

## HYPOGLYCEMIC AND HYPOLIPIDEMIC EFFECTS OF ETHANOL EXTRACT OF *CANSCORA PERFOIATA* LAM. (GENTIANACEAE) WHOLE PLANT IN ALLOXAN INDUCED DIABETIC RATS

SOUNDRAPANDIAN THANGAKRISHNA KUMARI<sup>1</sup>, GURUSAMY SAKTHIDEVI<sup>2</sup>, SABAPATHY MUTHUKUMARASWAMY<sup>1</sup> AND VEERABAHU RAMASAMY MOHAN<sup>2\*</sup>

<sup>1</sup>Department of Botany, Sri K.G.S.Atrs College, Srivaikuntam, <sup>2</sup>Ethnopharmacology Unit, Research Department of Botany, V. O. Chidambaram College, Tuticorin 628 008, Tamil Nadu, India. Email: vrmohanvoc@gmail.com

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### ABSTRACT

**Objective:** To evaluate the hypoglycemic and hypolipidemic effects of ethanol extract of *Canscora perfoliata* whole plant in alloxan induced diabetic rats.

**Methods:** Diabetes was induced in Albino rats by administration of alloxan monohydrate (150mg/kg i.p). The ethanol extracts of *Canscora perfoliata* whole plant at a dose of 150 and 300 mg/kg of body weight were administered at a single dose per day to diabetes induced rats for a period of 14 days. The effect of ethanol extract of *C. perfoliata* whole plant extract on blood glucose, plasma insulin, urea, creatinine, glycosylated haemoglobin, serum lipid profile [total cholesterol (TC), triglycerides (TG), low density lipoprotein-cholesterol(LDL-C), very low density lipoprotein-cholesterol (VLDL-C), high density lipoprotein-cholesterol (HDL-C) and phospholipid (PL), serum protein, albumin, globulin, serum enzymes [serum glutamate pyruvate transaminases] (SGPT), and serum glutamate oxaloacetate transaminases (SGOT) and alkaline phosphatase (ALP)] were measured in the diabetic rats.

**Results:** In the acute toxicity study, ethanol extract of *Canscora perfoliata* whole plant was non-toxic at 2000 mg/kg in rats. The increased body weight, decreased blood glucose, glycosylated haemoglobin and other biochemical parameters level was observed in diabetic rats treated with both doses of ethanol extract of *Canscora perfoliata* whole plant compared to diabetic control rats. In diabetic rats, ethanol extract of *Canscora perfoliata* whole plant administration, altered lipid profiles were reversed to near normal than diabetic control rats.

**Conclusion:** Ethanol extract of *Canscora perfoliata* whole plant possess significant hypoglycemic and hypolipidemic activity in diabetic rats.

**Keywords:** *Canscora perfoliata*, Hypoglycemic, Hypolipidemic, Alloxan, Glibenclamide, SGOT, SGPT and HbA<sub>1c</sub>.

### INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by high levels of glucose in the blood due to the impaired secretion of insulin or insulin insensitivity. The high concentration of blood glucose and other biochemical abnormalities result from a deficiency of  $\beta$ -cells of the endocrine pancreas and/or form sub-sensitivity to insulin in target cells [1-3]. DM is also associated with an increased risk for developing premature atherosclerosis due to independent risk factors such as hypertriglyceridemia and hypertension. It is also characterized by polyuria, albuminuria, renal enlargement and an increase in serum creatinine value [4]. DM affects approximately 4% of the population worldwide and is expected to increase by 5.4% in 2025 [5]. A worldwide survey reported that the estimated incidence of diabetes and projection for year 2030 is 350 million [6, 7].

Currently, the available therapy for diabetes includes insulin and various oral antidiabetic agents. Such as sulfonylureas, thiazolidinediones,  $\alpha$ -glucosidase inhibitors, etc. These drugs are used as monotherapy or in combination to achieve better glycemic control. Each of the above oral diabetic agents with a number of serious adverse effects [8]. Hence, antidiabetic drug discovery has shifted its focus to natural plant sources having minimal side effects. Plants have played a major role in the introduction of new therapeutic agents and have received much attention as sources of biologically active substances including antioxidants, hypoglycemic and hypolipidemic agents [9].

