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# EFFECACY OF *Strychnos colubrina* L. EXTRACT ON BLOOD GLUCOSE, PLASMA INSULIN AND TISSUE GLYCOPROTEINS IN STREPTOZOTOCIN INDUCED DIABETIC RATS

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**Abstract:**-The present study evaluated the anti-hyperglycemic activity of the Ethyl acetate extract of *Strychnos colubrina* L bark on blood glucose, Plasma insulin and glycoprotein contents of albino rats. SCEt was administered at doses of 150, 300 and 450 mg/kg body weight respectively on streptozotocin induced diabetic rats for 3 weeks. The aim of this study was to investigate the effect of Ethyl acetate extract of *strychnos coulubrina.*L (SCEt) in streptozotocin induced diabetic, which was induced in male albino Wistar rats by a single intraperitoneal injection of streptozotocin (55 mg/kg). After injection, the animals were divided into five groups viz., control group, SCEt (300mg/kg) treated group, diabetic group, diabetic + SCEt (450mg/kg) body weight for 15 days. The study shows a significant increase in the levels of blood glucose, and plasma glycoproteins, while plasma insulin levels were significantly decreased in diabetic rats. There was also a significant decrease in the level of sialic acid and elevated levels of hexose, hexosamine in the liver and kidney of diabetic rats. The diabetic animals also showed a reduction of body weights. Oral administration of SCEt reverted these changes. Thus the study indicates that *strychnos coulubrina* L. possesses a significant beneficial effect on glycoprotein's in addition to its antidiabetic action.

**Keywords:**Diabetes, *Strychnos coulubrina.*L, Glyco proteins, streptozotocin

## INTRODUCTION

Diabetes mellitus is a major public health problem in the world. The prevalence of diabetes mellitus is increasing with ageing of the population and lifestyle changes associated with rapid urbanization and westernization. According to the World Health Organization estimate 3% of the world's populations (194 million) have diabetes and is expected to double (6.3%) by the year 2025. It is well known that the incidence of diabetes mellitus is high all over the world, especially in Asia. Different types of oral hypoglycemic agents such as biguanides and sulphonylurea are avaiable along with insulin for the treatment of diabetes mellitus (Holman, R.R and Turner, R. C., 1991), but have side effects associated with their users (Kameswara Rao, et. al., 1997). There is growing interest in herbal remidies because of their effectiveness, minimal side effects in clinical experience and relatively low costs. Herbal drugs are their extracts are prescribed widely, even when their biological active compounds are unknown. Even the World Health Organization (WHO) approves the use of plant drugs for different diseases, including diabetes. There studies with plant extracts are useful to know their efficacy and mechanism of action and safety. Medicinal plants useful in diabetes were reviewed recently.(4,5)

Diabetes mellitus is a metabolic disorder of the endocrine system. The disease occurs worldwide and its incidence is increasing rapidly in most parts of the world. People suffering from diabetes are not able to produce or properly use insulin in the body, so they have a high level of blood glucose. Diabetes is becoming the third 'killer' of mankind, after cancer and cardiovascular diseases, because of its high prevalence, morbidity and mortality (Li et al.2004). Glycoproteins are carbohydrate linked protein macromolecules found in the cell surface, which form the principal component of animal cells. Hexose, hexosamine, and sialic acid are the basic components of the glycoproteins. They play an important role in membrane transport, cell differentiation and recognition, the adhesion of macromolecules to the cell surface, and the secretion and absorption of macromolecules (Mittal et al.1996). Impaired metabolism of glycoproteins plays a major role in the pathogenesis of diabetes mellitus (Knecht et al. 1990). It has been reported that alterations occur in the concentrations of

various glycoproteins in human diabetes (Sharma et al. 1987). Raised levels of glycoproteins in diabetics may also be an indicator of angiopathic complications (Konukoglu et al.1999). Several workers have suggested that elevated levels of glycoproteins in plasma, liver and kidney tissues in the diabetic condition could be a consequence of impaired carbohydrate metabolism. Insulin deficiency and high levels of plasma glucose in the diabetic condition may result in an increased synthesis of glycoproteins (Patti et al. 1999). This increase in plasma glycoproteins has been associated with the severity and duration of diabetes.

