

**HYPOGLYCAEMIC ACTIVITY OF CALLISTEMON LANCEOLATUS
LEAF ETHANOLIC EXTRACT IN STREPTOZOTOCIN INDUCED
DIABETIC RATS**

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Summary

The hypoglycaemic activity of the ethanolic extract, petroleum ether and chloroform fractions of leaves of *Callistemon lanceolatus* were carried out in streptozotocin induced diabetic rats for 30 days. The effect on biochemical parameters (lipid peroxidation, reduced glutathione levels, activity of antioxidant enzymes) were also assessed to evaluate their activity in controlling diabetes related metabolic conditions. The results indicate that the chloroform fraction at 150 mg/kg b.w. significantly lowers blood glucose level with insignificant ulceration compared to the standard drug glibenclamide (3 mg/kg b.w.). Also, there was reduction in lipid peroxidation level and reduced glutathione levels and elevation in the activity of antioxidant enzymes.

Keywords: *Callistemon lanceolatus* DC. Hypoglycaemic, lipid peroxidation, reduced glutathione, ethanolic extract.

Introduction

Diabetes mellitus (DM) is the most common endocrine disease and is always associated with complications like chronic hyperglycaemia and disturbances of carbohydrate, lipid and protein metabolism ^[1]. Currently available therapies for diabetes include insulin and various oral anti-diabetic agents which have a number of serious adverse effects; thus, managing diabetes without any side effects is still a challenge ^[2]. Therefore, many traditional plant treatments are used throughout the world due to their less toxicity and fewer side effects ^[3-4].

Callistemon lanceolatus DC (Myrtaceae) commonly known as 'red bottle brush'. The essential oils from leaves are reported to possess antibacterial and antifungal activity [5]. The antimicrobial, antistaphylococcal, antithrombin, anti inflammatory, strong DPPH scavenging and elastase inhibition activity of the plant are is well reported in traditional medicine [6-10]. It is also reported that piceatannol and scirpusin B isolated from the stem bark of *C. rigidus*, showed inhibitory effects on mouse α -amylase activity [11]. Despite of its ethnopharmacological properties, there are no reports about the hypoglycaemic activity of *C. lanceolatus* in the literature. *C. lanceolatus* DC is found to be rich source of phenolic acids, flavanoids, ellagic acid derivatives and tannins [12-14]. Recent investigations revealed that the polyphenols are found to be effective against the inhibitory activities of α -glucosidase and aldose reductase so they could lead to the development of new classes of possibly safer antidiabetic agents [15-16]. The present study is focussed to evaluate the hypoglycaemic activity of the ethanolic extract and fractions of *C.lanceolatus* in streptozotocin induced diabetic rats. The effect on biochemical parameters (lipid peroxidation, reduced glutathione superoxide dismutase and catalase enzymes) were also assessed to evaluate their activity in controlling diabetes related metabolic conditions.

Materials and methods

Collection of plant material

The leaves of *Callistemon lanceolatus* DC were collected from Saket Nursery, New Delhi, India in March 2009 and authenticated by Dr. H. B. Singh, Taxonomist, National Institute of Science Communication and Information resources, New Delhi. A voucher specimen (No.1386/188) has been deposited in the author's laboratory.

Preparation of plant extract

The leaves of *Callistemon lanceolatus* DC were shade dried for 2-3 days and coarsely powdered. The grounded leaves (10 kg) were extracted with 95% ethanol in a Soxhlet extractor. The ethanolic extract was concentrated under reduced pressure to yield a brown viscous mass (450 g). The ethanolic extract was then partitioned with petroleum ether and CHCl₃ to furnish petroleum ether fraction (100 g) and CHCl₃ fraction (150 g). The extract and its different fractions were kept under refrigeration until used for its biological and phytochemical screening.

Animals and housing condition

Albino wistar rats of either sex (150 to 200 g) were obtained from Central Animal House, Jamia Hamdard University, New Delhi. The animals were kept in cages at the room temperature and fed with food and water ad libitum. Fourteen hours before the start of the experiment the animals were sent to lab and fed only with water ad libitum. The experiments were performed in accordance with the rules of Institutional Animals Ethics Committee (registration number 173-CPCSEA).