*Canscora perfoliata* Lam. is one of the medicinally important plant belongs to Gentianaceae. The juice prepared from the plant is given to treat any poisonous bites by palliyar tribals of Grizzled Giant Squirrel Wildlife Sanctuary, Srivilliputhur, Western Ghats, Tamil Nadu [10]. The biological activities such as anti-inflammatory activity [11] and hepatoprotective activity [12] were reported.

There were no reports on the ability of *Canscora perfoliata* whole plant on hypoglycemic and hypolipidemic activities. Hence, this study was taken up to investigate the hypoglycemic and hypolipidemic activities of the whole plant of *Canscora perfoliata* in alloxan induced diabetic rats.

### MATERIALS AND METHODS

#### Plant Material

The well grown whole plant of *Canscora perfoliata* Lam. was collected from the natural forests of Western Ghats at Thanniparai, Srivilliputhur, Virudhunagar District, Tamil Nadu. With the help of local flora, voucher specimens were identified and preserved in the Ethnopharmacology Unit, Research Department of Botany, V. O. Chidambaram College, Tuticorin, Tamil Nadu for further references.

#### Preparation of plant extract for phytochemical screening and antidiabetic studies

The whole plant of *C. perfoliata* was shade dried at room temperature and the dried whole plants were powdered in a Wiley mill. Hundred grams of powdered *C. perfoliata* whole plant was packed in a Soxhlet apparatus and extracted with ethanol. The extract was subjected to qualitative test for the identification of various phytochemical constituents as per the standard procedures [13, 14]. The ethanol extracts were concentrated in a rotary evaporator. The concentrated ethanol extract were used for antidiabetic studies.

#### Animals

Normal healthy male Wistar albino rats (180- 240g) were housed under standard environmental conditions at temperature (25 $\pm$ 2<sup>o</sup> C) and light and dark (12: 12 h). Rats were fed with standard pellet diet (Goldmohur brand, MS Hindustan lever Ltd., Mumbai, India) and water *ad libitum*.

#### Acute Toxicity Study

Acute oral toxicity study was performed as per OECD – 423 guidelines (acute toxic class method), albino rats (n=6) of either sex selected by random sampling were used for acute toxicity study [15]. The animals were kept fasting for overnight and provided only with water, after which the extracts were administered orally at 5mg/kg body weight by gastric intubations and observed for 14 days. If mortality was observed in two out of three animals, then the dose administered was assigned as toxic dose. If mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for higher doses such as 50,100 and 2000 mg/kg body weight.

### Induction of Diabetes in Experimental animal

Rats were induced diabetes by the administration of simple intraperitoneal dose of alloxan monohydrate (150 mg/kg) [16]. Two days after alloxan injection, rats screened for diabetes having glycosuria and hypoglycemia with blood glucose level of 200-260 mg/100 ml were taken for the study. All animals were allowed free access to water and pellet diet and maintained at room temperature in plastic cages.

### Experimental Design

In the present investigation, a total of 30 rats (24 diabetic surviving rats and 6 normal rats) were taken and divided into five groups of 6 rats each.

**Group I:** Normal untreated rats

**Group II:** Diabetic control rats

**Group III:** Diabetic rats given ethanol extract of *C. perfoliata* whole plant (150mg/kg body weight)

**Group IV:** Diabetic rats given ethanol extract of *C. perfoliata* whole plant (300mg/kg body weight)

**Group V:** Diabetic rats given standard drug glibenclamide (600µg/kg body weight).

The animals were sacrificed at the end of experimental period of 14 days by decapitation. Blood was collected, sera separated by centrifugation at 3000g for 10 minutes.

### Estimation of insulin, glucose, urea, creatinine and glycosylated haemoglobin

Serum glucose was measured by the O-toluidine method [17]. Insulin level was assayed by Enzyme Linked Immunosorbant Assay (ELISA) kit [18]. Urea estimation was carried out by the method of Varley [19]; serum creatinine was estimated by the method of Owen et al [20]. Glycosylated haemoglobin (HBA<sub>1</sub>C) estimation was carried out by a modified colorimetric method of Karunanayake and Chandrasekharan [21].