Hyperglycaemia in experimental diabetic rats leads to a decreased utilization of glucose by insulin dependent pathways, thereby enhancing the formation of glycoproteins (Youngren et al. 1996). At the cell surface or inside the cells, the glyco-components such as fucose and sialic acid form specific structures, called glycanic chains covalently linked to lipids or proteins. An increase in the biosynthesis and or a decrease in the metabolism of glycoproteins could be related to the deposition of these materials in the basal membrane of pancreatic cells. In recent times, many traditionally important medicinal plants have been tested for their efficacy against impaired glycoprotein levels in diabetes (Ramkumar et al 2007).
Materials and methods:

**Chemicals:**

Streptozotocin was obtained from Himedia laboratory Limited , Mumbai, india. All other chemical were analytical grade laboratory reagents and were used as such without further testing.

**PLANT MATERIAL:**

*Strychnos coulubrina*.L .belongs to the family Loganiaceae . It is rare in the moist shades along ravins. *Strychnos coulubrina*.L. was collected freshly from Eastern ghats of Velugonda hills of Nellore district, Andhra Pradesh, India. The plant was identified and authenticated by Head of the department, Botany and the voucher specimen No. ASR 2609 was deposited in the Department of Botany, Sri Visovodaya Government Degree college (Botany Research center) Venkatagiri, Nellore District, Andhara Pradesh. The bark part of *strychnos coulubrina*.L was washed with distilled water, shade dried, powdered, and stored in and air tight container for further use.

**PREPARATION OF PLANT EXTRACTS:**

About 500g of the air dried powder of the plant material *strychnos coulubrina*.L was extracted successively with the following solvent in soxhlet extractor, and identified as fractions 1-3 as shown below; n-hexane-Fraction-1, Ethylacetate-Fraction-2, and Methonal-fraction-3. Every time before extracting with the next solvent, the plant material was dried in hot air oven below 500 C. The extracts were in rotary vaccume evaporator. Extracts were stored in air tight container in refrigerator at below 10c.

**Animals**

Adult male albino rats of the Wistar strain weighing approximately 200 to 230g were procured from department of zoology, Sri Venkateswara university, Tirupati. Andhra pradesh , India. They were acclimatized to animal house conditions, and fed with standard rat feed supplied by Hindustan Lever Ltd., Bangalore, India. All the animal experiments were conducted according to the ethical norms approved by the Ministry of Social Justice and Empowerment, Government of India and the guidelines of the Institutional Animal Ethics Committee S.V.University.

**Preliminary phytochemical screening:**

The bark extract of *strychnos coulubrina*.L were subjected to qualitative tests for the identification of various active constituents ViZ. Flavanoids, Steroids, glycoside, alkaloid, aminoacids tannins carbohydrate. (Venkateswara Rao et al., 2013).

**Acute toxicity study:**

The acute toxicity studies were conducted using adult swiss albino mice of both sixes taking the bark extract various dose levels (50, 450, 1000, 2000 mg/kg bw) by adopting fixed dose method as per the OECD guidelines (Veeraraghavan, guidelines no 420) . The animals were observed continuously for 2 hours and then occasionally for further 4 hours and finally overnight mortality / survival was recorded and LD 50 was extrapolated Graphically.

**Induction of experimental diabetes**

The animals were fasted overnight and diabetes was induced by a single intrapersonal injection of freshly prepared

solution of Streptozotocin (55 mg/kg body weight) in 0.1M cold citrate buffer pH 4.5 (Rakieten et al. 1963). The animals were allowed to drink 5% glucose solution overnight to overcome drug-induced hypoglycaemia. The control rats were injected with citrate buffer alone. After a week's delay for the development of diabetes, the rats with moderate diabetes, i.e. with glycosuria and hyperglycaemia (blood glucose range above 250 mg/dl) were considered as diabetic and used for the drug treatment. The bark ethyl acetate extract was administered orally througha gavages at a concentration of 150,300,450mg/kg body weight/rat/day for 15day.