Induction of diabetes

The rats were fasted overnight and diabetes was induced by injecting streptozotocin (STZ) (60 mg/kg body weight) intraperitoneally. STZ was prepared freshly in 0.1 M citrate buffer (pH 4.5). The animals were allowed to drink 5% glucose solution overnight to overcome the drug-induced hypoglycemia. Control rats were injected with citrate buffer alone. The animals were considered as diabetic, if their blood glucose values were above 250 mg/dl on the 3rd day after STZ injection. The treatment was started on the 4th day after STZ injection and this was considered as 1st day of treatment. The treatment was continued for 30 days.

Experimental design

The rats were divided into nine groups comprising of six animals in each group as follows: Group I: Control rats receiving 0.1 M citrate buffer (pH 4.5), Group II: Diabetic controls (STZ induced), Group III: Diabetic rats given *Callistemon* ethanolic extract (150 mg/kg bd wt) in aqueous suspension orally for 30 d, Group IV: Diabetic rats given *Callistemon* ethanolic extract (350 mg/kg bd wt) in aqueous suspension orally for 30 d, Group V: Diabetic rats given *Callistemon* petroleum ether fraction (80 mg/kg bd wt) in aqueous suspension orally for 30 d, Group VI: Diabetic rats given *Callistemon* petroleum ether fraction (150 mg/kg bd wt) in aqueous suspension orally for 30 d, Group VII: Diabetic rats given *Callistemon* chloroform fraction (80 mg/kg bd wt) in aqueous suspension orally for 30 d, Group VIII: Diabetic rats given *Callistemon* chloroform fraction (150 mg/kg bd wt) in aqueous suspension orally for 30 d, Group IX: Diabetic rats given glibenclamide (3 mg/kg bd wt) in aqueous solution orally for 30 d.

Biochemical Parameters

At the end of the experimental period, the rats were anaesthetized and sacrificed by cervical dislocation. Blood was collected in tubes containing EDTA. Organs (pancreas, kidney and liver) were removed for histopathological evaluation and biochemical parameters. Lipid peroxidation (LPO) was assayed by the method of Wright *et al.* ^[17], reduced glutathione (GSH) was assayed by the method of Jollow

et al. ^[18] Superoxide dismutase and Catalase activity was assayed by the method of Yen and Chen ^[19] and Claiborne ^[20] respectively.

Histopathological studies.

Animals were sacrificed by decapitation and tissues (liver, kidney and pancreas) were removed immediately for histopathological studies. The tissues were fixed with 10% phosphate-buffered neutral formalin, dehydrated in graded (50-100%) alcohol and embedded in paraffin. Thin sections (4-5 μ M) were cut and stained with routine hematoxylin and eosin (H & E) stain for photomicroscopic assessment.

Statistical analysis

Data was analyzed by one way ANOVA followed by Dunnett's 't' test (n=6), *p<0.05, **p<0.01 significant from control; ***P<0.001 extremely significant from control; ns- not significant.

Results

Phytochemical screening: Phytochemical screening of the ethanolic extract revealed the presence of flavanoids, tannins, steroids and phenolic glycosides.

Effect of *C. lanceolatus* ethanolic extract, petroleum ether and chloroform fractions on blood glucose levels of diabetic rats

The ethanolic extract of *C. lanceolatus* when administered to diabetic rats at 150 mg/kg b.w and 350 mg/kg b.w, caused a significant hypoglycaemic effect throughout the studies by reducing blood glucose level to 180 \pm 17.54 and 120 \pm 18.64 mg/dL respectively comparable to the standard glibenclamide which caused 90 \pm 8.80 reduction in blood glucose after 30 days of study (Figure I). Of the two fractions, petroleum ether and chloroform, the chloroform fraction was found to be more effective in reducing blood glucose level (135 \pm 18.79) than the petroleum ether fraction (145 \pm 19.56).

Effect of *C. lanceolatus* ethanolic extract, petroleum ether and chloroform fractions on lipid peroxidation and reduced glutathione (GSH) level in pancreas of diabetic rats

It was observed that the ethanolic extract, petroleum ether and chloroform fractions decrease the levels of lipid peroxidation in pancreas of diabetic rats (Figure 2A). Also, the ethanolic extract, petroleum ether and chloroform fractions and glibenclamide when administered to diabetic rats brought GSH levels to near normal level (Figure 2B).

