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Full Length Research Paper

Evaluation of anti-diabetic activity of *Strychonous potatorum* in alloxan induced diabetic rats

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In the present study, the anti-diabetic activity of *Strychonous potatorum* was evaluated. In Wister albino rat, the diabetic state was induced by intraperitonial injection of alloxan at a dose of 100mg/kg of body weight. Animal fasting blood glucose levels were evaluated and above 200mg/dl were considered as diabetic and used to screen the anti diabetic effect of *Strychonous potatorum*. Animals were grouped into normal rat (Control), diabetic rat (alloxan), diabetic but extract treated rat, only extract treated rat and diabetic but standard anti-diabetic drug tolbutamide treated rat groups. Animals were screened for the parameters such as body weight, blood glucose level, total proteins, cholesterol and enzyme levels such as AST, ALT and ALP for deducing the anti-diabetic activity of the test plant. The alloxan treated rat shows reduced body (26%) and liver (40%) weight. The plant extract plays a role in maintaining the liver weight as in case of normal but no much contribution towards body weight. The blood glucose level falls by 53% with extract treatment, demonstrating the anti-diabetic potential of the plant. The serum enzymes AST and ALT were increased 24 IU/1, 18 IU/1 from 60 IU/1, 65 IU/1 respectively whereas ALP was decreased to 5 IU/1 from 14 IU/1. The total serum protein level also increased up to 5 mg/ml in the extract treated animal. The insulin level also increased up to 61 µg /ml within 30 days of extract treatment compared to control with 51µg/ml. The plant extract efficiently decreased the initial cholesterol 219µg/ml level into 170µg/ml. In liver, the AST, ALT and ALP enzymes were decreased to 160 IU/1, 60 IU/1, 140 IU/1 from 178 IU/ml, 79 IU/ml and 156 IU/ml respectively.

Keywords: Anti-diabetic activity, rat and *Strychonous potatorum*.

INTRODUCTION

Medicinal plants played an important role in Indian culture since Rig Veda (5600 BC) where about 67 medicinal plants were recorded. It is estimated that 80 % of about 4 billion population have to rely on traditional medicines due to high cost of modern medicines, lack of availability of required medicines and personal preferences. Out of 250,000 higher plants, more than 80,000 have medicinal value and India occupies unique position among world's 12 biodiversity centers. It is identified that about 20,000 plants have good medicinal value and 7500 species are used by traditional communities (Miura et al., 2004)

Diabetes mellitus is one of the most common diseases affecting millions of people. At least, 30 million people throughout the world suffer from diabetes mellitus. Diabetes is becoming a major menace in the last 10 years. In Indian, the situation is expected to become much worse in the year to come because of food habit and sedentary life style. Life expectancy may be halved by this disease, especially in developing countries where its prevalence is increasing and adequate treatment is often unavailable. Diabetes not only kills, but it is a major cause of adult blindness, kidney failure, neuropathy, heart attack and strokes. It is a chronic disease characterized by deranged secretions and effects of insulin and glucagons, extensive disturbances of carbohydrates, proteins and lipid metabolism, thickening of capillary basement membrane through out the body leading to microangiopathy and macroangiopathy and

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long term complications will affect eye, kidney, nervous system and circulatory system.

Diabetes basically can be categorized in to two types. They are diabetes insipidus and diabetes mellitus (WHO, 1985). Diabetes insipidus is a condition where the urine is normal, of low specific gravity and excessive by the deficiency by the deficiency of ADH. It is caused due to tumor in posterior pituitary but may be idiopathic. Diabetes mellitus is essentially a metabolic disorder, characterized by high concentration of glucose in the blood. It is arising from insufficient insulin or impairment of its action. There are two types of diabetes mellitus, one is insulin dependent diabetes mellitus and the other is insulin independent diabetes mellitus. No drug is available in curing diabetes. But with a balanced diet and regular exercise we can control the consequences of diabetes. In critical and necessary situation, intake of hypoglycemic drugs solve the complications related with diabetes. But these are not curative and also combined with many negative effects. Therefore, searching for anti diabetic active fractions from plants gets greater importance nowadays.

