

Protective effect of aqueous suspension of dried latex of *Calotropis procera* against oxidative stress and renal damage in diabetic rats

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ABSTRACT: *Calotropis* species have been used in the traditional medicinal system for the treatment of diseases of the liver and abdomen. In view of the antioxidant and anti-hyperglycemic properties of an aqueous suspension obtained from the dried latex of *Calotropis procera*, the present study was carried out to evaluate its efficacy in affording protection against alloxan induced changes in rat kidney. A single intraperitoneal injection of alloxan (150 mg/kg) in rats produced hyperglycemia within 3 days and altered kidney functions over a period of 90 days. Daily oral administration of the aqueous suspension (100 and 400 mg/kg) in diabetic rats produced anti-hyperglycemic effect that was comparable to that of glibenclamide (10 mg/kg). Unlike glibenclamide, the aqueous suspension did not increase the serum insulin levels in diabetic rats. However, it produced a marked reduction in the levels of urinary glucose and protein and normalized the renal tissue levels of thiobarbituric acid-reactive substances (TBARS) and glutathione (GSH) in diabetic rats and the effect was comparable to that of glibenclamide. The protection afforded by the aqueous suspension was also evident from the histological analysis of the renal tissue. Our study shows that by exhibiting antioxidant and anti-hyperglycemic property the aqueous suspension of dried latex of *C. procera* affords protection against the complications associated with diabetes.

Introduction

Diabetes mellitus has assumed epidemic proportions worldwide and as per the estimate of the International Diabetes Federation there are about 41 million diabetics in India alone and this figure is expected to rise to 70 million by the year 2025 (Sicree *et al.*, 2006). It is a metabolic disorder associated with defect in insulin secretion or action thereby resulting in derangement in carbohydrate metabolism that is evident in the form of hyperglycemia. Chronic exposure to high glucose level leads to deterioration of pancreatic β -cell function and can induce both microvascular and macrovascular complications. The most common mi-

crovascular complications of diabetes mellitus include retinopathy, neuropathy and nephropathy where the role of reactive oxygen species is well established (Hakim and Pflueger, 2010; King and Loeken, 2004; Yamagishi and Imaizumi, 2005). About one third of diabetic patients develop significant nephropathy that may progress to end stage renal failure (Zipp and Schelling, 2003). In hyperglycemic conditions, presence of overt proteinuria correlates with the extent of mesangial hypertrophy and loss of glomerular function, thus suggestive of significant renal damage (Mauer, 1994). Experimental diabetes, induced by chemical agents like alloxan, destroys β -cells of pancreas by generating excess reactive oxygen species and produces kidney lesions that are similar to human diabetic nephropathy (Vander Jagt *et al.*, 2001).

Several medicinal plants and herbal formulations have been reported to lower blood glucose in diabetic

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patients by acting through different mechanisms. Their efficacy has also been demonstrated in various experimental models of diabetes (Hui *et al.*, 2009). *Calotropis* species are wild growing laticiferous plants of family Apocynaceae that have been used in the traditional medicinal system for the treatment of various diseases including those of spleen, liver and abdomen (Kirtikar and Basu, 1935). The latex of *C. gigantea* is one of the constituents of an Ayurvedic preparation called Swarnabhasma (gold ash), used for the treatment of asthma, rheumatoid arthritis, nervous disorders and diabetes mellitus (Mitra *et al.*, 2002). The latex of *C. procera* has earlier been demonstrated to exhibit anti-hyperglycemic effect in diabetic rats that is associated with its ability to restore hepatic glycogen and normalization of levels of oxidative stress markers (Roy *et al.*, 2005). The free radical scavenging and antioxidant property of the latex of *C. procera* has been shown to be comparable to standard antioxidant, Vitamin C (Mueen Ahmed *et al.*, 2003, 2004). The present study was carried out to study the effect of long term treatment with aqueous suspension of dried latex of *C. procera* on alloxan induced diabetic changes in rat kidney and to elucidate its mechanism of action. Effect on parameters like blood glucose, serum insulin, levels of oxidative stress markers and histology of renal tissue was evaluated.