Effect of *C. lanceolatus* ethanolic extract, petroleum ether and chloroform fractions on superoxide dismutase (SOD) and catalase (CAT) enzymes in pancreas of diabetic rats

Callistemon ethanolic extract, petroleum ether and chloroform fractions and the standard drug, glibenclamide recovered the activities of SOD and catalase enzymes to close to control values in rats in which diabetes had been induced (Figure 3A, B).

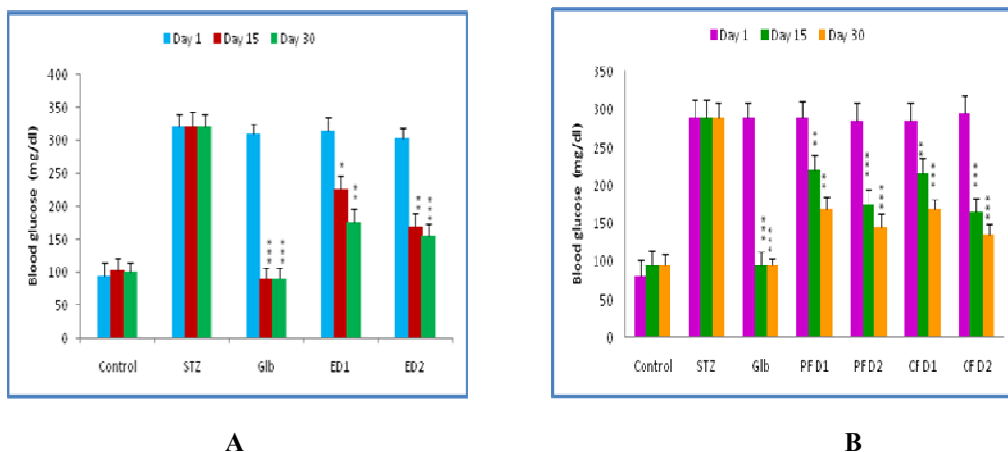


Figure I. Effect of (A) ethanolic extract and (B) petroleum ether and chloroform fractions of *Callistemon* on blood glucose levels of diabetic. Blood glucose was measured on days 1, 15 and 30 of induction of diabetes.

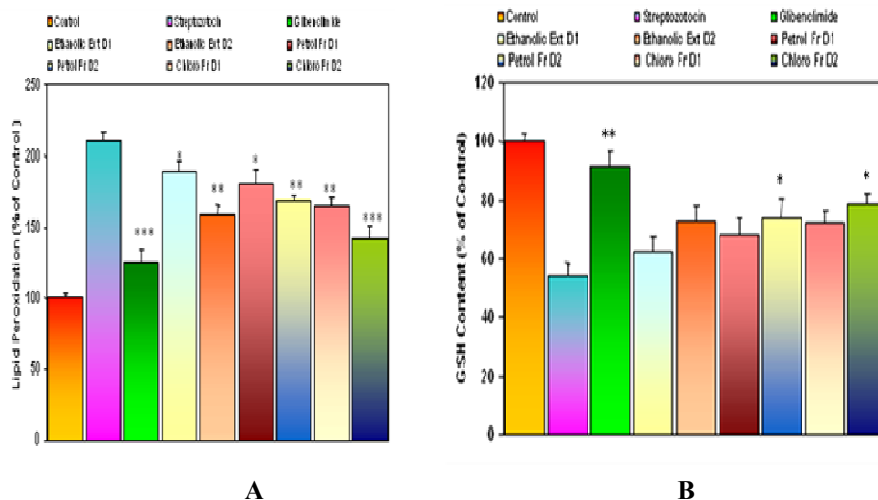


Figure II. Effect of ethanolic extract and petroleum ether and chloroform fractions of *Callistemon* on (A) lipid peroxidation and (B) reduced glutathione (GSH) level in pancreas of diabetic rats. Results are expressed as % of control. Each value is mean \pm S.E. ($n=5$)

