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Research article

ANTIDIABETIC ACTIVITY OF *Anisochillus dysophylloides* WALL. ex BENTH. (LAMIACEAE) IN STREPTOZOTOCIN INDUCED TYPE II DIABETES IN MALE WISTAR ALBINO RATS.

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ABSTRACT : The administration of *Anisochillus dysophylloides* (AD) ethanolic extract for 21 days on the functions of hyperglycemia, hyperinsulinemia and serum hepatic marker enzymes were evaluated with STZ induced diabetic mellitus in male wistar albino rats. The results showed that a significant increase in the levels of blood glucose, urea, creatinine and glycosylated Hb and decrease in the level of plasma insulin in the diabetic control rats were observed. A decrease in the activities of serum protein, albumin and globulin in diabetic rats and these levels were increased in AD treated groups III & IV. The marker enzymes SGPT, SGOT and ALP levels were significantly elevated in the STZ treated groups and compared to normal. The groups treated with AD extracts showed significantly decrease in the serum marker enzymes. The antidiabetic effect of AD extract was compared with a standard antidiabetic drug, glibenclamide.

Key words: *Anisochillus dysophylloides*, Hyperglycemia, hyperinsulinmia, Streptozotocin, Glibenclamide

INTRODUCTION

The Lamiaceae is characterized by square stem, aromatic leaves (gland-dotted) and zygomorphic flower. It comprises about 3500 species and 210 genera. It is a cosmopolitan family with many well-known members of horticulture and economic importance due to the presence of essential oil. The species are mainly herbs or shrubs of various sizes, rarely trees. The medical application of these aromatic oils range from skin treatments to remedies for cancer [1]. In several traditional medicinal systems, including ayurveda, roman, greek, siddha and unani medical system, various therapeutic properties of lamiaceae have been mentioned. It has been reported to possess anti-carcinogenic, anthelmintic, anti-septic, anti-rheumatic, anti-stress, anti-bacterial, antidiabetic and anti-inflammatory properties [2]. The genus *Anisochillus* contains the compounds like carvacrol, camphor and α -cis bergamotene [3]. The plant *Anisochillus dysophylloides* Wall. ex Benth. is an annual erect herb, distributed in Nilgiris district and an unexploited medicinal plant from Lamiaceae. Hence, an attempt was made to screen the extract of this plant for antidiabetic properties.

MATERIALS AND METHOD

PLANT MATERIAL

All parts of *Anisochillus dysophylloides* were collected from The Nilgiris and authenticated by a taxonomist. The whole plant materials were air-dried in shade at room temperature. The dried material macerated first in petroleum ether and followed by ethanol. The ethanolic extract was concentrated at room temperature in amber-coloured light protected bottles for the further experimental studies.

EXPERIMENTAL INDUCTION OF DIABETES

Male albino rats weighing about (150-200gm) were used in this study. They were kept in a 12:12hr L:D cycle and the temperature maintained at $22\pm 2^{\circ}\text{C}$. Standard laboratory pellet diet (Hindustan Lever, Bangalore) and water were given *ad libitum*. The care of the animals was as per the 'Guidelines for the care and use of animals in the scientific research' by the Indian National Science Academy New Delhi [3].

The animals were diabetic with an intraperitoneal injection of STZ at a dose of 40mg/kg body weight dissolved in citrate buffer (0.1M, pH 4.5). STZ injected animal exhibited massive glycosuria and hyperglycemia within a few days. Diabetes was confirmed in the overnight –fasted rats by measuring blood glucose concentration. The rats with blood glucose above 250mg/dl were considered to be diabetic and used further experiment.

EXPERIMENTAL DESIGN

30 adult male albino rats weighing about (150-200g) were divided into five groups, six rats each. Group I represented control, Group II diabetic rats, Group III diabetic rats treated with AD drug (100mg/Kg B.Wt.), Group IV diabetic rats treated with AD drug (200mg/kg B.Wt.) Group V diabetic rats treated with glibenclamide (600mg/kg B.Wt) daily for 21 days respectively.

The dose (100 & 200 mg/kg B.Wt.), orally by IGC was standardized by pilot study with different doses of ethanolic extract of *Anisochillus* to assess the antihyperglycemic effects in STZ induced diabetic rats. After the experimental period all the animals were sacrificed by cervical dislocation and biochemical studies conducted in Blood, Plasma and liver samples. The plasma and liver samples was assayed by ELISA method using Boehinger mannham GmbH kit. The data were expressed as mean \pm SD. Statistical comparisons were performed by one-way anova followed by Dunnelt's test. The results were considered statically significant if $P < 0.05$.

RESULTS

A significant increase in the level of blood glucose, urea, creatinine and glycosylated Hb in the diabetic rats and a significant decrease in the plasma insulin level at the end of the experiment in the diabetic control rats were observed. When the diabetic rats treated with AD drug at dose of 100mg/kg B.Wt. and 200mg/kg B.Wt. showed increase the insulin level whereas glucose, urea, creatinine and glycosylated Hb levels were significantly reduced than the group II. The AD extract showed antihyperglycemic effect in a manner similar to that of reference drug, glibenclamide in STZ induced diabetic rats (Table 1).

Table 1: Effect of AD extracts on the serum insulin, glucose, urea, creatinine and Glycosylated Hb levels of normal, diabetic induced adult albino rats .

