were prepared as follows. To 8 different test tubes added 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8 and 1ml of standard pyruvate solution. All the tubes were made up to 1.2ml with buffer. To all the tubes add 1ml of 2, 4 DNP, add 10ml of 0.4N NaOH. The colour developed was read at 540nm using a colorimeter. The activity of AST is expressed in IU/dl.

Alkaline phospatase (ALP) estimation: (King and Armstrong, 1980): The phosphate present in serum or substrate, disodium phenyl phosphate to yield phenol as a product. In the presence of alkaline oxidizing agents 4-amino antipyrine gives a purple color with phenol which is read at 520nm using colorimeter.

Two test tubes were taken and marked as test and blank. 2ml of buffered substrate was added to the each tube and placed in a water bath at 37˚C for a few minutes. To this 0.1ml of serum was added and incubated exactly for 15 minutes. To both tubes 0.8ml serum was added and simultaneously a serious of standard containing 0.2, 0.4, 0.6, 0.8 and 1ml of phenol standards were taken and made up to 1ml with double distilled water.1ml of water was taken as blank. To the test, blank and standard tubes 1ml of 4-aminoantipyrine and 1ml of potassium ferricyanate were added .The colour developed was read at 520nm using a colorimeter. The activity of ALP is expressed as IU/dl.

RESULTS AND DISCUSSION

In the present study, the effect of ethanol extract of *S. potatorum* leaves on normal and diabetic induced animals were screened and noted in the following

Blood glucose level: The level of blood glucose in rats maintained as control, alloxanized rats, and rats treated with lone effect of *S. potatorum* and in combination with Alloxan and also individual effect of diabetic control drug tolbudamide, was estimated and the result were reported in Table 1. In rats maintain as control there was no distinct variation in blood glucose level, during the exposure period.

In the present study, due to administration of Alloxan the blood glucose level was found to be increased by 330% on the fourth day of exposure period. However after the completion of exposure period (7 days) the tendency to increase in blood sugar level was not much and was found to be only 3.17%. Increase in blood glucose in swiss albino rats due to alloxan was reported by various researchers (Moram, 2001; Ilker *et al*,

2004 and Zulfiker *et al*., 2010). In the present study, due to administration of ethanol extract blood glucose level was found to be increased by 20% on the fourth day of exposure period. However, after the completion of exposure period (7 days) the tendency to increase in AST level was not much and was found to be only 5.8%. Similar kind of improvement in the AST level of rats due to ethanolic extract of *Cleome droserifolia* and *Helicteres isora* was reported by Abdo *et al*., 2006.

Aspartate aminotransferase (AST): The level of AST in rats maintained as control, alloxanized rats treated with lone effect of *S. potatorum* and in combination with Alloxan and also the effect of diabetic control drug tolbudamide, was estimated and the result were reported in Table 2. In rats maintained as control there was no distinct variation in AST level, during the exposure period.

In the present study, due to administration of Alloxan the AST level was found to be increased by 6.8% on the fifteenth day of exposure period. However after the completion of exposure period (30 days) the tendency to increase in AST level was not much and it was found to be only 2.1%. Similar trend in AST in Swiss albino rats due to alloxan was reported by various researchers (Moram, 2001). Further, due to diabetic control drug tolbudamide, AST level was reduced in rats by 2.9% on the fifteenth day of exposure and the same was 2.3% on the thirtieth day. In case of alloxanized rats due to administration of ethanol extract of *S. potatorum* the AST level was found to be decreased by 6.8% and 4.5% on the fifteenth and thirtieth day of exposure respectively. When compare the result of ethanol extract of *S. potatorum* in combination with alloxan the combined effect exhibited a favourable result. The result is in concordant with the reports of Chandrika *et.al*., 2010 in alloxanized rats due to the extracts of Artocarpus heterophyllus.

Alanine aminotransferase (ALT): The level of ALT in rats maintained as control, alloxanized rats, and rats treated with lone effect of *S. potatorum* and in combination with Alloxan and also individual effect of diabetic control drug tolbudamide, was estimated and the results were reported in Table 3. In rats maintained as control there was no distinct variation in ALT level, during the exposure period. In the present study, due to administration of Alloxan the ALT level was found to be decreased by 1% on the fifteenth day of exposure period. However, after the completion of exposure period (30 days) the tendency to increase in blood sugar level was not much varied and was found to be only 1.3% in ALT in Swiss albino rats due to alloxan was reported by various researchers (Jude *et.al*., 2010).

Further, due to diabetic control drug tolbudamide, ALT level was reduced in rats by 3.4% on the fifteenth day of exposure and 5.4% on the thirtieth day. In case of alloxanized rats due to aadminstration of ethanol extract of *S. potatorum* the ALT level was found to be decreased by 6% and 5% on the fifteenth and thirtieth days of exposure respectively. When compare the result of ethanol extract of *S. potatorum* in combination with alloxan the combined effect exhibited a favourable result.

