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Hypoglycemic Activity of Hydro-alcoholic Extract of *Calycopteris floribunda* Induced by Streptozotocin in Rats

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ABSTRACT

The present investigation was carried out to study the hypoglycemic effects of the hydro-alcoholic (70:30) extract of *Calycopteris floribunda,* in normal and streptozotocin induced diabetic model. *Calycopteris floribunda,* are reported to have medicinal and traditional values including hypoglycemic properties. Decreased blood glucose level of the test animals shows that the extract exhibit significant hypoglycemic activity when compared to diabetic control group, effect of various doses (100, 200 mg/kg, p.o) extract was studied on streptozotocin induced both diabetic and non diabetic rats. The results also indicated the dose dependent effect. The hypoglycemic activity produced by the extract may be due to increased uptake of glucose at the tissue level or by an increase in pancreatic beta cell function or due to inhibition of intestinal absorption of glucose. The present study supports the use of this herbal drug as hypoglycemic. The reduction in the glucose level in induced diabetic rats proved that *Calycopteris floribunda* having the hypoglycemic activity.

Keywords: *Calycopteris floribunda,* Streptozocin, hypoglycemic, diabetes.

INTRODUCTION

Diabetics have significantly accelerated levels of oxidative stress and this contributes massively to most neurological, cardiovascular, retinal, renal diabetic complications. [1] Diabetes mellitus is a metabolic disorder characterized by fasting hyperglycemia and alterations in carbohydrate, fat and protein metabolism, associated with absolute or relative deficiencies in insulin secretion and/or insulin action. [2] Experimentally, streptozotocin (STZ) or alloxan are used to induce diabetes in rodents. STZ is effective in triggering islet cell death by acute oxidative stress. STZ-induced diabetic rats are one of the animal models of insulin dependent diabetes mellitus characterized by high fasting blood glucose levels and drastic reduction in plasma insulin concentration. [3] Although different types of oral hypoglycemic agents are available along with insulin for the management of diabetes mellitus, there is a growing interest in herbal remedies due to the side effects associated with these therapeutic agents. [4] Thus plants have played a major role in the discovery of new therapeutic agents. The present study was undertaken to investigate the anti-hyperglycemic effect of the hydro-alcoholic of *Calycopteris floribunda* on the diabetes induced by a multiple dose of STZ in diabetic rats.

MATERIAL AND METHODS

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Plant Material

The leaves of *Calycopteris floribunda* used in the present study was collected from the natural habitat in and around Chennai, Tamilnadu and the plant material was authentified by Dr. P. Jayaraman Ph.D., Plant Anatomy Research Centre (PARC), Tambaram, Voucher number is **PARC/2010/803.**

Plant extraction

The fresh leaves of *Calycopteris floribunda* was defatted using petroleum ether. The marc obtained was dried and subjected to extraction by adding dried leaf powder of distilled water heated to 50-60°C under constant stirring conditions for 1hour and filtered. The hydro-alcoholic extract was prepared by using Soxhlet's apparatus.

Experimental Animals

Albino Wistar rats of either sex, weighing 150-200 g, were used in the study. They were kept in standard laboratory conditions under natural light and dark cycle, and are housed at ambient temperature (22±1°C), relative humidity (55±5%). Animals had access to standard pellet diet and water given *ad libitum*. The proposal number is **IAEC/131/2010.**

Induction of Diabetes

Streptozocin was obtained from Himedia Laboratories, Mumbai. All other chemicals used for this study were of analytical grade. Streptozotocin (55 mg/kg) was dissolved in 0.1M citrate buffer (pH 4.5). Six rats per group were administered by subcutaneous injection. After 48 hrs, fasting blood glucose levels as well as glycosuria were assessed to confirm the diabetic state. Only rats with a fasting blood

Table 1: Effects of *HACF* on the body weight

Group (n=6)	Body weight (g)	
	2days after STZ injection	14 days after administration of plant extract
Normal control rats	196.5 ± 3.821*	220.7±3.227**
Diabetic control rats	170.4 ± 3.541	162.8±3.227
Test I (*HACF* 100mg/kg)	184.6 ± 1.631*	172.0±4.708*
Test II (*HACF* 200mg/kg)	185.3 ± 1.631*	175.5±5.204**

Results are expressed as mean ± SEM, n=6

Table 2: Effects of *HACF* on the blood glucose level

Treatment	Blood glucose level in mg/dl at different time interval					
	0h	2h	4h	6h	8h	24h
Group–I Control	83.83±2.13	73.67±2.2	72.67±1.5	79.83±2.9	89.5±3.0	84.67±2.9
Group–II Negative Control	294.2±3.7*	304.5±9.1*	313±4.8*	306.5±13.6*	317.2±13.2*	321±4.4*
Group–III *HACF* 100mg/kg	309.5±5.3***	265±5.5***	188.3±6.7***	164.2±3.76***	222.8±4.07***	328.7±3.3***
Group –IV *HACF* 200mg/kg	304.8±4.7***	254±5.8***	184.2±3.7***	136.2±2.48***	215.7±4.03***	303.8±3.6***

Results are expressed as mean ± SEM, n=6

glucose level of at least 250 mg/dl and positive urine glucose were considered diabetic and were used in the experiment.