### Estimation of protein, albumin, globulin, SGPT, SGOT, ALP

Serum protein [22] and serum albumin were determined by quantitative colorimetrically method by using bromocresol green. The total protein minus the albumin gives the globulin, serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxaloacetate

transaminase (SGOT) was measured spectrophotometrically by utilizing the method of Reitman and Frankel [23]. Serum alkaline phosphatase (ALP) was measured by the method of King and Armstrong [24].

### Estimation of lipids and lipoprotein

Serum total cholesterol (TC) [25], total triglycerides (TG) [26], low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL- C) [27], high density lipoprotein cholesterol (HDL-C) [28] and phospholipids [29] were analyzed.

### Statistical analysis

The data were analyzed using student's t-test statistical methods. For the statistical tests a *p* values of less than 0.01 and 0.05 was taken as significant.

## RESULTS

### Phytochemical constituents

The phytochemical screening of ethanol extract of *C. perfoliata* whole plant revealed the presence of alkaloid, catechin, coumarin, flavonoid, phenol, saponin, steroid, tannin, terpenoid, sugar, glycoside and xanthoprotein.

### Acute toxicity study

The ethanol extract was safe upto a dose of 2000 mg/kg body weight. Behavior of the animals was clearly observed for the first 8 hours then at an interval of every 4 hours during the next 48 hours, the extract did not cause mortality on rats during 48 hours observation or any behavioral change.

### Body weight and fasting blood glucose (FBG) level changes in diabetic rats

In the present study, alloxan induced diabetic rats showed significant (*p*<0.05) reduction in body weight (Table-1). The administration of *C. perfoliata* and glibenclamide to diabetic rats restored the changes in the levels of body weight. Table-1 shows the dose dependent antihyperglycemic activity of *C. perfoliata* extracts. The FBG levels of diabetic rats were significantly (*p*<0.001) higher than those of normal control rats. When different doses of *C. perfoliata* were tested for their glucose lowering effects, the ethanol extract at a dosage of 300 mg/kg body weight produced the maximum fall in the FBG levels of diabetic rats after 2 weeks of treatment.

**Table 1: Effect of ethanol extracts of *Canscora perfoliata* whole plant on the Body weight and Fasting Blood Glucose in Normal, Diabetic induced and diabetic treated rats.**

Parameter	Mean initial Body weight (g)	Mean final Body weight (g)	Mean weight Gain (G↑) / Loss(L↓) (g)	Fasting Blood Glucose (mg/dl)	
				Initial	Final (after 2 wks)
Group I	201.56±8.32	219.35±6.42	17.81±0.39↑	78.36±2.17	74.53±1.51
Group II	194.11±8.32	170.14±5.84*	23.97±1.34↓*	214.33±7.56***	201.74±3.13***
Group III	196.10±8.46	181.32±4.36*	15.32±0.94↓	216.41±3.26**	138.30±3.06**
Group IV	189.56±2.51	178.33±3.49*	11.23±0.84↓	218.36±0.94**	123.51±5.33 <sup>ab</sup>
Group V	198.34±5.36	191.59±5.13*	6.75±0.34↓	193.98±3.46**	118.56±2.94 <sup>ab</sup>

Each Value is SEM of 5 animals: \**p* < 0.05 comparison with Normal Control vs Diabetic and Drug treated; \* *p* < 0.05; \*\**p* <0.01; \*\*\**p* <0.001; ns - Not significant a - *p* < 0.05 Diabetic Control vs Drug treated; b - *p*<0.05 comparison with initial vs final

**Table 2: Effect of ethanol extracts of *Canscora perfoliata* whole plant on the Serum Insulin, Glucose, Urea, Creatinine and Glycosylated Haemoglobin level of Normal, Diabetic induced and diabetic treated rats.**