Strychonous potatorum Linn (Loganiaceae) commonly known as katakam in Ayurveda, Tettankottai in Tamil and Tettamparal in Malayalam. It is a moderate sized tree found in southern and central parts of India, Srilanka and Burma (Kritikar and Basu, 1993). The ripe fruit is emetic, diaphoretic and alexiteric; it cures inflammation, anemia, and jaundice and causes biliousness (Kritikar and Basu, 2000). In ayurvedic system of medicine, it is known to be used in vitiated conditions of kappa and vata, hepatopathy, nephropathy, gonorrhea, gastropathy, bronchitis, chronic diarrhea, dysentry, renal and vesicule calculi, diabetes, burning sensation, dipsia, conjunctivitis etc. Therefore, in the present study the evaluation of anti diabetic activity of *Strychonous potatorum* in using alloxan induced rats was carried out.

MATERIALS AND METHODS

Sample collection

The plant sample *Strychonous potatorum* was collected from ayurveda market, Kollam, Kerala and was identified by a taxonomist. The collected plant was immediately transported to the lab and a voucher specimen is submitted to our lab.

Preparation of ethanol extracts of *Strychonous potatorum*

The ethanol extract of *Strychonous potatorum* was prepared by Yajninik (2003) method. Approximately 500 g of fresh plant material was shade dried and then powdered using a blender. It was soaked in 1500 ml of 95 % ethanol at room temperature over night. The above soaked contents were filtered through Whatman no 1 filter paper. The residue was again resuspended with equal volume of 95 % ethanol and incubated at room temperature for 48 hours, and filtered again. The filtrates were pooled and evaporated at 40°C -50 °C and the residue weighed. The extract was made into a semisolid by mixing with 80 % ethanol and stored below 10 °C until use. The extract was brown in color.

Experimental animals

Wister albino rats, the experimental animals were acclimatized to lab condition. The toxicity analysis of plant extract was done by Spraque (1963) method. Diabetes was induced by injecting alloxan. Animals are divided into five groups and treated as follows: A total number of 30 animals were divided into 2 lots with 12 and 18 animals respectively. The first lot of animals served as control and such divided into two groups of each. One group did not receive any treatment and the other one received just plant extract for (100 mg / kg bw) 30 days. The second lot were induced with alloxan monohydrate (100 mg / kg bw) after overnight fast for 12 hours, sub divided into 3 groups of each. Group I did not received anything (other than alloxan), group II received plant extract (100 mg / kg bw) and group III received standard antidiabetic drug, tolbutamide (100 mg / kgbw).

Glucose estimation

The glucose in urine sample was qualitatively tested using Benedict's method. In the animals tested positive, glucose level in liver tissue was quantitatively estimated by the method of O-toluidine using the modified reagent of Sasaki *et al.* (1972). Separately drawn 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. 1 ml of the supernatant was mixed with 4 ml of O-toluidine reagent and kept in boiling water bath for 15 minutes. The green colour developed, was read colorimetrically at 620 nm. Amount of glucose present in the sample was calculated using standard graph.

Insulin estimation: The plasma insulin was analyzed by ELISA method using Boehringer Mannheim kit. 0.1 ml of plasma protein was injected into the plastic tubes coated with anti-insulin antibodies. Phosphate buffer and anti-insulin POD conjugate was added to form anti-insulin antibody-POD conjugate. Substrate-chromogen solution was then added to form indicator reaction. A set of standards were also treated in the similar manner. After the development of color, the absorbance was read at 420 nm. The values were expressed as IU/ml of plasma.