Materials and Methods

Experimental animals

The study was carried out in Wistar rats of either sex ranging from 150 to 200 g that were obtained from the Experimental animal facility of All India Institute of Medical Sciences after getting the approval of Institutional Animal Ethics Committee and the experiments were carried out as per the guidelines of Animal Ethics Committee. The rats were acclimatized for a period of one week before starting the experiments.

Plant material

The latex was collected from the aerial parts of the plant *Calotropis procera* that was identified by the Raw materials, Herbarium & Museum Division, National Institute of Science Communication, New Delhi where a voucher specimen is preserved (Voucher No. PID 1739). It was dried under shade at ambient temperature with a yield of 20 g per 100 ml. The dry latex was tritu-

rated with normal saline and the residual rubber like material that separated was discarded. The aqueous suspension thus obtained was administered orally to rats.

Experimental design

Diabetes was induced in rats by an intraperitoneal (i.p.) injection of freshly dissolved alloxan in normal saline at a dose of 150 mg/kg body weight. Fasting blood glucose levels were measured on day 3 and rats with blood glucose levels greater than 175 mg/dl were included in the study (Yadav *et al.*, 2002).

Rats were divided into five groups comprising of six animals each: Group I served as normal control while

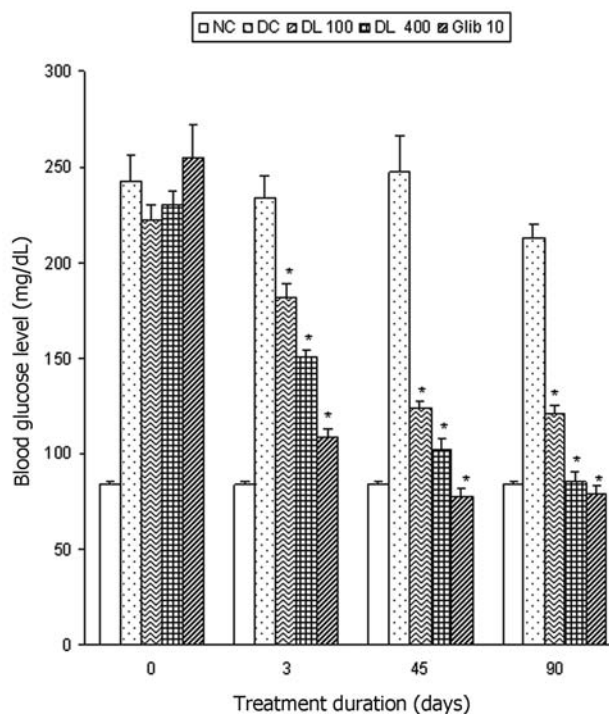


FIGURE 1. Effect of an aqueous suspension of dried latex of *C. procera* on blood glucose level in diabetic rats. Diabetes was induced in rats by a single intraperitoneal injection of alloxan. Both the aqueous suspension and glibenclamide were administered orally and daily at the indicated doses. Blood glucose levels were measured at different time intervals over 90 days. Each bar represents the values as mean \pm SEM (n=6). Abbreviations: NC, normal control; DC, diabetic control; DL 100, latex suspension, 100 mg/kg; DL 400, latex suspension, 400 mg/kg; Glib 10, glibenclamide, 10 mg/kg. Stars indicate statistically significant differences from the diabetic control group.

alloxan diabetes was induced in all other groups. Group II: diabetic control; Groups III-IV: the aqueous suspension was administered in doses of 100 or 400 mg/kg/day, respectively, for 90 days; Group V: the standard anti-diabetic drug glibenclamide (10 mg/kg) was orally administered for 90 days. Blood glucose levels were measured on day 0, 3, 45 and 90 following drug treatment. Urine samples of the rats were collected several times during the study using a metabolic cage and glucose and protein were detected to ensure that the renal functions are altered by alloxan in the diabetic control group. Rats were sacrificed on day 90 when the urinary levels of glucose and protein were significantly higher in the diabetic control group as compared to normal control group. Their serum was collected for determining the levels of insulin. The kidneys were excised immediately and a portion was fixed by immersion in buffered formalin while the rest was stored at -70°C for the measurement of parameters of oxidative stress.

