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Leucas cephalotes regulates carbohydrate and lipid metabolism and improves antioxidant status in IDDM and NIDDM rats

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ABSTRACT

Ethnopharmacological relevance: Leucas cephalotes (Roth.) Spreng. (Laminaceae) is an ayurvedic traditional medicinal plant used in India, Nepal and Pakistan to treat several ailments including diabetes.
Aim of the study: The aim of the present study is to investigate the antidiabetic, antihyperlipaemic and antioxidant activities of *Leucas cephalotes* for its purported use in diabetes.
Materials and methods: The ethanol extract of leaves of *Leucas cephalotes* was administered (150, 300 and 450 mg kg^{-1} bw) to diabetes induced (IDDM and NIDDM) rats and carbohydrate, lipid, antioxidant, urea and creatinine profiles were assessed.
Results: All the three doses of extract decreased plasma glucose and lipid profiles and, improved the antioxidant status of both types of diabetic rats. The extract administration improved hepatic glycogen content and hexokinase activity, decreased glucose-6-phosphatase activity, blood urea, creatinine contents and decreased lipid peroxidation in diabetic rats. Of the three doses used, 450 mg kg^{-1} bw dose was found to be more potent in its effects comparable to those of glibenclamide and metformin.
Conclusion: Leucas cephalotes regulates both carbohydrate and lipid metabolism and, improves body antioxidant defense systems in both types of diabetes.

1. Introduction

Although, non-insulin dependent diabetes mellitus (NIDDM) is more prevalent than insulin dependent diabetes mellitus (IDDM), both are chiefly characterized by chronic hyperglycemia with disturbances of carbohydrate, lipid and protein metabolism resulting from defects in insulin secretion, insulin action or both (Brownlee, 2001; Clark and Pierce, 2000). Hyperglycemia due to insulin deficiency and/insulin resistance has been shown to be associated with the pathogenesis of diabetic dyslipidaemia, micro-and macrovascular complications (Brownlee, 2001). Hyperglycemia is also known to bring about an increased production of superoxides and lowers the antioxidant enzyme activities compromising with body antioxidant defense systems (Brownlee, 2001; Kamalakkannan and Prince, 2006). Due to several limitations of currently available drugs including side effects and failure of response after prolonged use, plant based medicines are gaining prominence in treatment of metabolic diseases like diabetes (Bailey and Day, 1989; Grover et al., 2002; Mukherjee et al., 2006). *Leucas cephalotes* (Roth.) Spreng. (Family: Lamiaceae) is a common weed plant associated with agricultural fields in India. According to Ayurveda, the Indian medicinal system, this plant is a mild stimulant, diaphoretic, anti-inflammatory and has been used in treatment of jaundice, asthma, paralysis, scabies, fever, coughs and colds, edema, urinary complications and diabetes (Chandel et al., 1996; Miyaichi et al., 2006; Dash, 1987). A recent report on *Leucas cephalotes* indicated that it contains terpenes (three types of diterpenes and two types of triterpenes), sterols (five types) and flavones (eight types) (Miyaichi et al., 2006) besides glycosides and alkaloids (Chandel et al., 1996). As no detailed reports are available on the effect/s of *Leucas cephalotes* on diabetes or its associated metabolic disorders, we have examined the effect/s of *Leucas cephalotes* on carbohydrate and lipid metabolism and body antioxidant defense system in both types of diabetic (IDDM and NIDDM) animal models in the present study.

2. Materials and methods

2.1. Animals

Three-month-old male Charles Foster rats (weighing 200–250 g) were used for the present investigation. The animals were provided standard diet (Pranav Agro Industries, Vadodara, India) and water *ad libitum*, and were housed individually in well-ventilated animal unit (26 ± 2 °C, humidity 62%, and normal light/dark cycles).

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The Institutional Animal Ethics Committee approved the present investigation.

2.2. Leaf extract preparation

Leucas cephalotes leaves were collected from Saurashtra region of Gujarat. The material was authenticated by our faculty taxonomist Dr. A.S. Reddy (Voucher specimen No. JHB-03, S.P. University). Leaves were air-dried, ground to powder and stored in an airtight container. The leaf powder was soxhlet-extracted with 95% ethanol. The alcoholic leaf extract (LCLEt) was filtered and dried at room temperature (yield 20.49%, w/w) and the residue was stored at 4 °C until used for experiments.

2.3. Induction of insulin dependent diabetes mellitus (IDDM)

Overnight fasted rats were given a single intraperitoneal injection of alloxan monohydrate (120 mg kg^{-1} bw; Loba Chemie, Mumbai, India) dissolved in normal saline. Rats exhibiting hyperglycemia (i.e. fasting blood glucose levels >140 mg dl^{-1}) after 2 weeks were considered IDDM diabetic animals.

2.4. Induction of non-insulin dependent diabetes mellitus (NIDDM)

The model was developed according to the description of Bonner-Weir et al. (1981). Newly born males aged 48 ± 2 h were injected intraperitonealy with streptozotocin (Sisco Research Laboratories, Mumbai, India) in citrate buffer (pH 4.5) at a dose of 100 mg kg^{-1} bw. The animals showing fasting blood glucose levels >140 mg dl^{-1} after 12 weeks, were considered NIDDM diabetic animals.