Parameter	Insulin (µu/ml)	Glucose (mg/dl)	Urea (mg/dl)	Creatinine (mg/dl)	Glycosylated Hb
Group I	18.59±1.53	76.99±1.26	10.53± 1.45	0.74±0.05	5.03±0.04
Group II	5.26±1.41***	201.56±14.33***	14.91±1.68*	0.88±0.02	9.36±1.16*
Group III	8.14±1.06 <sup>a</sup>	154.30±8.32 <sup>a</sup>	12.51±1.36 <sup>a</sup>	0.78±0.04 <sup>a</sup>	7.20±1.36 <sup>a</sup>
Group IV	12.81±1.31 <sup>a</sup>	126.39±2.56 <sup>a</sup>	11.84±0.93 <sup>a</sup>	0.72±0.01 <sup>a</sup>	5.27±1.07 <sup>a</sup>
Group V	17.91±1.26 <sup>aa</sup>	102.63±2.62 <sup>aa</sup>	9.43±0.14 <sup>a</sup>	0.79±0.05	4.97±0.84 <sup>aa</sup>

Each Value is SEM of 5 animals: \* *P* < 0.05; \*\*\**P* <0.001. Comparison made between Normal Control and Diabetic Control and Drug treated groups. <sup>a</sup> - *P*<0.05; <sup>aa</sup> - *P*<0.01- Comparison made between Diabetic Control and Drug treated groups.

### Blood glucose and the other parameters levels of diabetic rats

Table-2 shows the levels of blood glucose, serum insulin, urea, creatinine and glycosylated haemoglobin of normal, diabetic control and drug treated rats. There was a significant ( $p<0.001$ ) increase in blood glucose level in alloxan induced diabetic rats (Group-II) when compared with normal rats (Group-I). The administration of whole plant extract of *C. perfoliata* (Group-III and IV) and glibenclamide (Group-V) tends to bring the parameters ( $p<0.05$ ) towards the normal.

Serum insulin level of diabetic control group was significantly ( $p<0.001$ ) decreased when compared to normal control group (Group-I). The plant extract and glibenclamide group of diabetic rats significantly ( $p<0.05$ ;  $p<0.01$ ) increased insulin. A significantly elevation in urea and creatinine was observed in alloxan induced diabetic rats when compared to control rats. The *C. perfoliata*

extracts were administered orally to diabetic rats for 14 days reversed the urea and creatinine level to near normal. The *C. perfoliata* whole plant and glibenclamide ( $p<0.05$ ;  $p<0.001$ ) reduced HbA<sub>1c</sub>.

### Biochemical parameters levels in diabetic rats

The decreased total protein, albumin and globulin levels were noticed in diabetic control rats (Group-II) (Table-3). The administration of *C. perfoliata* whole plant extract 150 and 300 mg/kg and glibenclamide significant ( $p<0.05$ ) increased total protein, albumin and globulin levels compared to diabetic control rats. Also, the SGPT, SGOT and ALP levels were elevated in alloxan induced diabetic rats compared to control rats. Oral administration of *C. perfoliata* whole plant extract 300 mg/kg and glibenclamide treatment reduced above parameters compare to diabetic control rats.

**Table 3: Effect of ethanol extracts of *Canscora perfoliata* on the Serum protein, Albumin, Globulin, SGOT, SGPT and ALP levels of Normal, Diabetic induced and diabetic treated rats.**

Parameter	Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	SGPT (u/l)	SGOT (u/l)	ALP (u/l)
Group I	7.93±0.14	4.13±0.23	3.80±0.67	19.56±1.14	17.39±0.36	116.34±2.56
Group II	5.98±0.26*	3.48±0.14	2.50±0.24	20.66±2.01	21.42±0.88	124.56±1.93
Group III	6.12±0.16	3.65±0.26	2.47±0.14	20.06±1.32	20.33±0.46	112.16±1.26
Group IV	6.43±0.21*	3.75±0.39	2.68±0.16	18.31±1.24	18.16±0.14	108.33±2.84
Group V	8.24±0.19 <sup>a</sup>	4.20±0.16	4.04±0.36 <sup>a</sup>	17.56±0.84	18.54±0.91	108.51±1.88

Each value is SEM of 5 animals. \*  $P<0.05$ . Comparison made between Normal Control and Diabetic Control and Drug treated groups. <sup>a</sup>  $P < 0.05$ : - Comparison made between Diabetic Control and Drug treated groups

**Table 4: Effect of ethanol extracts of *Canscora perfoliata* on the Serum Lipid profile of normal, Diabetic induced and Diabetic treated rats.**