**Experimental procedure**

In the experiment, a total of 30 rats (24 diabetic surviving rats, six normal rats) were used. The rats were divided into six groups and labeled as below:-

| | | |
|---|---|---|
| Group 1 | : | Normal untreated rats. |
| Group2 | : | Diabetic control rats |
| Group 3 | : | Diabetic rats given SCEt (150 mg /kg body weight) in 1 ml of Ethyl acetate extract solution daily using an intra gastric tube v for 15 days. |
| Group 4 | : | Diabetic rats given SCEt (300 mg /kg body weight) in 1 ml of Ethyl acetate extract solution daily using an intra gastric tube v for 15 days. |
| Group 5 | : | Diabetic rats given SCEt (450 mg /kg body weight) in 1 ml of Ethyl acetate extract solution daily using an intra gastric tube v for 15 days |

For every 5 days the blood sample were taken for the glucose monitoring of blood glucose level monitoring from the tail vein at 0, 5, 10, 15 days interval. The blood glucose level was determined through an electric semi auto analyzer and data so obtained was used for analysis

**Biochemical assays**

After 15 days of treatment, the rats were fasted overnight and sacrificed by cervical dislocation and the blood was collected using EDTA as an anticoagulant. The whole blood was used for the estimation of glucose (Sasaki et al. 1972), glycosylated haemoglobin (Nayak and Pattabiraman 1981) and urea (Natelson et al. 1961). Plasma insulin and C-peptide assays were performed using a radio immunoassay (RIA) kit for rats supplied by Linco Research Laboratories, USA. The plasma protein levels were estimated according to themethod of Lowry et al. (1951) and serum creatinine was estimated by the method of Brod and Sirota (1948).

**Extraction of glycoproteins**

The tissue samples were defatted before estimation. A weighed amount of defatted tissue was suspended in 3.0 ml 2M HCl and heated at 90 °C for 4h. The sample was cooled and neutralized with 3.0 ml 2M NaOH. Samples from this were used for the estimation of hexose, hexosamine, fucose and sialic acid. The plasma and tissue hexose content was estimated by the method of Niebes (1972) Sialic acid in plasma and tissues were estimated by the method of Warren (1959) and hexosamine by the method of Wagner (1979).

**Statistical analysis**

All the grouped data were statistically evaluated with SPSS/11.5 software. Hypothesis testing methods included one-way analysis of variance (ANOVA) followed by the Duncan multiple analysis test. All the results were expressed as the Mean ± Standard Deviation (SD) for six animals in each group.

**RESULTS**

Table 1 illustrates the levels of blood glucose, plasma insulin, and body weight in normal and experimental animals. The levels of blood glucose was significantly increased Where as the levels of plasma insulin were significantly decreased in diabetic rats when compared with normal rats. Body weights were also significantly reduced in diabetic rats when compared to normal rats while it was significantly recovered in the SCEt treated animals. The recovery effect with plant extract (450 mg/kg) was signficant .

Tables 2, 3 and 4 show the levels of plasma and tissues glycoproteins in normal and experimental animals. There was a significant increase in the level of plasma glycoproteins in diabetic rats. In liver and kidney of diabetic rats, the levels of hexose and hexosamine were significantly raised, but the level of sialic acid was significantly decreased. Oral administration of SCEt significantly restored the changes in plasma, liver and kidney glycoproteins of diabetic rats. The recovery effect with plant extract (450 mg/kg) was significant normal rats treated with SCEt did not show significant changes.

**TABLE-1**
**Effect of alcoholic extract of *strychnos coulubrina.L* on fasting blood glucose of normal and diabetic rats**

| Groups | Blood glucose | Plasma insulin |
|---|---|---|
| **Control** | 76.75±5.88[a] | 15.99±1.51[c] |
| **D. Control** | 272.42±5.88[d] | 4.78±1.28[a] |
| **D. 150 mg/bw** | 248.25±18.24[c] | 5.30±0.74[a] |
| **D.300 mg/bw** | 174.83±14.32[b] | 7.05±0.66[b] |
| **D. 450 mg/bw** | 84.46±8.00[a] | 14.73±1.20[c] |
| **F-Value** | 326.241 | 135.136 |

