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Pharmacological Evaluation of Fractioned Extracts of *Callistemon lanceolatus* for Antidiabetic and Hypolipidemic Activities in Diabetic Rats

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ABSTRACT

The present study was carried out to evaluate antidiabetic and hypolipidemic activities of *Callistemon lanceolatus* ethyl acetate and hexane fractions of dichloromethane extracts in streptozotocin and streptozotocin-nicotinamide induced diabetic wistar rat by administering graded oral doses (200 and 400 mg kg^{-1} b.wt.) for 21 days. Daily oral treatment with for 3 weeks resulted in significantly reduction in blood glucose, serum cholesterol ($p<0.05$) and triglycerides whereas HDL-cholesterol and serum insulin levels were found to be improved ($p<0.05$) as compared to diabetic control group. Present results indicate that ethyl acetate and hexane fractions have prominent antidiabetic effect at dose 400 mg kg^{-1} b.wt. in experimental diabetes and can therefore be used as an alternative remedy for the treatment of diabetes mellitus and its complications. In conclusion, the present study demonstrated that *C. lanceolatus* could be useful in management of type -1 and 2 diabetes associated with abnormalities in lipid profiles. The study needs to be validated in human volunteers to claim for its further usage in human volunteers and further studies are required to identify the active constituents.

Key words: *Callistemon lanceolatus*, hypolipidemic, serum cholesterol, streptozotocin, triglycerides

INTRODUCTION

Diabetes mellitus is a group of metabolic disorders characterised by high blood glucose level, resulting from insulin secretion defects, action or both (Okokon *et al*., 2007). Long-term complications of this illness contribute to increase mortality and morbidity. Conventional treatment regimens include insulin, oral antihyperglycemic agents and inhibitors of α-glycosidase enzyme. However, alternative therapies are also used, including plant products (Sabo *et al*., 1999) but these synthetic agents can produce serious side effects like hematological effects, coma, disturbance in kidney and liver and in addition they are not suitable for use during pregnancy (Rao *et al*., 2001; Valiathan, 1998; Radhika *et al*., 2010).

Diabetes mellitus is considered as one of the five leading causes of death in the world. Globally the number of people with diabetes is projected to rise from 171 million in 2000 to 366 million in 2030. Herbal treatment for diabetes has been a part of traditional medicine for thousands of years. Traditional Medicines derived from medicinal plants are used by about 60% of the world's population (Joseph and Jini, 2011; Latha and Daisy, 2010). India has about 45000 plant species and among them, several thousands have been claimed to possess medicinal properties

(Grover *et al.*, 2002). The available literature indicates that there are more than 800 plant species showing hypoglycaemic activity (Rajagopal and Sasikala, 2008; Jeyachandran and Mahesh, 2007). There has been increasing demand for the use of plant products with antidiabetic activity due to low cost, easy availability and lesser side effects. Therefore, plant materials are continuously scrutinized and explored for their effect as hypoglycemic agents.

Callistemon is a genus of more than 20 species of trees and shrubs, of which a few are cultivated in India as ornamental plants. *Callistemon lanceolatus* (Family: Myrataceae) commonly known as bottle brush, is shrub or small tree grow upto 7.5 m in height. Because of its ornamental value, it is cultivated in gardens (Sharma *et al.*, 2006; Anonymous, 1992). An aqueous extract of the leaves and flowers shows antifungal and antibacterial activity. The essential oils of the leaves of this plant possess antimicrobial, fungitoxic, antinociceptive and anti-inflammatory activities (Sudhankar *et al.*, 2004; Jeong *et al.*, 2009). Many people have been using *Callistemon lanceolatus* leaves in diabetes problems nearby area. There is no previous research wok available on antidiabetic use of the plant. So, the present work was performed to explore the antidiabetic and hypolipidemic activities of isolated fractions of dichloromethane extract of *Callistemon lanceolatus*.

MATERIAL AND METHODS

Chemicals: Streptozotocin was purchased from Sigma-Aldrich, India and nicotinamide was purchased from Hi-Mediam, India. Total cholesterol, High Density Lipoprotein (HDL)-cholesterol and triglyceride (TC) were assayed by autoanlyser (Erba Chem 7, Mannheim, Germany) using standard kits from Erba diagnostics Mannheim Gambh, Germany and Blood glucose level was measured using Elegance glucose meter (CT-X10) of Convergent Technologies, Germany. All reagents used in study were analytical grade.

Animals: Fifty four Wistar rats of either sex, weighing about 150-250 g were used in the study. Animals were maintained under standard environmental conditions i.e. ambient temperature of 22±2°C and at 45-55% relative humidity for 12 h, each of dark and light cycle and fed with a standard pellet rats diet obtained from Ashirwad Industries, Chandigarh, India and water was supplied *ad libitum*. All the studies were conducted in accordance with the Animal Ethical Committee of the University.

Plant material: *Callistemon lanceolatus* leaves were collected from the campus of Kurukshetra University, Kurukshetra, India during July, 2010 and were identified were identified by Dr. H.B. Singh, Scientist F and Head, Raw Material Herbarium and Museum, NISCAIR, New Delhi, India. A voucher specimen of the plant is preserved in the herbarium (NISCAIR/RHMD/Consult/-2009-10/1381/182/2).