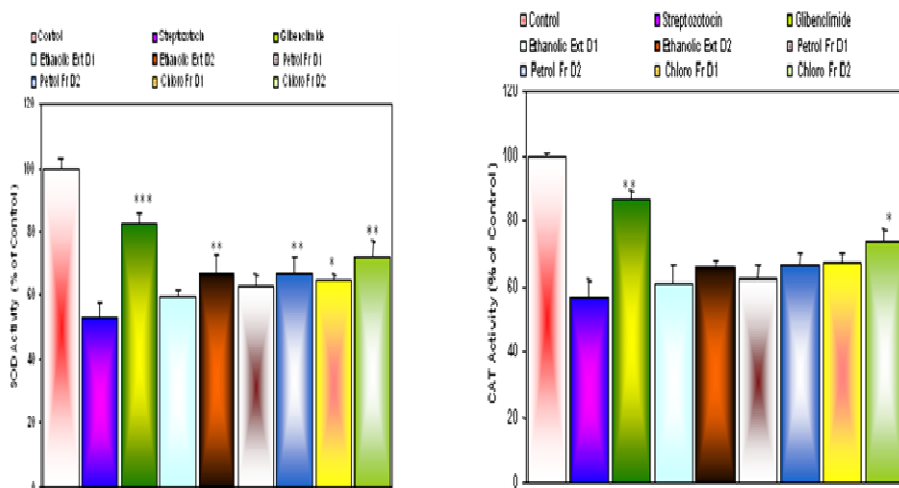


Figure III. Effect of ethanolic extract and petroleum ether and chloroform fractions of *Callistemon* on (A) SOD and (B) CAT activity in pancreas of diabetic rats. Results are expressed as % of control. Each value is mean \pm S.E. ($n=5$)

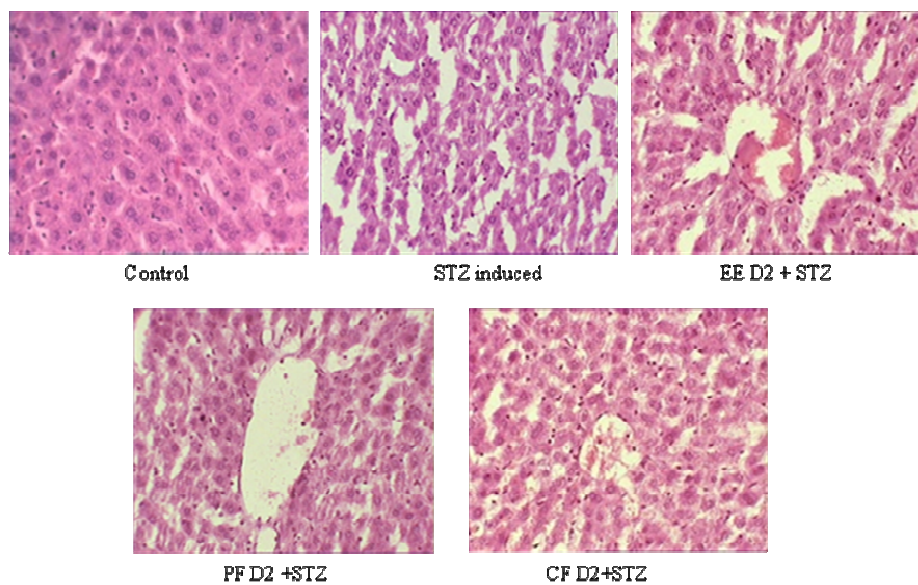


Figure IV. Effect of *Callistemon* extract and its fractions on histopathological changes in liver of rats in which diabetes had been induced by STZ.