2.5. Experimental design

The animals were randomly divided into 11 groups of six rats each: normal control (rats without treatment, NC), IDDM control (IDDM diabetic rats given only vehicle treatment), IDDM-LC I (IDDM diabetic rats given LCLEt 150 mg kg^{-1} bw), IDDM-LC II (IDDM diabetic rats given LCLEt 300 mg kg^{-1} bw), IDDM-LC III (IDDM diabetic rats given LCLEt 450 mg kg^{-1} bw), IDDM-GB (IDDM diabetic rats given glibenclamide 600 μg kg^{-1} bw) (Aristo Pharmaceuticals, Mumbai, India; Ananthan et al., 2003), NIDDM control (NIDDM diabetic control rats given only vehicle treatment), NIDDM-LC I (NIDDM diabetic rats given LCLEt 150 mg kg^{-1} bw), NIDDM-LC II (NIDDM diabetic rats given LCLEt 300 mg kg^{-1} bw), NIDDM-LC III (NIDDM diabetic rats given LCLEt 450 mg kg^{-1} bw), NIDDM-ME (NIDDM diabetic rats given metformin 500 mg kg^{-1} bw, USV Ltd., Daman, India; Pari and Ashokkumar, 2005).

The plant extract, glibenclamide and metformin were given in 1 ml of 2% (v/v) Tween 80 solution daily using an intragastric tube for 4 weeks. At the end of experimental period, the animals were deprived of food overnight and sacrificed under mild anesthesia. Blood and liver tissues were collected immediately for biochemical analyses.

2.6. Plasma glucose, hepatic glycogen, hexokinase and glucose-6-phosphatase

Plasma glucose levels were measured by o-toluidine method (Webster et al., 1971). Hepatic glycogen was extracted with 30% KOH and the yield was determined by anthrone–sulphuric acid method (Seifter et al., 1950). Hepatic hexokinase (EC 2.7.1.1) and glucose-6-phosphatase (EC 3.1.3.9) activities were determined following the methods of Brandstrup et al. (1957) and Baginsky et al. (1974), respectively.

2.7. Plasma and hepatic lipid profiles

Plasma and hepatic cholesterol (TC) and high-density lipoprotein cholesterol (HDL-C) were estimated by Wybenga and Pileggi's method with a cholesterol kit (Eve's Inn Diagnostics, Baroda, India). Plasma and hepatic triglycerides (TG) were estimated by GPO method with a Triglyceride kit (Eve's Inn Diagnostics, Baroda, India). Plasma low-density lipoprotein cholesterol (LDL-C), very low-density lipoprotein cholesterol (VLDL-C) were calculated by Friedewald formula (Friedewald et al., 1972). Plasma and hepatic total lipids (TL) were extracted by the method of (Folch et al., 1957) and were estimated by gravimetric method. The same extract was also used for estimating total cholesterol and triglyceride content using appropriate kits (Eve's Inn Diagnostics, Baroda, India).

2.8. Blood urea and creatinine

Blood urea and creatinine levels were determined (using diacetyl monoxime and alkaline picrate reagents) with urea and creatinine kits (Eve's Inn Diagnostics, Baroda, India).

2.9. Lipid peroxidation and enzymatic and non-enzymatic antioxidants

The hepatic lipid peroxidation (LPO) was determined by TBARS assay (Ohkawa et al., 1979). Total ascorbic acid was assayed by the method described by Schaffert and Kingsley (1955). Antioxidant enzymes-Superoxide dismutase (SOD) (EC 1.15.1.1) and Catalase (EC 1.11.1.6) were estimated according to the methods described by Kakkar et al. (1984) and Aebi (1974), respectively. Reduced Glutathione (GSH) was measured by the method of Jollow et al. (1974). The glutathione peroxidase (GPx) (EC 1.11.1.9) activity was determined by the method of Flohe and Gunzler (1984).

2.10. Statistical analysis

Results are expressed as means ± SEM. Significant differences among the groups were determined by one-way ANOVA using 12th version of SPSS with Tukey's test as post hoc analysis. Differences were considered significant if $P < 0.05$.

3. Results

3.1. Effect of LCLEt on plasma glucose, hepatic glycogen, hepatic hexokinase and glucose-6-phosphatase

The plasma glucose concentration was significantly higher in IDDM and NIDDM rats compared to the normal controls. LCLEt administration to both groups of rats produced a significant drop in plasma glucose level even at low dose ($P < 0.05$). The effect of maximum dose was comparable to that of glibenclamide and metformin administered groups. The IDDM and NIDDM diabetic rats showed significant decline in hepatic glycogen content ($P < 0.05$). LCLEt administration with all doses increased glycogen content in a dose dependent manner in both the diabetic models. However, the IDDM rats responded to LCLEt administration more vigorously than NIDDM animals. A significant decrease in hepatic hexokinase level was found in both IDDM and NIDDM rats ($P < 0.05$). The enzyme activity increased significantly upon LCLEt administration in a dose dependent manner and, with maximum dose the increase in the enzyme level was comparable to normal rats. A significant increase in glucose-6-phosphatase level was found in both types of