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ANTIDIABETIC AND ANTIHYPERLIPIDEMIC ACTIVITY OF BARK OF *CASUARINA EQUISETIFOLIA* ON STREPTOZOTOCIN INDUCED DIABETIC RATS

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ABSTRACT

The present study was aimed to evaluate the anti diabetic - activity potential of *Casuarina equisetifolia* leaves against streptozotocin (STZ) induced experimental rats. Ethanolic extract of bark of *Casuarina equisetifolia* (EECE) was administered to streptozotocin induced rats. Glibenclamide was used as a standard drug. Blood glucose levels were determined after oral administration of a dose of *Casuarina equisetifolia* (400 mg/kg b. wt) in diabetic groups. Blood glucose levels were determined on 0, 7th, 14th and 21st day after oral administration of ethanolic extracts of *Casuarina equisetifolia* (400 mg/kg). An ethanolic extract of *Casuarina equisetifolia* was found to reduce blood sugar in streptozotocin induced diabetic rats. Reduction in blood sugar could be seen from 7th day after continuous administration of the extract. The effect of extracts of *Casuarina equisetifolia* on serum lipid profile like Total cholesterol, triglycerides, low density, very low density and high density lipoprotein were also measured in the diabetic and non diabetic rats. There was significant reduction in Total cholesterol, LDL cholesterol, VLDL cholesterol and improvement in HDL cholesterol in diabetic rats. These results indicated that *Casuarina equisetifolia* possesses a hypoglycemic and antihyperlipidemic effect.

Key words: *Casuarina equisetifolia,* Glibenclamide, Hypoglycemia, Antihyperlipidemic, Streptozotocin.

Introduction

Diabetes mellitus is a complex disorder that characterized by hyperglycemia resulting from malfunction in insulin secretion and/or insulin action both causing by impaired metabolism of glucose, lipids and protein [1]. The chronic hyperglycemia of diabetes is associated with long term damage, dysfunction and failure of various organs [2]. In diabetic rats, the utilization of impaired carbohydrate leads to accelerate lipolysis, resulted in hyperlipidemia [3, 4]. Despite the presence of known antidiabetic medicine in the pharmaceutical market, diabetes and the related complications continued to be a major medical problem. Recently, some medicinal plants have been reported to be useful in diabetes worldwide and have been used empirically as antidiabetic and antihyperlipidemic remedies [5-10]. Diabetes mellitus is known to cause hyperlipidemia through various metabolic derangements. Among several metabolic derangements, insulin deficiency has been known to stimulate lipolysis in the adipose tissue and gives rise to hyperlipidemia and fatty liver. Thus, in diabetes hypercholesterolemia and hypertriglyceridemia often occurs [11]. More than 400 plant species having hypoglycemic activity have been available in literature [12, 13]; however, searching for new antidiabetic drugs from natural plants is still attractive because they contain substances which take alternative and safe effect on diabetes mellitus. Most of plants contain glycosides, alkaloids, terpenoids, flavonoids, cartenoids, etc., that are frequently implicated as

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having antidiabetic effect [14]. *Casuarina equisetifolia* is a herbaceous member of the family Casuarinaceae. It is common along the coast on beaches, rocky coasts, limestone outcroppings, dry hillsides and open forests in both wet and dry zones from sea-level to mid-montane. It is native to South-East Asia, Australia and Polynesia. It is also cultivated as an ornamental, for wind-breaks, or as a medicinal plant in some tropical countries in the South Pacific. It contains Ellagic acid, beta-sitosterol, kaempferol and glycosides, quercetin, cupressuflavone, isoquercitrin, several common triterpenoids, trifolin, catechin and epicatechin, cholesterol, stigmasterol, campesterol, cholest-5-en-3-beta-ol derivatives, tannin, proantho-cyanidins, juglanin, citrulline and amino acids, afzelin, casuarine, gallicin, catechol derivatives, gentisic acid, hydroquinone, nictoflorin, rutin, trifolin [15-17]. Phytosterol from the leaves of the plant shows antibacterial activity, hypoglycemic, antifungal, molluscicidal, cytotoxic [16-19]. In Tahiti, the plant is used to treat nervous disorders, diarrhoea and gonorrhoea. Tongans use it to treat coughs**,** ulcers, stomachaches and constipation. Dysuria and menorrhagia are treated with an infusion of the leaves. Secondary amenorrhoea is treated with a decoction of the leaves. An infusion of the leaves, in Tonga, is used as an emetic to treat throat infections. The plant's uses in treating throat infections, coughs and stomach-aches are also noted in Fiji and India. In Samoa, an infusion of the leaves is used as a remedy for coughs, asthma and diabetes. Cook Islanders use an infusion of the grated leaves to treat mouth infections and urinary tract infections [21-22]. However, no simultaneous antidiabetic and antihyperlipidemic activity on the bark of *Casuarina equisetifolia* was scientifically available. Therefore, the present study has been carried out to explore the antidiabetic and antihyperlipidemic activity of *Casuarina equisetifolia.*

MATERIALS AND METHODS

Materials:

The bark of *Casuarina equisetifolia* was collected from Tirunelveli District, in the Month of August 2008. The plant was authenticated by Dr.V. Chelladurai, Research Officer Botany. C.C.R.A.S., Govt. of India, by carrying out macroscopic and microscopic evaluation.