Parameter	TC (mg/dl)	T G(mg/dl)	LDL-C (mg/dl)	VLDL (mg/dl)	HDL (mg/dl)	PL (mg/dl)
Group I	118.31±2.63	83.67±1.84	24.31±1.13	77.27±1.27	16.73±0.98	173.29±3.14
Group II	184.16±1.98**	116.26±1.80*	49.64±1.73	141.27±2.14**	13.25±1.03*	231.90±2.59*
Group III	156.34±0.38	108.42±1.76	38.26±1.24	101.96±1.76	15.26±1.11	198.14±2.36
Group IV	129.31±1.63 <sup>a</sup>	101.34±1.67	29.66±1.84 <sup>a</sup>	79.39±1.93 <sup>a</sup>	20.26±1.21 <sup>a</sup>	183.0±3.46 <sup>a</sup>
Group V	122.33±1.42 <sup>a</sup>	98.26±0.94 <sup>a</sup>	29.56±1.61 <sup>a</sup>	73.12±1.83 <sup>aa</sup>	19.65±0.78 <sup>a</sup>	176.87±3.04 <sup>a</sup>

Each Value is SEM of 5 animals: \* $P < 0.05$ ; \*\* $P<0.01$  Comparison made between Normal Control and Diabetic Control and Drug treated groups. <sup>a</sup>  $P<0.05$ ; <sup>aa</sup>  $P<0.01$  - Comparison made between diabetic control and drug treated groups.

### Lipid profiles

Table-4 shows the levels of TC, TG, LDL-C, VLDL-C, HDL-C and PL in the serum of diabetic rats showed significantly ( $p<0.05$ ) increased serum lipid profiles except HDL-C when compared with normal rats. The ethanol extract of *C. perfoliata* whole plant treated rats showed a significant ( $p<0.05$ ) decrease in the content of lipid profiles when compared with diabetic induced rats. Similarly HDL-C level decreased in alloxan induced diabetic rats when compared with normal rats. The administration of ethanol extract of *C. perfoliata* whole plant and glibenclamide to the diabetic rats, HDL-C level found to be restored to normal.

### DISCUSSION

Diabetes mellitus is one of the common metabolic disorders with micro and macro vascular complications that results in significant morbidity and mortality. In the treatment of diabetes mellitus, non pharmacologic measures remain a critical component of therapy. The purpose of choosing alloxan monohydrate as the diabetes-inducing agent was known to produce diabetes mellitus irreversibly with a single dose administration by relative necrotic action on the beta cells of pancreas leading to insulin deficiency. Insulin deficiency leads to various metabolic aberrations in animals viz., increased blood pressure level, decreased protein content, increased level of cholesterol and triglycerides were reported [30].

Dietary management includes the use of traditional medicines that are mainly derived from plants [31]. Even now, approximately 80% of the third world population is almost entirely dependent on traditional medicines. There are numerous traditional medicinal plants reported to have hypoglycemic properties [32-34].

The present study indicates the hypoglycemic and hypolipidemic potential of *C. perfoliata* whole plant ethanol extract on alloxan induced diabetic rats. In the present study, induction of diabetes by alloxan, decreased the body weight. In diabetic rats, observed reduction in body weight was possible due to catabolism of fats and proteins [35]. The administration of ethanol extract of *C. perfoliata* whole plant improves the body weight compared to diabetic control rats which indicates preventive effect of *C. perfoliata* whole plant extract on degradation of structural proteins. Ethanol extract of *C. perfoliata* whole plant showed a dose dependent effect on FBG upto a dose of 300 mg/kg.

Administration of alloxan led to more than 1.5 fold elevation of fasting glucose level which was maintained over a period of two weeks. Two weeks of daily treatment of *C. perfoliata* whole plant extract (300 mg/kg) make fall in blood glucose level by 43.2%. The present findings indicate the hypoglycemic and potential antihyperglycemic nature of the extract.