Alkaline Phosphatase (ALP): The level of ALP in rats maintained as control, alloxanized rats, and rats treated with lone effect of *S. potatorum* and in combination with Alloxan and also individual effect of diabetic control drug tolbudamide, was estimated and the results were reported in Table 4. In rats maintained as control there was no distinct variation in ALP level, during the exposure period. Due to administration of Alloxan the ALP level was found to be increased by 20% on the fifteenth day of exposure. However, after completion of the exposure period of 30 days the tendency to increase in ALP level was not much and was found to be only 1%. Increase in the level of ALP in Swiss albino rats due to alloxan of the present study supported by earlier works of Moram, 2001 in rats due to the plant extract.

Further, due to diabetic control drug tolbudamide ALP level was reduced in rats by 2% on the fifteenth day of exposure and the same was 1% on the thirtieth day. In case of alloxanized rats due to administration of ethanol extract of *S. potatorum* the level of AST was found to be increased by 1% both on the fifteenth and thirtieth days of exposure respectively. When compare the result of ethanol extract of *S. potatorum* in combination with alloxan the combined effect showed a positive result. The result is in confirmation with the reports of Jude *et al*., (2010) in alloxanized rats due to the extract of *Croton zambesicus*.

CONCLUSION

Diabetes mellitus is a major disease affecting nearly 10% of the population. Inspite of the introduction of hypoglycemic agents, diabetes and the related complications continue to be a major medical problem. This metabolic disorder is characterized by hyperglycemia and disturbances of carbohydrate, protein and fat metabolism, secondary to an absolute or relative lack of the hormone insulin. Diabetes in India is slowly becoming a killer disease next to coronary heart disease. Studies suggest that between 10-13% of the urban population and 4 to 6% of the rural populations of India have diabetes. There are already a few good reviews antidiabetes and hypoglycemic plants. In the traditional system of Ayurvedic treatment medicines consist of plant products, either single drug or in combination to be less toxic and from side effects compared to synthetic drugs. A search for traditional drugs used for diabetes in remote villages and tribal pockets of India could reveal many useful plants.

Table 1 Effect of continued administration of ethanol extract of *S. potatorum* leaves on blood glucose levels in alloxan induced diabetic rats.

Groups	Treatment	Blood sugar (mg / 100ml)		
		Initial	Day 4	Day 7
I	Normal			
II	Alloxan	84.1 ± 1.2	85.2 ± 0.3	85.3 ± 1.6
III	Alloxan + *S. potatorum*	85.2± 2.0	356 ± 20	378 ± 21
IV	extract	85.0 ± 2.0	189 ± 16	176 ± 15
V	*S. potatorum* extract only	81.2 ± 1.5	80.1 ± 0.2	80.4± 2.1
	Tolbutamide	82.3 ± 2.0	24.0 ± 0.8	19.6 ± 9.5

All values are mean ± SD of six animals in each group

Table 2 Effect of ethanol extract *S. potatorum* leaves on serum AST activity in alloxan induced diabetic rats.

Groups	Treatment	AST (IU / l)		
		Initial	15 days	30 days
	Normal			
I	Alloxan	84.5 ± 1.5	84.6 ± 0.53	12.5 ± 2.3
II	Alloxan + *S. potatorum*	84.2 ± 0.54	88.4 ± 1.8	90.3 ± 2.1
III	extract	80.8 ± 1.07	76.5 ± 1.0	91.2± 1.5
IV	*S. potatorum* extract only	89.5 ± 0.47	87.8 ± 1.4	79.0 ± 1.8
V	Tolbutamide	85.2 ± 2.1	8031 ± 1.6	

Table 3 Effect of ethanol extract *S. potatorum* leaves on serum ALT activity in alloxan induced diabetic rats.

Groups	Treatment	ALT (IU / l)		
		Initial	15 days	30 days
I	Normal	83.12 ± 1.2	82.3 ± 0.37	85.1 ± 0.4
II	Alloxan	82.3 ± 0.5	83.8 ± 1.18	84.5 ± 2.2
III	Alloxan + *S. potatorum* extract	85.1± 1.2	78.9 ± 1.75	75.8± 1.2
IV	*S. potatorum* extract only	85.6 ± 1.2	84.4 ± 1.25	84.1± 1.2
V	Tolbutamide	85.2 ± 1.1	87.0 ± 2.6	84.2 ± 1.4

Table 4 Effect of ethanol extract *S. potatorum* leaves on serum ALP activity in alloxan induced diabetic rats

Groups	Treatment	ALP (IU / l)		
		Initial	15 days	30 days
I	Normal			
II	Alloxan	5.18 ± 1.1	5.1 ± 1.12	5.8 ± 2.1
III	Alloxan + *S. potatorum* extract	6.1 ± 0.4	18.2 ± 0.58	18.2 ± 0.2
IV	*S. potatorum* extract only	7.1 ± 0.9	8.0 ± 0.92	8.8 ± 1.3
V	Tolbutamide	6.1 ± 0.5	7.1 ± 0.39	7.2 ± 0.6
		6.2 ± 1.0	4.2 ± 1.1	3.2 ± 1.0

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