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Short communication

Antioxidant and protective effect of latex of *Calotropis procera* against alloxan-induced diabetes in rats

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Abstract

In the present study, dry latex (DL) of *Calotropis procera* possessing potent anti-inflammatory activity was evaluated for its antioxidant and anti-hyperglycemic effects against alloxan-induced diabetes in rats. Daily oral administration of DL at 100 and 400 mg/kg doses produced a dose-dependent decrease in the blood glucose and increase in the hepatic glycogen content. DL also prevented the loss of body weight in diabetic rats and brought down the daily water consumption to values comparable to normal rats. DL also produced an increase in the hepatic levels of the endogenous antioxidants, namely superoxide dismutase (SOD), catalase and glutathione, while it brought down the levels of thiobarbituric acid-reactive substances (TBARS) in alloxan-induced diabetic rats. The efficacy of DL as an antioxidant and as an anti-diabetic agent was comparable to the standard anti-diabetic drug, glibenclamide.

Keywords: *Calotropis procera*; Latex; Antioxidant; Anti-diabetic; Anti-inflammatory

1. Introduction

Calotropis procera (Ait.) R. Br. (Asclepiadaceae), a wild growing tropical plant possesses various medicinal properties. In Indian traditional medicine, different parts of the plant have been used as purgative, antihelminthic and also in the treatment of diseases, such as leprosy, ulcers, tumors and piles (Kirtikar and Basu, 1935). Experimentally, the latex of this plant has been shown to possess potent anti-inflammatory, analgesic and weak antipyretic properties (Kumar and Basu, 1994; Dewan et al., 2000a,b). The latex exhibits anti-diarrhoeal properties possibly due to its desensitizing effect on the smooth muscles of the gastrointestinal tract (Kumar et al., 2001; Kumar and Shivkar, 2004). It is also known for its free radical scavenging and antioxidant property that is comparable to standard antioxidant, Vitamin C (Mueen Ahmed et al., 2003, 2004).

Diabetes mellitus (DM) is the most common endocrine disorder with over 150 million people suffering from it worldwide (King et al., 1998). It is associated with hyperglycemia, dyslipidemia and abnormal levels of amino acids due to defective carbohydrate, lipid and protein metabolism (Fajans, 1996). Besides endocrine factors, a number of other factors are also associated with the pathogenesis of DM and its complications that include oxidative stress, altered levels of antioxidants and release of inflammatory cytokines (Rabinovitch and Suarez Pinzou, 1998; Zhang and Tan, 2000). Inflammatory cellular infiltration occurs in Type-I DM, while raised levels of C-reactive protein and other acute phase reactants has been reported in Type-II DM. (Pickup and Crook, 1998; Rabinovitch and Suarez Pinzou, 1998). In view of the above pathogenic mechanisms involved in the progression of DM and pleotropic effects of the latex of *Calotropis procera*, the present study was carried out to evaluate the anti-diabetic and antioxidant potential of the latex against alloxan-induced diabetes in rats.

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2. Materials and methods

2.1. Experimental animals

Animals used for the study were obtained from the experimental animal facility of the All India Institute of Medical Sciences after getting approval from Institute Animal Ethics Committee. Wistar rats of either sex ranging from 150 to 200 g were used for the study after acclimatization for a period of 2–3 days in a new environment.

2.2. Plant material

Latex was collected from the aerial parts of plant *Calotropis procera* and dried under shade (DL). The plant 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). The dry latex (DL) was triturated in normal saline (NS) and administered orally.

2.3. Drugs and chemicals

Alloxan (Allx) was purchased from Calbiochem, Glibenclamide (Glb) was obtained from Arbro Pharmaceuticals, India. All the other chemicals used for this study were of analytical grade.

2.4. Experimental design

Rats of either sex were made diabetic with an intraperitoneal (i.p.) injection of alloxan (150 mg/kg body weight) dissolved in NS. Fasting blood glucose levels were measured on day 3 and rats with blood glucose level greater than 9.7 mmol/l (175 mg/dl) were included in the study (Yadav et al., 2002). The standard anti-diabetic drug and test drugs were administered at 10 a.m. to the diabetic rats once daily at doses that were considered safe based on previous work (Sangraula et al., 2002; Mahdi et al., 2003).

Rats were divided into five groups ($n = 6$ per group)—Group I: normal control (NS, 2 ml); Group II: diabetic control (NS, 2 ml); Group III: diabetic rats given glibenclamide (Glb 10 mg/kg); Group IV: diabetic rats given DL (100 mg/kg; DL 100); Group V: diabetic rats given DL (400 mg/kg; DL 400). Fasting blood glucose, body weight and daily water consumption were recorded for day 0, 3, 7 and 31. Rats were sacrificed after 31 days and the liver was dissected out for the measurement of lipid peroxidation and antioxidant levels.

2.5. Blood glucose estimation

Blood glucose was estimated by commercially available glucose kit based on glucose oxidase method (Trinder, 1969)—Glucocare, India.

2.6. Assay of hepatic glycogen, antioxidant levels and protein

Glycogen was estimated by the method of Kahan (1953). The activity of superoxide dismutase (SOD) was determined by the method of Kakkar et al. (1984). Catalase was assayed by the method of Aebi (1974). The levels of glutathione (GSH) were determined by the method of Ellman (1959). Protein content in the sample was determined by the method of Bradford (1976).

2.7. Estimation of thiobarbituric acid-reactive substances (TBARS)

The TBARS levels measured as an index of malonyldialdehyde (MDA) production were determined by the method of Okhawa et al. (1979).

2.8. Statistical analysis

The values are given as mean ± S.E.M. ($n = 6$). The data was analyzed by ANOVA followed by post hoc test using computerized software SPSS (Version 10).

3. Results

3.1. Anti-hyperglycemic effect of DL in diabetic rats

Treatment of rats with alloxan produced a significant increase in blood glucose within 3 days. The levels of blood glucose in diabetic control rats were 12.9 ± 1.3 mmol/l as compared to 5.4 ± 0.3 mmol/l in normal rats ($p < 0.01$). Treatment of diabetic rats with DL produced a dose-dependent decrease in the level of blood glucose. The anti-hyperglycemic effect of DL was discernible within 3 days of treatment, comparable to standard anti-diabetic drug glibenclamide and was maintained throughout the study period of 31 days (Fig. 1).

3.2. Effect of DL on body weight and water consumption in diabetic rats

Alloxan treatment produced a significant decrease in the body weight of the rats from 174.4 ± 7.8 to 138.3 ± 9.2 g

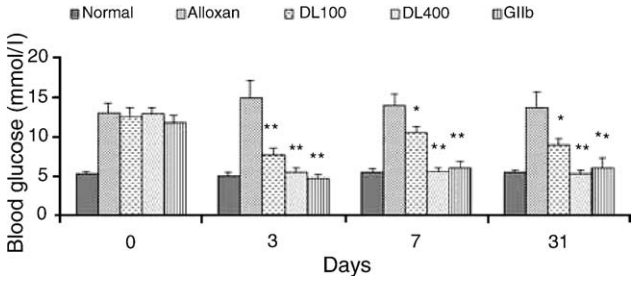

Fig. 1. Effect of DL on blood glucose in diabetic rats. $^{*}p < 0.05$; $^{**}p < 0.01$ vs. Alloxan control