**TABLE - 2**
**Effect of alcoholic extract of *strychnos coulubrina.L* on fasting Plasma of normal and diabetic rats**

| Groups | Hexose | Hexosamine | Sialic acid |
|---|---|---|---|
| **Control** | 85.75±5.37[a] | 69.91±3.08[a] | 49.88±5.02[a] |
| **D. Control** | 140.52±7.36[d] | 102.24±2.93[c] | 73.74±3.26[c] |
| **D. 150 mg/bw** | 137.15±3.09[d] | 99.27±2.44[c] | 50.40±2.95[a] |
| **D.300 mg/bw** | 130.98±4.56[c] | 86.03±3.84[b] | 59.32±3.85[b] |
| **D. 450 mg/bw** | 91.91±3.20[b] | 70.10±3.38[a] | 49.29±4.35[a] |
| **F-Value** | 167.161 | 141.495 | 41.859 |

**TABLE 3**
**Effect of alcoholic extract of *strychnos coulubrina.L* on fasting Liver of normal and diabetic rats.**

| Groups | Hexose | Hexosamine | Sialic acid |
|---|---|---|---|
| **Control** | 29.24±2.74[a] | 8.89±1.93[a] | 14.06±1.42[a] |
| **D. Control** | 54.19±3.10[e] | 18.35±2.17[c] | 27.18±2.29[c] |
| **D. 150 mg/bw** | 32.84±2.73[b] | 10.91±2.05[a] | 14.70±2.32[a] |
| **D.300 mg/bw** | 36.66±2.12[c] | 16.12±2.36[b,c] | 19.19±2.99[b] |
| **D. 450 mg/bw** | 48.17±3.07[d] | 14.56±1.76[b] | 25.31±2.62[c] |
| **F-Value** | 86.691 | 20.748 | 37.709 |

**TABLE-4**
**Effect of alcoholic extract of *strychnos coulubrina.L* on fasting Kidney of normal and diabetic rats**

| Groups | Hexose | Hexosamine | Sialic acid |
|---|---|---|---|
| **Control** | 20.88±1.90[a] | 12.35±1.55[a] | 11.93±2.44[a] |
| **D. Control** | 42.51±2.36[d] | 25.67±3.87[d] | 26.86±1.95[d] |
| **D.150mg/bw** | 38.58±4.88[c] | 21.90±1.85[c] | 23.27±1.91[c] |
| **D.300mg/bw** | 32.28±3.47[b] | 17.88..1.44[b] | 16.05±1.43[b] |
| **D.450mg/bw** | 19.05±1.24[a] | 13.50±2.45[a] | 12.15±1.52[a] |
| **F-Value** | 70.062 | 32.536 | 75.262 |

## DISCUSSION

In the present study STZ-induced diabetes was chosen as the animal model because it resembles many of the features of human diabetes mellitus (Tomlinson et al. 1992). The results of the present study showed that the oral administration of *strychnos coulubrina.L* extract significantly decreased the levels of blood glucose and glycosylated haemoglobin in STZ-induced diabetic rats *strychnos coulubrina.L* may bring about its hypoglycaemic action through stimulation of the surviving or remnant β- cells of islets of Langerhans to release more insulin. This was further evidenced by the observed increase in the levels of plasma insulin and bloodglucose in the diabetic rats treated with *strychnos coulubrina.L* extract.

In diabetes, the glycation and subsequent browning reaction are enhanced by increased glucose levels, and there is some evidence that glycation itself may induce the formation of oxygen-derived free radicals (Gupta et al. 1997). The levels of glycosylated haemoglobin are monitored as a reliable index of glycaemic control in diabetes (Cerami et al. 1978).

Increased glycosylation of various proteins in diabetic patients has been reported (Rahman et al. 1990). In this study, we have observed increased levels of hexose, hexosamine and sialic acid in the plasma and tissues of streptozotocin induced diabetic rats. The increase in plasma glycoprotein components has been associated with the severity and duration of diabetes. In hyperglycaemia, free amino groups of proteins react slowly with the carbonyl groups of reducing sugars such as glucose, to yield a Schiff's-base intermediate (Maillard reaction). These Schiff-base intermediates undergo Amadori rearrangement to a stable ketoamine derivative (fructosamine) (Bucala 1999).

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