Animals:

Male Wistar rats of body wt. 180–200 g were obtained from Smt. Sarojini Ramulama College of Pharmacy, Mahabub Nagar, Andhra Pradesh-509 001. The animals were fed on standard pellet diet (Hindustan Lever, Mumbai, India) and water ad libitum. The rats used in the present study were maintained in accordance with guidelines of the CPCSEA, India and the study approved by the ethical committee.

Preparation of the root extract:

The shade dried bark was powdered to get a course granule. About 250 g of dried powder were extracted with 90% ethanol by continuous hot percolation, using soxhlet apparatus. The resulted dark – brown extract was concentrated up to 100 ml on Rota vapour under reduced pressure. The concentrated crude extracts were lyophilized in to powder and used for the study.

The preliminary phytochemical analysis:

The preliminary phytochemical studies were performed for testing different chemical groups present in ethanolic extract of *Casuarina equisetifolia* [23]. Phytochemical screening gave positive test for alkaloids.

Toxicity studies:

The animals were divided into six groups separately and were treated orally with ethanolic extracts of *Casuarina equisetifolia* at 100, 200 and 400 mg/kg, body weight doses. The animals were continuously observed for 1 hr., then frequently for 14 days. The parameters observed were grooming, hyperactivity, sedation, loss of righting reflex, respiratory rate and convulsion [24].

Streptozotocin-induced diabetic rats:

Streptozotocin (STZ), was dissolved in ice-cold normal saline immediately before use. Diabetes was induced in rats by intraperitoneal (i.p) injection of streptozotocin at a dose of 50 mg/kg [25]. Forty eight hours after streptozotocin administration, blood samples were drawn from tail and glucose levels determined to confirm diabetes. The rats were divided into 4 groups as follows, first group served as normal control, received food and water. Second group served as diabetic control, received 0.5 ml of 5% Tween 80; third group served as (diabetic control), received glibenclamide (0.5 mg/kg p.o.), and fourth groups, (diabetic rats) received 400 mg/kg, b.wt. of ethanolic extracts of *Casuarina equisetifolia*. The treatment was continued daily for 21days. Blood drop was collected from the tail for glucose estimation, just before drug administration on 1st day and 1 h after sample administration on days 7, 14 and 21 (Table 1).

Biochemical parameters:

Triglycerides, cholesterol, HDL-cholesterol, and LDL-cholesterol were estimated from the serum by using standard kits [26-28].

Statistical evaluation:

All the data are presented as mean ± SEM. The differences between group were evaluated by one-way analysis of variance (ANOVA) followed by the Dunnette multiple comparisons test's <0.01 was considered to be significant.

RESULTS AND DISCUSSION

Phytochemical screening:

Phytochemical screening of both the plant extracts revealed that the presence of alkaloids, phytosterols, carbohydrates and saponins.

Toxicity studies:

In performing preliminary test for pharmacological activity in rats, ethanolic extract did not produce any significant changes in the behavioral or neurological responses upto 400 mg/kg
body weight. Acute toxicity studies revealed the non-toxic nature of the ethanolic extracts of
Casuarina equisetifolia. The result obtained from the LD50 study indicates that ethanolic extract of *Casuarina equisetifolia* is safer to use in animals even at a dose of 400 mg/kg p.o.

Antidiabetic Effects:

Effect of ethanolic extract of *Casuarina equisetifolia* on serum glucose levels in diabetic rats was depicted in Table 1. In animals treated with streptozotocin (50 mg/kg i.p) (Group II), a significant increase in serum glucose level was observed on 7th, 14th, 21st, and 28th day when compared with normal rats (Group I). Group III received glibenclamide (0.5 mg/kg p.o.) showed decrease in serum glucose level when compared with diabetic control rats (Group II). After the oral administration of ethanolic extract of *Casuarina equisetifolia* in diabetic control rats, a significant reduction in blood glucose level was observed on the 7th, 14th, 21st, and 28th day compared with diabetic control rats (Group II).