Blood glucose estimation

Glucose level was estimated in tail vein blood sample by commercially available glucose kit (Glucocare, India) that is based on glucose oxidase method (Trinder, 1969).

Estimation of serum insulin levels

The level of insulin was measured in serum samples of rats by ELISA (Mercodia, Sweden).

Detection of urinary glucose and protein

Both glucose and protein were detected in urine with the help of commercially available strips (TECO Diagnostics, USA) and their levels were graded 0, trace, 1+, 2+, 3+ and 4+ as per the details provided by the manufacturer.

Estimation of GSH and TBARS in renal tissue

The levels of GSH and TBARS were measured in kidney tissue by the methods of Ellman (1959) and Ohkawa *et al.* (1979) respectively.

Histological analysis of kidney

The sections of formalin fixed kidney specimens were stained with periodic acid-Schiff stain and observed under a light microscope.

Statistical analysis

The values are expressed as mean \pm SEM of six observations. Statistical analysis was carried out by SPSS program, version 10 (SPSS Inc, USA). ANOVA followed by post hoc test (LSD) was used to compare the groups and values of $p < 0.05$ were considered as statistically significant.

Results

Effect of the aqueous suspension of dried latex on blood glucose levels in diabetic rats

Diabetes was induced in rats by a single injection of alloxan where a significant increase in the level of blood glucose was obtained on day 3. The level of blood glucose in the diabetic control group was 242.44 ± 13.70 mg/dl against 83.51 ± 2.45 mg/dl in the normal control group ($p < 0.001$). Treatment of diabetic rats with the aqueous suspension produced a dose-dependent decrease in the levels of blood glucose. The anti-hyperglycemic effect of the aqueous suspension was discernible as early as third day of treatment and was observed throughout the study period of 90 days. Blood glucose levels in rats treated with the higher dose of the aqueous suspension (400 mg/kg) and glibenclamide treated rats on day 90 were comparable to the level in the normal rats (Fig. 1).

Effect of the aqueous suspension of dried latex on serum insulin levels

Induction of diabetes with alloxan produced a significant decrease in the serum levels of insulin in rats from 87.00 ± 20.09 pmol/l to 14.79 ± 0.50 pmol/l over a period of 90 days. Treatment of diabetic rats with the aqueous suspension produced a marginal increase in the levels of insulin while glibenclamide produced a significant increase ($p < 0.001$) in the insulin level to 162.98 ± 2.09 pmol/l (Fig. 2).

Effect of the aqueous suspension of dried latex on urinary glucose and protein level

The study was carried out for a period of 90 days till a marked increase in the urinary level of glucose and protein was observed in the alloxan treated rats as compared to that of normal rats. The level of urinary glucose was 4+ in alloxan treated rats against 0 in the

normal control group and that of protein was 3+ against trace in the normal control group over a period of 90 days. The urine samples of rats treated with both doses of the aqueous suspension, as well as those treated with glibenclamide were negative for both glucose and protein.

Effect of the aqueous suspension of dried latex on renal tissue GSH and TBARS levels

In view of the key role of lipid peroxidation and oxidative stress in the pathogenesis of diabetes, the levels of GSH and TBARS were estimated in the rat renal