*C. perfoliata* whole plant ethanol extract (150 and 300 mg/kg body weight) significantly ( $p<0.05$ ) decreased blood glucose level in alloxan induced diabetic rats. Similarly, ethanol extract of leaves of *Dalbergia sisoo* (250 mg/kg) showed reduction in blood glucose level was reported [36]. Similar antidiabetic activity was seen in the ethanol extract of leaves of *Eugenia singampattiana* [37]. There were two possible explanations for this finding. First, *C. perfoliata* whole plant may exert its effect by preventing the death of  $\beta$ -cells and/or second, it may permit recovery of partially destroyed  $\beta$ -cells. Like *Pterocarpus marsupium* [38], *C. perfoliata* may also have initiated cell proliferation. Hypoglycemic effects have been reported with other plants such as *Pithecolobium dulce* [39], *Aloe vera* [40],

*Sphaeranthus indicus* [41], *Wattakaka volubilis* [42] and *Polygala rosmarinifolia* [43] well known for their antidiabetic activities.

In diabetes, elevated levels of serum urea and creatinine are observed which may be due to renal damage caused by abnormal glucose regulation or elevate glucose and glycosylated protein tissue levels [44]. In the present study, significant increase in serum urea and creatinine levels was observed in diabetic rats. The treatment with ethanol extract of *C. perfoliata* whole plant decreased the above parameters significantly ( $p < 0.05$ ) compared to diabetic control rats and it showed protective effect of ethanol extract of *C. perfoliata* whole plant on the kidneys.

HbA<sub>1c</sub> is used as a marker for estimating the degree of protein glycation in diabetes mellitus. HbA<sub>1c</sub> was found to increase in patients with diabetes mellitus and the amount of increase is directly proportional to the fasting blood glucose level. In diabetic condition, the excess glucose present in the blood reacts with haemoglobin to form HbA<sub>1c</sub> [1]. Hence HbA<sub>1c</sub> levels were elevated. HbA<sub>1c</sub> levels were well regulated near to normal levels in *C. perfoliata* whole plant extract treated diabetic rats. This could be due to an improvement in insulin secretion upon *C. perfoliata* treatment.

In diabetic condition, occurrence of reduction in protein and albumin may be due to proteinuria, albuminuria or increased protein catabolism which is clinical markers in diabetic nephropathy [45]. The protein and albumin level were reduced after the induction of diabetes and treatment of ethanol extract of *C. perfoliata* whole plant increased both levels considerably in diabetic rats towards normal level. This action possibly is through increase in the insulin mediated amino acid uptake, enhancement of protein synthesis and/or inhibition of protein degradation [46]. Also, increased serum GOT, GPT and ALP levels were reported in diabetes and it may be due to liver dysfunction [47]. Many workers have reported increase in transaminase activity in liver and serum of diabetics. The increase level of transaminase which is active in the absence of insulin because of availability of amino acid in the blood of diabetics is responsible for the increased gluconeogenesis and ketogenesis observed in diabetics. In this study, increased levels of SGOT, SGPT and ALP was observed in alloxan induced diabetic rats which may have occurred by leakage of enzymes from the liver cytosol into the blood stream. It represents the toxicity of alloxan on liver. Diabetic rats treated with ethanol extract of *C. perfoliata* whole plant significantly reduced enzyme level which represents the protective action of ethanol extract of *C. perfoliata* whole plant on liver in diabetic condition.

The most common lipid abnormalities in diabetes are hypertriglyceridemia and hypercholesterolemia [48]. The abnormal high concentrations of serum lipids in diabetic animals are mainly due to an increased mobilization of free fatty acids from peripheral fat depots [49]. In the present study, significantly increased levels of serum TC, TG, VLDL and LDL as well as marked reduction in serum HDL level were observed in diabetic rats. Administration of both the doses of ethanol extract of *C. perfoliata* whole plant decreased levels of TC, LDL, VLDL and TG levels as well as increased the level of HDL in diabetic rats. The above action could be beneficial in preventing diabetic complications like coronary heart diseases and atherosclerosis in diabetic condition. Increased phospholipids level in serum was reported by Anitha *et al.*, [50] in alloxan induced diabetic rats. Administration of ethanol extract of *C. perfoliata* whole plant and glibenclamide decreased the levels of phospholipids.

The present study demonstrated that the ethanol extract of *C. perfoliata* whole plant could be useful in management of diabetes associated with abnormalities in lipid profiles. Further study need to be isolated, identified the active compounds and formulation.

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