Vol : No. XXVII (2) October, Novermber, December - 2007

Effect of *Syzygium calophyllifolium* Walp. seed extract on transaminases and phosphatases in alloxan induced diabetic rats

Received : 18.03.2007
Accepted : 14.06.2007

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Abstract

The antidiabetic effect of an aqueous seed extract of *Syzygium calophyllifolium* Walp.was studied in alloxan induced diabetic rats. Changes in serum and tissue AST, ALT LDH, ACP and ALP, enzymes activities in alloxan induced rats were studied and found to be reversed by the oral administration of *S.calophyllifolium* seed extract.

Introduction

Diabetes is a group of related diseases, all of which are characterized by hyperglycemia secondary to deranged secretion or action of insulin. The concentration of glucose in blood depends on insulin production and utilization in normal as well as in diabetic condition by different tissues [1]. Absence of insulin effect leads to glucose over production and under utilization by liver. However the insulin dependent tissues such as kidney show over utilization of glucose during diabetes because of hyperglycemia [2].

Hyperglycemia directly contributes to the complications through increased glycosylation leading to biochemical and morphological abnormalities due to altered protein structure [3]. Diabetes complications include thickening of the capillary basement membranes, retinopathy and nephropathy. If diabetes is left untreated it almost develops into complications such as irreversible hepatic and renal deterioration [4].

Insulin fails to prevent the serious vascular and other complications of diabetes inspite

of effective control of diabetic symptoms. Thus, there is a need to seek newer and alternative approaches for effective therapy in diabetes management. Traditional medicine such as Ayurveda and Unani in India and other countries since ancient days have employed hypoglycemic plants [5]. The present study investigates the antidiabetic property of *S.calophyllifolium* seed extract in alloxan induced rats which was assessed by measuring the levels of tissue and serum marker enzymes.

Materials and methods :

Plant material and preparation of plant extract

S. calophyllifolium, fruits were collected from Ooty, Nilgris District, Tamilnadu, India[6]. They were identified and authenticated by Department of Botany, Kongunadu Arts and Science College, Coimbatore. The seeds were separated from the fruits and shadow dried in laboratory at room temperature and powdered in a mixer grinder.

Aqueous extract was prepared by taking 100g of seed power and added 500ml of distilled water and heated for 3 hours below 50ÚC. The extract was filtered with Whatmann No.2 filter paper. The filtrate was used for the oral administration.

Selection of animals

Male albino rats weighing at an average of 150-200g were obtained from the Vetinary College, Thrissur, Kerala were used for the study. They animals were fed with standard pelleted diet from Hindustan Lever Ltd., Bangalore, India and water *ad libitum*. The pellet composition was found to be similar to RDA (Recommended Dietary Allowances) for laboratory animals.

Experimental induction of diabetes mellitus

The rats were injected intraperitonialy with alloxan monohydarte dissolved in sterile saline at dose level of 80 mg/Kg body weights. After two weeks rats with moderate diabetes having glycosuria (indicated by benedict's qualitative test) and hyperglycemia (i.e with a blood glucose of 200-300 mg/ dl) were used for the experiment [7].

Experimental setup

Rats were divided in to four groups comprising of six animals each groups as follows.

Group I : Control group fed with normal diet.

Group II : Toxic group animals induced with alloxan monohydrate

Group III : Diabetic rats treated with herbal drug 1.0 ml /rat/day, orally for 30 days and

Group IV : Control animal treated with herbal drug 1.0ml/rat/day, orally for 30 days.

Collection of rat serum, liver and kidney samples

After the experimental regimen the animals were sacrificed under mild chloroform anesthesia. Blood was collected and serum was separated by centrifugation for 20 min. at 2000 rpm. The liver and kidney were excised immediately, washed with cold saline.

Preparation of tissue homogenate

The liver was cut into small pieces. A 10% homogenate of liver and kidney tissues were

prepared by using 0.1M Tris HCl buffer (pH 7.4). This serum and total tissue homogenate were then subjected to biochemical analysis.

Biochemical stuides

Aminotransferase were estimated by the method Reitmann and Frankel (1957) [8]. Acid and alkaline phosphatase were estimated by King and Amstrong (1934)[9]. Lactate dehydrogenate was estimated by King (1965)[10].

Statistics

The results were expressed as mean ± S.D and the data obtained were analyzed by one-way analysis of variance (ANOVA). The significant difference between the means of treated and untreated groups estimated by Duncan's Multiple Range Test (DMRT). $P<0.05$ was considered significant.

Results

Effect of aqueous extrat of S.calophyllifolium on serum marker enzymes such as Aspartate transaminase (AST), alanine transaminase (ALT), lactate dehydrogenase (LDH) acid phosphatase (ACP) and alkaline phosphatase (ALP) of control and experimental rats shown in table 1.

The level of all the enzymes in serum were markedly elevated in alloxan induced diabetic rats when compared with control. Oral administration of S.calophyllifolium seed extracts for 30 days brought back to the activity of the above enzymes to near normal levels. However herbal drug alone treated rats did not show any significant changes when compared with control (Group IV).