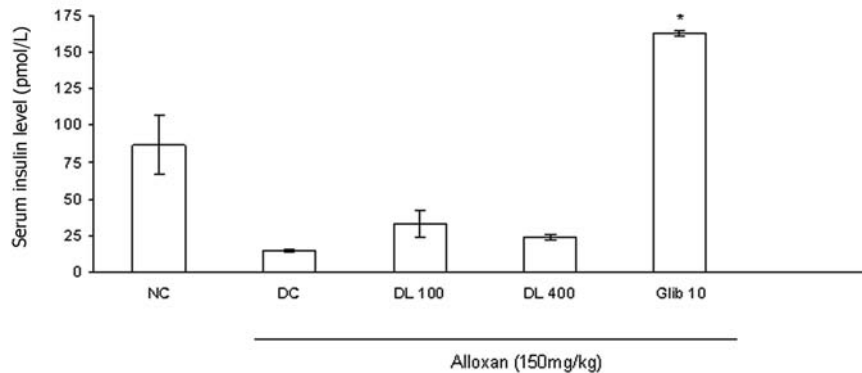


FIGURE 2. Effect of an aqueous suspension of dried latex of *C. procera* on the serum level of insulin in diabetic rats. Diabetes was induced in rats by a single intraperitoneal injection of alloxan. Both the aqueous suspension and glibenclamide were administered orally and daily at the indicated doses. Serum insulin levels were measured on day 90. Each bar represents the values as mean \pm SEM (n=6). *Abbreviations:* NC, normal control; DC, diabetic control; DL 100, latex suspension, 100 mg/kg; DL 400, latex suspension, 400 mg/kg; Glib 10, glibenclamide, 10 mg/kg. Stars indicate statistically significant differences from the diabetic control group.

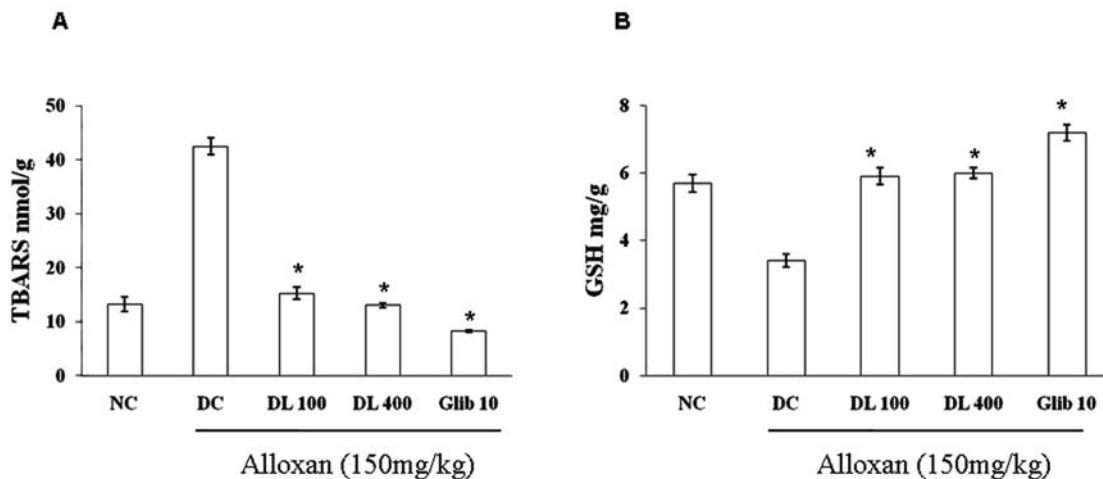


FIGURE 3. Effect of an aqueous suspension of dried latex of *C. procera* on the levels of TBARS and GSH in renal tissue of diabetic rats. Diabetes was induced in rats by a single intraperitoneal injection of alloxan. Both the aqueous suspension and glibenclamide were administered orally and daily at the indicated doses. TBARS and GSH levels were measured in the kidney tissue on day 90. Each bar represents the values as mean \pm SEM (n=6). *Abbreviations:* NC, normal control; DC, diabetic control; DL 100, latex suspension, 100 mg/kg; DL 400, latex suspension, 400 mg/kg; Glib 10, glibenclamide, 10 mg/kg. Stars indicate statistically significant differences from the diabetic control group.

tissue. Alloxan diabetes was found to be associated with a significant increase in the levels of TBARS and a decrease in the levels of GSH. Treatment of diabetic rats with the aqueous suspension normalized the renal tissue levels of TBARS and GSH and the effect was dose-dependent and similar to that of glibenclamide (Fig. 3).

Effect of the aqueous suspension of dried latex on renal histology

Induction of diabetes with alloxan was associated with marked histological changes in the kidney over a period of 90 days as revealed by tubular epithelial hypertrophy, glomerulosclerosis and glycogen accumulation. Treatment of diabetic rats with the aqueous suspension afforded significant protection from renal

damage whereas tubular damage was more pronounced in rats treated with glibenclamide (Fig. 4).