Cholesterol estimation: 0.1 ml of the serum was added to 9.5 ml of ferric chloride acetic acid reagent and centrifuged. It was then stoppered and condensed. Mixed well and allowed to stand for 10 mins to precipitate proteins. Then centrifuged and 5 ml of supernatant was used as test solution. To another set of tubes named standards 0.5, 1.0, 1.5, 2.0 and 2.5 ml using ferric chloride acetic acid reagent and blank was also maintained. To all tubes 3 ml con H2SO4 was added and mixed by complete inversion. The tubes were allowed to stand for 30 minutes and the colour developed was read at 550 nm.

Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) estimation: Three test tubes were taken and marked as test 1, test 2 and blank. To all 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 1 and 2; 0.2ml of serum was added. All the tubes were incubated at 37˚C for 30 minutes after incubation 0.2 ml serum was added to blank, 0.1ml of phenyl hydrazine was added to test 1, 2, 4 dinitrophenyl hydrazine was added to test 2 and allowed to stand for 20 minutes followed by the addition of 10 ml of 0.4N NaOH and colour developed was read at 540nm. The activity of ALT and AST were expressed as IU/dl.

Alkaline phosphatase (ALP) estimation: Two test tubes were taken and marked as test and blank. 2 ml of buffered substrate was added to each tube and placed in a water bath at 37 degree Celsius for few minutes. To this 0.1 ml of serum was added and incubated

Table 1: Blood glucose level in different animal groups

Groups	Treatment	Blood sugar (mg/100ml)		
		Initial	Day 4	Day 7
I	Normal	85.1±1.8	86.0±0.2	86.0±1.8
II	Alloxan	86.0±2.0	370±20	383±21
III	Alloxan + *Strychonous potatorum* extract	85.0±2.0	190±16	180±15
IV	*Strychonous potatorum* extract only	82.0±1.5	81.0±0.2	81.0±2.1
V	Tolbutamide	83.0±2.0	72.0±0.8	68.0±9.5

Table 2: showing the serum AST level in different animal groups

Groups	Treatment	AST (IU/l)		
		Initial	15 Days	30 Days
I	Normal	85.5±1.5	86.46±0.53	82.5±2.3
II	Alloxan	81.08±1.07	65.5±1.0	60.1±1.1
III	Alloxan + *Strychonous potatorum* extract	85.2±0.54	83.4±1.8	84.3±2.1
IV	*Strychonous potatorum* extract only	85.95±0.47	88.0±1.4	90.0±1.5
V	Tolbutamide	82.8±2.1	80.1±1.6	78.2±1.8

for 15 minutes. To both tubes 0.8 ml serum was added and simultaneously a series of standard containing 0.2, 0.4, 0.6, 0.8 and 1 ml of phenol standards were taken and made up into 1 ml with distilled water and 1ml of water was taken in another tube as blank. 1 ml of 4-aminoantipyrine and 1 ml of potassium ferricyanide were added to all tubes. The colour developed was measured at 520 nm. The activity is expressed as IU/dl.

RESULTS

Strychonous potatorum is being used traditionally by diabetic patients in India and are taken as water decoction. In the present study an effort was made to confirm its anti diabetic activity. The effect of extracts of *Strychonous potatorum* on glucose tolerance was estimated. The ethanol extract influenced blood glucose level significantly compared to other extracts (data not shown). Based on this observation, the ethanol extract was selected for further study. The results in table 1 demonstrate the effect of the ethanol extract on blood sugar level. It was effective even at a lower dose (100 mg/kg) in decreasing blood sugar level in alloxan treated rats. The plant extract almost brought down blood glucose level by 50 % in diabetic animals. Thus, it may be effective like tolbutamide.

This study reports for the first time, the anti hyperglycemic effect of *Strychonous potatorum* and suggests that the active principle from this plant could be effective in the treatment of diabetes. Since the blood glucose lowering effect of ethanol extract of *Strychonous potatorum*, was observed in alloxan diabetic rats as well as in fasted normal rats, this effect could, possibly, be due to increased peripheral glucose utilization. Inhibition of the proximal tubular re-absorption mechanism for glucose in the kidney, if any, can also contribute towards blood glucose lowering effect (Sharma et al., 1983; Subramoniun et al., 1998).