Table 2 and 3 show the levels of marker enzymes in liver and kidney of control and experimental animals. Significant elevations in liver marker enzyme were observed in diabetic rats when compared with control rats. Oral administration of S.calophyllifolium reversed these enzymes to near normal value. Rats administered with S.calophyllifolium alone treated did not produce any significant alteration in liver marker enzymes.

Discussion

Diabetes mellitus (DM) is an endocrine disorder in which glucose metabolism is impaired because of total loss of insulin after destruction of pancreatic beta cells (type I diabetes mellitus or IDDM) or because of inadequate release of insulin from the pancreatic beta cells or insentitivity of target tissue to insulin (type II diabetes mellitus or NIDDM). Alloxan is a valuable agent for the experimental production of diabetes. The user of lower dose of Alloxan (80mg/dl) produced an incomplete destruction of pancreatic beta cells even though rats become permanently diabetic [11].

A marked increased in the activities of AST, ALT, and LDH in serum and tissues of group II rats indicates injury caused in tissues. When cell membrane gets damaged these enzymes which are normally located in the cytosol leak in to the blood stream thus manifesting damage effected to liver and other tissues. The attainment of near normalcy was observed in the activities of AST, ALT, and LDH in *S.calophyllifolium* administered group III rats [12].

Acid phosphatase and alkaline phosphatase are frequently employed as marker enzymes

to assess the lysosomal changes in vivo because it is localized almost exclusively in the particles and its release parallels that of lysosomal hydrolases. A significant reduction in these phosphatases as a result of *S.calophyllifolium* treatment against alloxan induced diabetes indicates protection against the rupture of lysosomal and the leakage of these enzymes. These altered biochemical profiles due to Alloxan induced diabetes were significantly reversed towards normalization by the treatment with *S.calophyllifolium* aqueous seed extract.

In conclusion the result of the present study demonstrates that *S.calophyllifolium* seed extract has potent antidiabetic activity against alloxan induced rats. The result also implies that the antidiabeteic effect of *S.calophyllifolium* may be due to it secondary metabolites.

Acknowledgement

The authors would like to thank the Secretary & Director Dr.M.Aruchami, & Principal Dr.A.A.Sivakumar for their constant encouragement and providing laboratory facilities.

Table 1

Effect of *S. calophyllifolium* on marker enzyme in serum of control and alloxan induced diabetic rats.

Parameters	Group I	Group II	Group III	Group IV
AST	44.51±3.52	74.53±3.20 a*	48.44±2.53b*	43.38±3.56 c^{ns}
ALT	98.46±7.73	199.22±12.09a*	105.33±7.42b*	97.67±6.56 c^{ns}
LDH	109.11±6.82	143.50±8.17a*	124.98±8.42b*	104.02±8.68 c^{ns}
ACP	56.05±2.38	72.63±2.37a*	60.59±2.11b*	54.55±1.87 c^{ns}
ALP	65.54±3.75	163.68±3.93a*	80.99±5.43b*	63.88±3.60 c^{ns}

Values are expressed as mean ± SD of six animals (n = 6)

Statistical comparison
a: group I and group II
b: group II and group III
c^{ns}: group I and group IV

Statistical significance: * p <0.05, ns – not significant

Units:

AST, ALT, LDH, ACP and ALP – IU/L.

Table 2 : Effect of *S. calophyllifolium* on marker enzyme in liver of control and alloxan induced diabetic rats

Parameters	Group I	Group II	Group III	Group IV
AST	6.34±0.16	15.16±0.92 a*	8.06±0.52b*	5.79±0.52 c^{ns}
ALT	8.48±0.67	24.77±1.46a*	13.30±0.97b*	7.74±0.75 c^{ns}
LDH	50.13±0.81	123.19±4.33a*	87.78±2.90b*	46.99±1.93 c^{ns}
ACP	18.87±0.83	30.17±1.29a*	20.59±0.64b*	18.13±0.56 c^{ns}
ALP	15.30±0.18	31.03±0.82a*	17.78±0.34b*	14.34±0.28 c^{ns}

Values are expressed as mean ± SD of six animals (n = 6)

Statistical Comparison
a: group I and group II
b: group II and group III
c^{ns}: group I and group IV

Statistical significance: * p <0.05, ns – not significant

Units : AST, ALT, LDH, ACP and ALP – IU/L.

Table 3 : Effect of *S. calophyllifolium* on marker enzyme in kidney of control and alloxan induced diabetic rats

Parameters	Group I	Group II	Group III	Group IV
AST	7.28±0.65	15.26±0.89 a*	8.96±0.41b*	6.81±0.57 c^{ns}
ALT	6.31±0.18	21.46±2.01a*	8.28±0.62b*	5.83±0.35 c^{ns}
LDH	29.84±0.94	66.05±1.77a*	34.99±1.31b*	30.03±1.43 c^{ns}
ACP	16.45±0.33	32.14±0.85a*	18.08±0.27b*	15.69±0.20 c^{ns}
ALP	15.81±0.34	30.52±0.96a*	17.94±0.36b*	15.60±0.81 c^{ns}

Values are expressed as mean ± SD of six animals (n = 6)

Statistical Comparison
a: group I and group II
b: group II and group III
c^{ns}: group I and group IV

Statistical significance: * p <0.05, ns – not significant

Units : AST, ALT, LDH, ACP and ALP – IU/L.

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