Discussion

A number of experimental and clinical studies have shown the efficacy of various herbs in lowering blood glucose in diabetes. These herbal preparations exhibit their beneficial effects by different mechanisms which may or may not affect insulin release (Hui *et al.*, 2009). In view of the traditional use of *Calotropis* species in treating diabetes and lowering blood glucose levels in experimental conditions, the present study was carried out to evaluate the efficacy of aqueous suspension of dried latex of *C. procera* in affording protection against

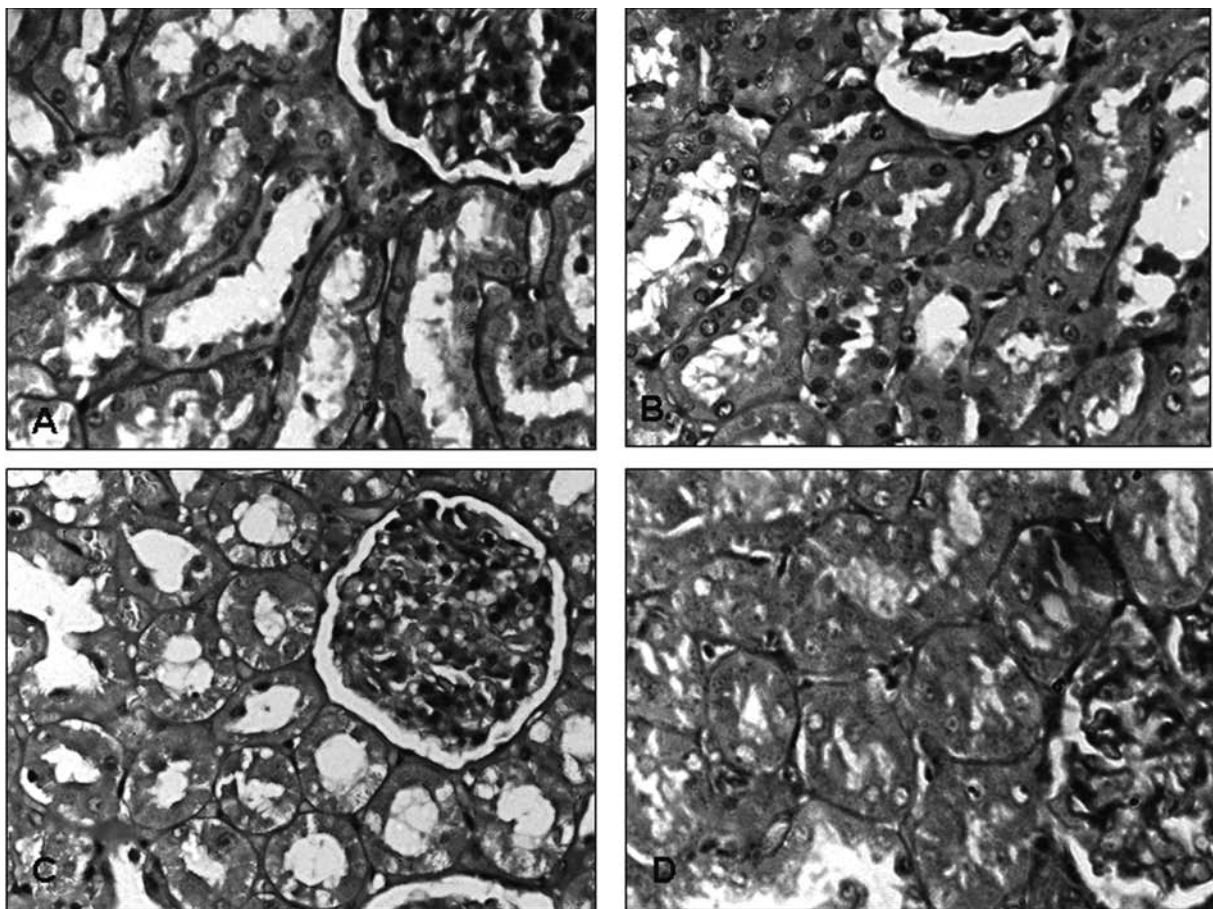


FIGURE 4. Composite pictomicrograph showing the effect of an aqueous suspension of dried latex of *C. procera* on kidney tissue histology in diabetic rats. Diabetes was induced in rats by a single intraperitoneal injection of alloxan. Both the aqueous suspension and glibenclamide were administered orally daily at the doses mentioned. The histological analysis of kidney tissue was carried out on day 90. (A) normal control; (B) diabetic control (C) treated with 400 mg/kg of the aqueous suspension (D) treated with 10 mg/kg of glibenclamide.