Parameter	Insulin (MIu/ml)	Glucose (mg/dl)	Urea (mg/dl)	Creatinine (mg/dl)	Glycolyted Hb
Group I	19.39 \pm 1.34	68.35 \pm 1.59	12.35 \pm 1.21	0.63 \pm 0.84	3.91 \pm 0.15
Group II	05.26 \pm 0.81**	221.41 \pm 5.34**	31.45 \pm 2.36*	0.93 \pm 0.34	9.36 \pm 0.23*
Group III	07.15 \pm 0.93	193.56 \pm 5.91	29.11 \pm 2.14	0.81 \pm 0.21	9.04 \pm 0.14
Group IV	12.11 \pm 0.83a	121.31 \pm 4.86a	24.14 \pm 1.96	0.84 \pm 0.11	7.11 \pm 0.21a
Group V	18.22 \pm 0.93 aa	81.15 \pm 3.84aa	22.05 \pm 1.83	0.79 \pm 0.32	6.28 \pm 0.33a

Values are expressed as mean \pm SEM of triplicate observations. N=6

*Comparison made between normal control to diabetic control and drug treated groups * $P < 0.05$;** $P < 0.01$

^aComparison made between diabetic control to drug treated groups Level of significance a: $P < 0.05$;aa $P < 0.01$

The table 2 represents a significant decrease in serum protein, albumin and globulin concentrations in diabetic condition, while AD ethanolic extract treated groups III and IV significantly ($P < 0.05$) improved the protein, albumin and globulin levels. It indicates control over the breakdown of body protein by the AD extract. However, these values levels after the treatment of rats with glibenclamide showed recovery almost equal to control. The activities of serum hepatic marker enzymes viz. SGPT, SGOT and ALP levels were significantly ($P < 0.05$) elevated in STZ treated group when compared to control (Table 2). The groups III & IV administered with AD at two concentrations (100mg/kg B.Wt. and 200mg/kg B.Wt.) showed significant decrease in the levels of serum marker enzymes. These effects were also comparable to glibenclamide treated group.

Table 2: Effect of AD extracts on the serum protein, albumin, globulin, SGOT, SGPT and ALP levels of normal, diabetic induced adult albino rats.

Parameter	Protein(g/dl)	Albumin(g/dl)	Globulin(g/dl)	SGPT(u/l)	SGOT(u/l)	ALP (u/l)
Group I	8.14±0.21	4.54±0.34	3.60±0.11	17.26±2.25	23.7±3.37	184.15±4.49
Group II	6.91±0.61*	3.81±0.35*	3.10±0.08	45.3±4.29*	45.33±4.93	206.25±6.44
Group III	8.34±0.42*	4.94±0.47	3.40±0.10	27.17±2.22	17.64±3.15	123.12±3.45
Group IV	8.24±0.30a	4.46±0.39	3.78±0.04	26.36±3.85	28.34±3.29	153.59±5.25
Group V	8.74±1.24aa	4.95±0.67a	3.79±0.04	15.35±3.84	19.49±1.26	138.23±4.75

Values are expressed as mean ± SEM of triplicate observations. N=6

*Comparison made between normal control to diabetic control and drug treated groups * $P < 0.05$;** $P < 0.01$

aComparison made between diabetic control to drug treated groups Level of significance a: $P < 0.05$;aa $P < 0.01$

DISCUSSION

STZ is a commonly employed compound for the induction of diabetes mellitus in experimental rats [5]. It causes DNA strands break in pancreatic islets, stimulates nuclear poly (ADP ribose) synthetase, and thus depletes the intracellular NAD^+ and $NADP^+$ levels, which inhibits proinsulin synthesis and induces diabetes [6]. The fundamental mechanism underlying hyperglycemic in diabetic mellitus involve over production of excessive hepatic glycogenolysis, gluconeogenesis and decreased utilization of glucose by tissues [7]. This study revealed a significant elevation ($P < 0.01$) in blood glucose in diabetic controls when compared with normal animals at the end of the third weeks of experimental period (Table1). This increase indicates in the controlled hyperglycemia in STZ treated rats.

The previous study reports have shown that protein synthesis decreased in all tissues due to decrease products of ATP and absolute or relative deficiency of insulin which may be responsible for the decreased level of Hb in diabetic rats [8]. Glycosylated Hb is increased in patients of overt diabetes [9]. A marked reduction in the glomerular filtration rate which was accompanied by an increase in the serum creatinine level indicating induction acute renal failure [10]. Alternations in the values of creatinine levels are taken as the indication of abnormal glomerular functions and these changes in the renal correlated with the nephrotoxic effects of negative controls which affect the renal function [11]. Excessive breakdown of body protein in conjugation either inadequate supply or defective utilization observed in uncontrolled diabetes may be accompanied by hypoalbuminemic [12]. The administration of STZ developed various symptoms of diabetes such as hyperglycemia, hypoinsulinemia, loss of body weight, polyphagia, polyurea and polydipsia. AD treatment significantly prevented the loss in body weight in diabetic rats and gain in body weight in non-diabetic animals. The treatment with AD extract prevented hyperglycemic and hyperinsulinemia. Serum glucose levels in STZ diabetic rats were significantly higher than the control. In the present study, there was a significant increase in glucose and a significant decrease in insulin values in STZ diabetic rats as compared to control rats. In spite of this the AD and glibenclamide administered rats have shown that these values were altered in a significant manner and these results are as comparable to previous works [13, 14].

Since the changes associated with STZ induced liver damage are similar to that of acute viral hepatitis [15], STZ mediated hepatotoxicity was taken here as the experimental model for liver injury. The hepatotoxic compounds such as STZ are known to cause marked elevation in serum transaminases. In agreement with results obtained in previous investigations [16,17], our present study elicited a significant increase in the activities of SGOT, SGPT and ALP. Pretreatment with the AD extract attenuated these increased enzyme activities produced by STZ and a subsequent recovery towards normalization of these enzymes strongly suggests the possibility of AD extract being able to condition the hepatocytes so as to cause accelerated regeneration of parenchyma cells, thus protecting against membrane fragility decreasing the leakage of marker enzymes into the circulation.

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