Anti-hyperlipidaemic activity:

The lipid profiles in control and experimental rats are depicted in Table 2 in STZ induced diabetic rats. The diabetic control rats (Group II) showed significant increase in serum triglycerides, Total cholesterol, very low density lipoproteins (VLDL), low density lipoproteins (LDL), and High density lipoproteins (HDL) when compared with normal (Group I). Standard glibenclamide (Group III) also reduced triglycerides, Total cholesterol, very low density lipoproteins (VLDL), low density lipoproteins (LDL), and increased High density lipoproteins (HDL) when compared with normal (Group I). The ethanolic extract showed significant decrease ($p<0.001$) in Total cholesterol, LDL, VLDL, Triglycerides and significant increase ($p<0.001$) in HDL when compared with diabetic control group (Group II). All these effects were observed on day 14th, 21st, and 28th .The present experimental result indicated that ethanolic extracts exhibited a potent blood glucose lowering properties in STZ diabetic rats.

Tab 1: Anti-hyperglycemic activity of ethanol extracts of *Casuarina equisetifolia* on STZ induced diabetic rats.

Groups	0 day (mg/ml)	After 7days (mg/ml)	After 14days (mg/ml)	After 21days (mg/ml)	After 28days (mg/ml)
Normal control	64.57±1.47	98.87±1.06	87.35±2.25	81.57±2.09	68.17±2.13
Diabetic control	226 ±1.47	216.5±2.60*	213 ±2.45*	210.12 ± 1.54*	204± 3.12*
Glibenclamide (0.5mg/kg)	234.32±1.92***	186.23 ±2.84***	131.54 ±1.68 ***	94.57±2.06 ***	94.14±2.21***
EECE 400mg/kg	235 ±1.24***	192±1.15***	135.67 ±1.57 ***	100.47±2.24 ***	90.04±2.25***

The values are mean ±SEM, n=6, When compared with diabetic control *p<0.05, **p<0.001, ***p<001 (One way ANOVA followed by Dennett's, multiple comparison test).

Tab 2: Antihyperlipidemic activity of ethanol extracts of *Casuarina equisetifolia* on STZ induced diabetic rats.

Groups	TC	TG	HDL C	LDL C	VLDL C
Normal control	80.50 ±1.35	69.33 ±0.75	39.83±0.69	42.00±2.79	19.83±0.75
Diabetic control	135.83 ±1.97*	138±1.68*	28.67±1.15*	89.50±2.29*	29.34±1.67*
Glibenclamide (0.5mg/kg)	98.57 ±3.73**	88.52 ±2.17**	34.23±5.52**	59.23±1.49**	23.67±0.77**
EECE 400mg/kg	99.19 ±2.26**	89 ±2.69**	35.57±1.33**	58.13±1.62**	24.93±0.37**

The values are mean ± SEM n= 6, when compared with diabetic control, * = p<0.05, ** = p<0.01, (One way ANOVA followed by Dennett's, multiple comparison tests).

DISCUSSION

Diabetes mellitus is one of the leading causes of death, illness and economic loss all over the world. Insulin-dependent (Type I, IDDM) diabetes is characterized by juvenile onset and by absolute insulin deficiency. Non-insulin- dependent (Type II, NIDDM) diabetes is

characterized by mature onset, by varying basal insulin levels and a frequent association with obesity. We found an elevated blood glucose concentration accompanied by increase in total cholesterol, triglycerides, LDL,VLDL and decrease in HDL cholesterol in streptozotocin induced diabetic rats as compared to control animals. Oral administration of ethanolic extract of *Casuarina equisetifolia* normalized the levels of blood glucose. The potent antidiabetic effect of the plant extract suggests the presence of potent antidiabetic active principles, which produced antihyperglycemic effect in diabetic rats. In recent years, considerable interest has been directed towards the investigation of plasma lipids and lipoproteins pattern in diabetes mellitus due to the fact that abnormal lipid level leads to the development of coronary artery disease in diabetic patients [29]. Reduced insulin secretion and defect in insulin function resulted in enhanced metabolism of lipids from adipose tissue to the plasma. Impairment in insulin sensitivity due to high concentration of lipids in the cells is responsible for the elevated cardiovascular risk in diabetes mellitus [30]. Thus, the altered lipid and lipoprotein pattern observed in diabetic rats could be due to defect in insulin secretion and/or action. Hypercholesterolemia and hypertriglyceridemia have been reported to occur in alloxan induced diabetic rats. Accumulation of cholesterol and phospholipids in liver due to elevated plasma free fatty acids has been reported in diabetic rats. In the present study, ethanolic extract of *Casuarina equisetifolia* had significantly decreased Total Cholesterol, Triglycerides, VLDL, and LDL with increase in HDL which is having a protective function for the heart compared with diabetic control group [31].

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