Experimental Design

Male Wistar albino rats weighing 150-200 g (90 to 110 days old) were used. The animals were randomly divided into four groups of six animals each. Group 1: Normal control (non-diabetic, untreated, 5ml/kg, p.o) rats. Group 2: Diabetic control (diabetic, untreated, 8ml/kg, s.c) rats. Group 3: Diabetic test rats given *Calycopteris floribunda* extracts at the dose of 100 mg/kg. Group 4: Diabetic test rats given *Calycopteris floribunda* extracts at the dose of 200 mg/kg. Treatment of experimental animals with plant extracts was initiated 2 days post streptozotocin injection and was carried out once daily, by orally, for 14 days. Food and water were made freely available.

Measurement of body weight gain, food, water intake and blood glucose

Body weight gain, food and water intakes were monitored daily during the 14 days experimental period. Blood samples for glucose determination were obtained from the tail tip of 12 hrs fasted rats on day 0 (before streptozotocin administration), days 2 (48 h post streptozotocin injection), 5, 8, 11 and 14th day of the experiments. [5] Blood glucose level was determined using a glucometer. Urine glucose was also assessed in fresh urine using glucose indicator sticks before and 48 hrs after streptozotocin administration, for the confirmation of the diabetic state of animals.

Statistical Analysis

Mean values were obtained by one-way analysis of variance (ANOVA) followed by Dunnet's't' test, using the computer software, Graph pad Prism 5. The significance of difference between and within various groups was determined. The results are expressed as mean ± S.E.M. Values of $p < 0.05$ were taken to imply as statistically significant.

RESULTS AND DISCUSSION

The effects of the *Calycopteris floribunda* extract on the body weight of diabetic rats are shown in the following;

Effects of *HACF* on the body weight and Blood glucose level

During the 2 weeks of observation of the extract treated diabetic rats at doses of 200 mg/kg, there were very significant ($p < 0.01$) weight gains relative to day 2 showed a very significant ($p < 0.01$) weight increase in the body compared to untreated diabetic rats (Table 1). When compared to the untreated diabetic rats, untreated diabetic rats had severe polyphagia and polydipsia at the end of the second week of the experiment with respective increase in food and fluid intakes. However, in the presence of *Calycopteris floribunda* extracts extract (100mg/kg and 200 mg/kg), food intake was reduced when compared with diabetic control rats but it is not statistically significant ($p>0.05$). Fluid intakes showed decrease in *Calycopteris floribunda* extracts treated diabetic rats at doses of both 100 mg/kg and 200 mg/kg when compared with diabetic control rats (Table 2). Following a 48 h post streptozotocin injection, all diabetic rats exhibited hyperglycemia, which ranged between 330 and 400 mg/dl while normal control rats showed a normal blood sugar level of 110 mg/dl. After 2 weeks of treatment with the extracts, the glycemic level of 100 mg/kg *Calycopteris floribunda* extract treated diabetic rats dropped significantly from day 2 to day 14. In diabetes, oxidative stress is due to both an increased production of plasma free radical concentration and a sharp reduction of antioxidant defenses. GSH, being the most important bio-molecule against chemically induced toxicity [6] can participate in the elimination of reactive intermediates by reduction of hydro peroxides in the presence of Glutathione peroxidase. Glutathione (GSH) also functions as free radical scavenger and in the repair of free radical caused biological damage. The important mechanism implicated in the diabetes-genic action of STZ is by increased generation of oxygen free radicals [7], which causes a decrease in plasma GSH concentration, and plasma GSH/GSSG ratio. Our results suggest that the hydro-alcoholic *Calycopteris floribunda* extracts have dose-dependent hypoglycemic activities on streptozotocin-induced diabetes. [8] The metabolic disturbances were corrected after the plant extracts were administered for 2 weeks, as shown by the normalization of fasting blood glucose levels, reduction in polyphagia and polydipsia and weight gain by diabetic-treated rats but reduction in polyphagia and polydipsia are not statistically significant. [9] The mechanisms by which streptozotocin brings about its diabetic state include selective destruction of pancreatic insulin secreting beta cells, which make cells less active [10] and lead to poor glucose utilization by tissues. *Calycopteris floribunda* significantly reduced the high fasting glucose levels in streptozotocin-induced diabetic rats. [11] This suggests that the extracts may possess insulin like effect on peripheral tissues by either promoting glucose uptake and metabolism, by inhibiting hepatic gluconeogenesis [12] or absorption of glucose into the muscles and adipose tissues by the stimulation of a regeneration process and revitalization of the remaining beta cells. [13]

In conclusion the present investigation showed that *Calycopteris floribunda* extract possess hypoglycemic

activity. *Calycopteris floribunda* extract showed the effect due to enhancing effect on cellular antioxidant defenses to protect against oxidative damage.

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