Strychonous potatorum seed extract made a significant increase in serum AST and ALT in alloxan treated animals (table 2 and 3). Suggesting that the reduction in diabetes is by inhibiting intestinal peristalsis.

The activity of ALP in normal and experimental animals was also observed (table 4). In diabetic rats, the administration of ethanol extract exhibited a remarkable increment in ALP level in treated animals when compared to normal rats.

The serum insulin and cholesterol level were not modified much upon treatment with the plant extract (table 5 and 6). Suggesting that the hypoglycemic activity of *Strychonous potatorum* is not related to the insulin secretion. Hence, the hypoglycemic effect may probably be brought about by extra pancreatic mechanism. There was significant increase in liver, AST, ALT and ALP due to exposure to alloxan. The effect of ethanol extract on diabetic induced rat liver AST, ALT and ALP levels reduced to almost normal level. The administration of alcoholic extract caused a highly significant effect on enzymes of liver such as AST, ALT and ALP in 15th and

Table 3: showing the serum ALT level in different animal groups

Groups	Treatment	ALT (IU/l)		
		Initial	15 Days	30 Days
I	Normal	84.12±1.2	84.23±0.37	85.12±0.4
II	Alloxan	85.1±1.2	70.9±1.75	65.8±1.2
III	Alloxan + *Strychonous potatorum* extract	83.2±0.5	81.88±1.18	83.0±2.2
IV	*Strychonous potatorum* extract only	85.7±1.2	85.4±1.25	84.6±1.2
V	Tolbutamide	85.1±1.1	88.0±2.6	84.8±1.4

Table 4: showing the serum ALP level in different animal groups

Groups	Treatment	ALP (IU/l)		
		Initial	15 Days	30 Days
I	Normal	5.58±1.1	5.56±1.12	6.00±2.1
II	Alloxan	6.12±0.4	18.8±0.58	14.0±0.2
III	Alloxan + *Strychonous potatorum* extract	7.01±0.6	8.03±0.92	9.0±1.3
IV	*Strychonous potatorum* extract only	6.81±0.5	7.16±0.39	7.8±0.6
V	Tolbutamide	6.7±1.0	4.46±1.1	3.8±1.0

Table 5: showing the level of insulin in different animal groups

Groups	Treatment	Insulin (µg/ml)		
		Initial	15 Days	30 Days
I	Normal	136.47±3.6	136.33±4.4	137.2±1.3
II	Alloxan	135.2±1.4	55.16±3.38	51.0±2.4
III	Alloxan + *Strychonous potatorum*extract	137.1±2.1	90.17±2.4	112.0±1.8
IV	*Strychonous potatorum*extract only	136.8±1.9	129.16±2.27	132.0±1.8
V	Tolbutamide	135.9±2.1	130.0±2.8	140.0±2.1

Table 6: showing the cholesterol level in different animal groups

Groups	Treatment	Cholesterol (µg/ml)		
		Initial	15 Days	30 Days
I	Normal	140.0±1.5	142.5±2.6	143.0±2.1
II	Alloxan	141.0±2.4	212.3±6.2	219.0±2.1
III	Alloxan+ *Strychonous potatorum*extract	142.0±1.9	161.0±3.9	170.0±4.2
IV	Only Strychnos potatorum extract	143.0±1.4	146.2±3.1	151.0±3.9
V	Tolbutamide	145.0±1.2	138.0±3.4	127.0±4.6

30th days of treatment.

CONCLUSION

The acute treatment with *Strychonous potatorum* extract caused a significant decrease in the blood glucose level of diabetic rats but no effect was observed in an induced (alloxan) rats. Measurement of enzymatic activities of aminotransferases and alkaline phosphates are of clinical and toxicological importance as changes in their activities are indicative of tissue damage by toxicants or in diseased conditions. Detection of hypoglycemic activity in ethanol extract along with protective effect against alloxan challenge, lipid peroxidation provides scientific rationale for the use of *Strychonous potatorum* as anti diabetic plant.

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