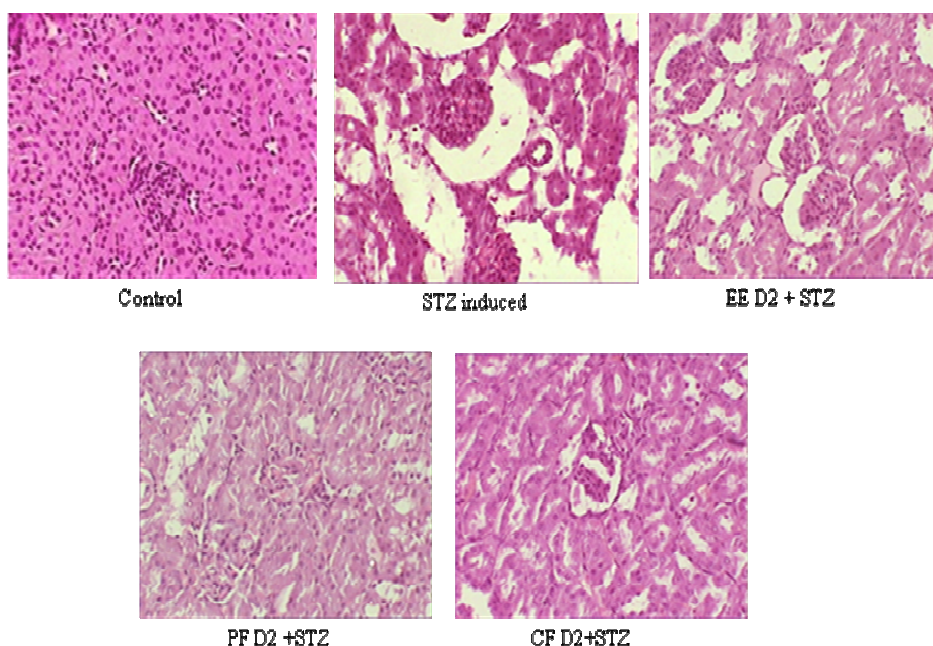


Figure V. Effect of *Callistemon* extract and its fractions on histopathological changes in kidney of rats in which diabetes had been induced by STZ

Discussion

The results of the study indicate that the tested ethanolic extract, petroleum ether and chloroform fractions exhibited significant hypoglycaemic activity. It was observed that there was a significant elevation in blood glucose levels 48 h after administration of streptozotocin (STZ), which persisted until the end of the study, i.e., after 1 month indicating persistent hyperglycemia. In diabetic rats the blood glucose level remained high (320 ± 13.76 mg/dL) and the administration of ethanolic extract of 350 mg/kg b.w caused a significant hypoglycaemic effect throughout the studies by reducing blood glucose level to 155 ± 12.08 mg/dL comparable to the standard glibenclamide which caused 90 ± 8.80 reduction in blood glucose after 30 days of study (Figure I). Of the two fractions, petroleum ether and chloroform, the chloroform fraction was found to be more effective in reducing blood glucose (135 ± 18.79) than the petroleum ether fraction (145 ± 19.56). The phytochemical analysis of the ethanolic extract of *C.lanceolatus* revealed the constituents that have been reported to have antidiabetic potential e.g. flavanoids^[21-22] and tannins^[23] Thus, one or more of these constituents present in the leaf extract of *C.lanceolatus* are likely to contribute to the observed hypoglycaemic activity.

Hyperglycaemia is associated with elevation in lipid peroxidation which represents a risk factor for coronary heart diseases [24]. Administration of the *Callistemon* extract and its fractions to the diabetic rats suggest that the extract not only possess significant hypoglycaemic ability but also has a remarkable hypolipidemic effect in STZ induced diabetic rats (Figure II). A significant decrease in the concentration of GSH was observed in diabetic rats when compared to control group of rats. Feeding of *Callistemon* extract or its petroleum ether/chloroform fractions or glibenclamide to diabetic rats brought GSH levels to near normal level.

SOD and CAT enzymes have important effects on the control of oxidation reactions in the body, impair cellular function and contribute to the pathophysiology of diabetes [25]. The concentration of SOD and CAT enzymes in the diabetic rats was significantly lowered than in the normal rats (Figure III). The level of SOD and CAT enzymes was increased after treatment with the ethanolic extract and its fractions suggesting that the extract and its fractions have effective antioxidant properties and could scavenge excess free radicals. It suggests that the *C. lanceolatus* has an obvious antioxidant effect and may prevent the oxidative damage to the tissue and increase the protective effect against diabetic complications.

Histopathology studies also supported our findings. The histopathological examination revealed extensive alterations in liver and kidney of STZ-induced diabetic rats. The liver of control rat showed normal architecture. The liver of diabetic rat showed perivenular inflammatory infiltration filling over the sinusoidal vacuolation of the hepatocyte nuclei. The pathomorphological changes observed in STZ-induced diabetes became apparently normal after treatment with *Callistemon* extract or its petroleum ether /chloroform fractions or glibenclamide (Figure IV). The kidney of control rat showed normal glomeruli and tubules. The kidney of diabetic rat showed thickening of vesicles, glomeruli showed some cellular proliferation with fibrosis (Figure V). *Callistemon* extract or its petroleum ether/chloroform fractions or glibenclamide treated diabetic rat showed normal glomeruli.

From this study, it can be concluded that the tested ethanolic extract, petroleum ether and chloroform fraction of *C. lanceolatus* possess hypoglycaemic potential in Streptozotocin induced diabetic rats. The treatment of diabetic rats with *C. lanceolatus* extract recovered the activities of antioxidant enzymes, lipid peroxidation and GSH levels without causing any toxicity.

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