renal damage in diabetic rats and to elucidate its mechanism of action. Diabetes was induced in rats by single intraperitoneal injection of alloxan, an agent that brings about reactive oxygen species mediated β -cell toxicity and produces hyperglycemia (Lenzen, 2008; Srinivasan and Ramarao, 2007). In present study alloxan produced hyperglycemia in three days and its effect was maintained throughout the study period of 90 days when a marked increase in the level of urinary glucose and protein was observed. This was accompanied by a decline in serum insulin level. Treatment of diabetic rats with the aqueous suspension of dried latex produced a dose-dependent anti-hyperglycemic effect as reported earlier in a short term study of one month (Roy *et al.*, 2005). It is interesting to note that the aqueous suspension of dried latex produced only a marginal change in serum insulin levels in diabetic rats while glibenclamide treatment produced a marked increase in insulin level that was higher than that in the normal rats. Our findings suggest that the anti-hyperglycemic effect of the aqueous suspension of dried latex could be mediated through its extra-pancreatic effects while glibenclamide is well known to stimulate insulin release from the surviving β -cells in addition to its extra-pancreatic actions (Tasaka, 1999). However, in an earlier study, both the aqueous suspension of dried latex and glibenclamide have been shown to restore the depressed hepatic glycogen levels in diabetic rats possibly due to their antioxidant properties (Roy *et al.*, 2005; Mueen Ahmed *et al.*, 2004; Chugh *et al.*, 2001).

Nephropathy is one of the serious complications of diabetes that is associated with the excretion of albumin in urine and is the leading cause of end-stage renal failure (Remuzzi *et al.*, 2006; de Zeeuw, 2007). The development and progression of renal disease is indicated by the appearance of proteinuria (Zipp and Schelling, 2003). In the current study, a single alloxan injection affected kidney functions and produced a marked increase in urinary protein and glucose levels over a period of 90 days. Treatment with both the aqueous suspension of dried latex and glibenclamide prevented urinary excretion of glucose and proteins. Importantly, marked reduction in proteinuria brought about by the aqueous suspension of dried latex indicates its protective effect on the renal functions (de Zeeuw, 2007; Lee, 2005). In our study the protective effect of the aqueous suspension of dried latex on renal functions was further substantiated by histological findings on the kidney tissue while the tubular architecture of glibenclamide treated rats revealed some tubular damage. Despite anti-oxidant properties, the use of

glibenclamide has been contraindicated in renal disease (Hartmann *et al.*, 2010). It is also important to note that the aqueous suspension of dried latex as used in present study does not affect the kidney and liver functions of normal rats and is free from rubber like poly-isoprene known to produce toxic effects (Alencar *et al.*, 2006; Singhal and Kumar, 2009).

Persistent hyperglycemia associated with diabetes has been shown to increase the production of free radicals through glucose auto-oxidation and protein glycation (Zhang and Tan, 2000). High level of glucose is known to induce reactive oxygen species and upregulate TGF- β 1 and extracellular matrix expression in glomerular mesangial cells. Inhibition of these changes by anti-oxidants strengthens the role played by reactive oxygen species in mediating glucose-induced renal injury (Lee *et al.*, 2003; Ha *et al.*, 1997). In view of the established role of oxidative stress and altered antioxidant levels in the pathogenesis of diabetic complications, we have evaluated the effect of the aqueous suspension of dried latex on the levels of TBARS and GSH in the kidney tissue of the rats. Like glibenclamide, the aqueous suspension of dried latex also afforded antioxidant protection to the kidney. The antioxidant and free radical scavenging properties of the latex have earlier been demonstrated and attributed to its constituents like cardinolides, lignans and flavonol glycosides (Roy *et al.*, 2005; Mueen Ahmed, 2003, 2004).

Thus, our study indicates that by exhibiting anti-hyperglycemic and antioxidant effect, the aqueous suspension of dried latex of *C. procera* affords protection against renal complications associated with diabetes.

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