Extract preparation: The collected leaves were washed with distilled water and shade-dried. The dried leave were powdered by using dry grinder and passed through sieve. Previously defatted powder material with petroleum ether (60-80°C) was packed into soxhlet apparatus and extracted with dichloromethane. The extract was evaporated to dryness under reduced pressure at 45°C to give solid residues. It is macerated with hexane for 48 h, filtered and dried to get hexane fraction (CLH). The marc was dried and again macerated with ethyl acetate. It was filtered and dried to obtained ethyl acetate fraction (CLE). Both fractions were stored in airtight containers in refrigerator below 10°C for subsequent experiments.

Induction of type-1 diabetes: Animals were made diabetic by single intraperitoneal administration of Streptozotocin (60 mg kg^{-1}) dissolved in 0.1M citrate buffer, pH 4.5. The blood glucose level was checked before and 72 h after streptozotocin injection to confirm the development of diabetes. Only those animals which showed hyperglycemia (blood glucose levels >250 mg dL^{-1}) were used in the experiment.

Type-2 diabetes: Type-2 diabetes mellitus (NIDDM) was induced in overnight fasted animals by a single intraperitoneal injection of 60 mg kg^{-1} STZ, 15 min after the i.p., administration of 120 mg kg^{-1} nicotinamide. Hyperglycemia was confirmed by the elevated blood glucose levels determined at 72 h and then on day 7 of the injection. Only rats confirmed with permanent NIDDM were used in the antidiabetic study (Masiello *et al.*, 1998).

Biochemical parameters: Blood samples were taken from the tip of the tail of each rat of different groups under mild ether anesthesia and glucose levels were determined by using blood glucose test strips with elegance glucometer (Frankenberg, Germany) at weekly intervals i.e., 0, 7, 14 and 21 day after daily administration of extract orally. Serum cholesterol, triglycerides and HDL-cholesterol were also evaluated in normal and streptozotocin induces diabetic rats by autoanalyser using Erba diagnostic kits (Rifai *et al.*, 1999; Burstein *et al.*, 1970).

Experimental design

Effect of CLE on diabetic rats: The study was carried out in the Institute of Pharmaceutical Sciences, Kurukshetra University, Kurukshetra, Haryana, India as follows:

Overnight fasted normoglycemic and diabetic rats were divided into nine groups with six animals each and treated orally daily for 21 days are as follows:.

- **Group I** : Normal healthy control: received only vehicle (Tween 80, 2% v/v)
- **Groups II** : Type-1, Diabetic control: received only vehicle
- **Groups III** : Type-2, Diabetic control: received only vehicle
- **Groups IV** : Type-1, Diabetic rats treated with CLE (400 mg kg^{-1} b.wt.)
- **Groups V** : Type-1, Diabetic rats treated with CLH 400 mg kg^{-1} b.wt.
- **Groups VI** : Type-2, Diabetic rats treated with CLE (400 mg kg^{-1} b.wt.)
- **Groups VII** : Type-2, Diabetic rats treated with CLH 400 mg kg^{-1} b.wt.
- **Groups VIII** : Type-1, Diabetic control: received Glibenclamide (10 mg kg^{-1})
- **Groups IX** : Type-2, Diabetic control: received Glibenclamide (10 mg kg^{-1})

Statistical analysis: All the results are presented as Mean±standard error of mean (S.E.M.) The statistical analysis involving two groups was evaluated by means of Student's t-test whereas one way analysis of variance (ANOVA) followed by Dunnet's multiple comparison post-test was used for statistical comparison between control and various treated groups. Statistical significance was accepted at the $p<0.05$ values (Kumar *et al.*, 2010b).

RESULTS AND DISCUSSION

The present study was undertaken to investigate the anti-diabetic and hypolipidemic effects of isolated fractions of dichloromethane extract of *C. lanceolatus* in type -1 and 2 diabetic rats model. Streptozotocin (60 mg kg^{-1}, i.p.) induced type -1 (IDDM) diabetes in rats with fating blood

Table 1: Long term effect of CLE and CLH on the blood glucose levels in diabetic rats

	Blood glucose level (mg dL^{-1})			
Groups/Treatment	Initial day	Day 7	Day 14	Day 21
I:NC	110.23±5.60	108.23±4.80	112.50±3.40	115.47±3.90
II:DC-1	261.50±3.22	294.50±3.71	355.60±1.32	405.00±3.58
III:DC-2	278.30±3.52	304.49±4.25	375.31±2.42	415.20±3.97
IV: DM-1+CLE	258.34±2.50	232.45±1.42*	168.43±2.16**	119.89±2.41**
V: DM-1+CLH	265.54±3.21	245.37±2.18*	176.32±3.28*	138.18±2.57**
VI: DM-2+CLE	272.25±2.45	263.47±2.27	182.25±2.46*	128.42±3.37**
VII: DM-2+CLH	281.23±3.23	256.42±4.49	126.32±2.28*	139.28±2.87*
VIII: DM-1+Std.	294.25±2.84	216.50±5.45*	128.50±5.24**	117.72±4.3**
IX:DM-2+Std.	283.42±2.34	224.80±4.27*	145.60±3.45*	130.56±2.47**

Data represent Mean±SEM. *$p<0.05$: When groups IV, V and VIII compared with diabetic control i.e., group II, **$p<0.001$: When groups VI, VII and IX compared with normal control i.e., group III

sugar level more than 250 mg dL^{-1}. CLH and CLE at doses of 200 mg kg^{-1} didn't show much significant results. Higher dose of 400 mg kg^{-1} was taken for the whole study. Daily orally administration of CLH and CLE at a dose 400 mg kg^{-1} significantly ($p<0.001$) reduced blood glucose level in diabetic rats from 258.34±2.5, 265.54±3.21 (initial day) to 119.89±2.41, 138.18±2.57 mg dL^{-1} (day 21), respectively (Table 1).

Streptozotocin-nicotinamide induced NIDDM diabetes. At the dose of 400 mg kg^{-1}, CLH and CLE brought about a significant reduction in the blood glucose levels in type-2 diabetic rats. CLE reduced 52.83% blood glucose level ($p<0.001$) whereas CLH showed 50.47% glucose level reduction ($p<0.05$) at the end of study as compared to the initial day as shown in Table 1.

Thus the isolated fractions, ethyl acetate and hexane brought significant reduction in blood glucose level in both types of diabetes. But ethyl acetate fraction (CLE) showed more reduction in blood glucose level than hexane fraction (CLH). Glibenclamide (a long lasting sulfonylurea) acts mainly by stimulating insulin secretion was taken as standard antidiabetic drug. Plants may act on blood glucose through different mechanisms, some of them may have insulin-like substances and some may inhibit insulinase activity (Collier *et al*., 1987; Chakravarthy *et al*., 1980). Stimulation of β-cells to produce more insulin and others may increase β-cells in the pancreas by activating regeneration of pancreatic cells (Bopanna *et al*., 1997) Abnormalities in lipid profile are one of the most common complications in diabetes mellitus which is found in about 40% of diabetics (Nagappa *et al*., 2003). In our study, the isolated fractions showed antidiabetic activity in both types of diabetes. This suggested that the plant may act as antidiabetic by stimulation of β-cells to produce more insulin of regeneration of the cells. Insulin deficiency or insulin resistance is associated with hypercholesterolemia and hypertriglyceridemia (Shanmugasundaram *et al*., 1990; Kumar *et al*., 2010a). There was also abnormal lipid level in diabetic rats as shown in Table 2. Treatment with fractions CLH and CLE at 400 mg kg^{-1} caused significant improvement in HDL-cholesterol level in type-1 diabetes from 28.23±2.2 to 36.38±2.4 ($p<0.05$) and 34.24±3.8 mg dL^{-1} ($p<0.001$), respectively. The HDL-cholesterol was also enhanced significantly ($p<0.05$) by CLE in type-2 diabetes from 24.37±2.5 to 32.32±4.3 mg dL^{-1} as shown in Table 2. Serum cholesterol and triglyceride levels were decreased significantly by both fractions in type-1 diabetes and type-2 diabetes. CLE showed better results as compared to CLH. All the results were compared to diabetic

Table 2: Effect of CLE and CLH on lipid profile (mg dL^{-1}) in diabetic rats

Groups	Cholesterol	Triglycerides	HDL-cholesterol
I:NC	87.28±3.8	82.42±5.16	37.32±2.9
II:DC-1	254.73±7.6	150.52±4.71	28.23±2.2
III:DC-2	257.54±5.4	156.43±3.25	24.37± 2.5
IV: DM-1+CLE	115.34±5.2**	95.24±3.43*	36.38±2.4**
V: DM-1+CLH	125.49±3.7*	104.20±4.79*	34.24±3.8*
VI: DM-2+CLE	117.56±4.9*	96.28±3.76**	32.32±4.3*
VII: DM-2+CLH	127.75±3.4*	108.30±4.52	30.23±3.5
VIII: DM-1+Std.	98.72±5.3**	83.47±4.5*	45.28±4.8**
IX:DM-2+Std.	95.43±4.5**	81.25±3.7*	39.24±3.7**

Data represent Mean±SEM. *$p<0.05$: When groups IV, V and VIII compared with diabetic control i.e., group II, **$p<0.001$ When groups VI, VII and IX compared with normal control i.e., group III

controls (Table 2). In CLH and CLE treated diabetic rats, there was a reduction in total cholesterol and triglycerides which shows the hypolipidemic effect of this plant. There was also beneficial effect on HDL-cholesterol. The hypolipidemic effect may be due to inhibition of fatty acid synthesis (Chi *et al.*, 1982).

CONCLUSION

The repeated administration of the CLH and CLE for a period of 21 days resulted in a significant decreased blood glucose level and enabled maintenance lipid profile in the diabetic rats. The present study demonstrated that *C. lanceolatus* could be useful in management of both types diabetes associated with abnormalities in lipid profiles.

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