Antidiabetic, Antioxidant and Antihyperlipidemic Status of Heliotropium zeylanicum Extract on Streptozotocin-Induced Diabetes in Rats

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The potential role of the methanolic extract of Heliotropium zeylanicum (Burme.) Lamk (MEHZ) in the treatment of diabetes along with its antidiabetic and antihyperlipidemic effects was studied in streptozotocin-induced diabetic rats. Oral administration of (MEHZ) 150 and 300 mg/kg/d for 14 d significantly decreased the blood glucose level and considerably increased the body weight, food intake, and liquid intake of diabetic-induced rats. MEHZ significantly decreased thiobarbituric acid reactive substances and significantly increased reduced glutathione, superoxide dismutase and catalase in streptozotocin-induced diabetic rats at the end of 14 d of treatment. The study also investigated the antihyperlipidemic potential of MEHZ. The results show that the active fraction of MEHZ is promising for development of a standardized phytomedicine for the treatment of diabetes mellitus.

Key words Heliotropium zeylanicum; streptozotocin-induced diabetic rat; antihyperglycemic activity; antioxidant potential; antihyperlipidemic effect

Over the last three to four decades, the use of alternative systems of medicines based on Ayurveda, Siddha, Unani, in India and similar traditional systems in other countries has been increasing at a rapid pace. Based on ancient scriptures, these medicines are derived from natural sources, such as plants, herbs, and minerals. In comparison with modern allopathic medicines, these traditional medicines have no appreciable toxic side effects and this unique characteristic has contributed to the worldwide interest in herbal medicines. Due to the expanded use of herbal drugs, intensive R & D is now being carried out to evolve suitable process equipment or technology.1)

Diabetes mellitus is a noncommunicable disease considered to be one of the five leading causes of death world wide. About 100 million people around the world have been diagnosed with diabetes and by the year 2010, it is projected that 215 million people will have the disease.2) Recently, the search for appropriate hypoglycemic agents has been focused on plants used in traditional medicine partly because of leads provided by traditional medicine, to natural products that may be better treatments than currently used drugs.3)

Heliotropium species (Boraginaceae) constitute a rich source of pyrrolizidine alkaloids, some of which have antitumour and hepatotoxic activities.4,5) The wide range of biological activities exhibited by these alkaloids prompted us to carry out systematic chemical studies on various local plants of the genus Heliotropium. Heliotropium zeylanicum (Burme.) Lamk is an erect herb with a stout rootstock and long twiggy branches bearing conspicuously long slender racemes of distant flowers, especially in fruit. The flowers are rather large, the limb white, the tube yellow, and the leaves very narrow.6,7)

MATERIALS AND METHODS

Chemicals Tolbutamide and streptozotocin were obtained from Hoechst Pharmaceuticals, Mumbai, India and Sigma Chemical Company, St. Louis, MO, U.S.A. respectively. All other chemicals and reagents used were of analytical grade and were obtained from commercial sources: thiobarbituric acid, nitro blue tetrazolium (NBT), nicotinamide adenine dinucleotide (NADH) (Loba Chemie, Mumbai, India); 5,5’dithio bis-2-nitro benzoic acid (DTNB), and reduced glutathione (GSH) (SISCO Research Lab, Bombay, India).

Plant Material H. zeylanicum (Burme.) Lamk Boraginaceae was collected from Trichy district, Tamil Nadu, India, of June 2004. It was identified by the Botanical Survey of India, Coimbatore. The voucher specimen number is BSI/SC/5/21/04-05/Tech.1403. The whole plant was then dried under air, shade-dried and mechanically powdered separately to obtain a coarse powder, which was then subjected to extraction.

Preparation of Extract H. zeylanicum was initially defatted with petroleum ether (60 to 80 °C) followed by chloroform and methanol, using the method of continuous hot extraction with a Soxhlet apparatus. The chloroform and methanol extract (MEHZ) was distilled and dried a in vacuum, with a yield of 3.5% w/w and 16% w/w, respectively.

Animals Male Wistar albino rats (150—180 g) were obtained from the Indian Institute of Chemical Biology (IICB), Kolkata, India. The animals were grouped and housed in polycrystalline cages and maintained under standard laboratory conditions (temperature 25±2 °C) with a 12-h/12-h dark and light cycle. They were allowed free access to a standard dry pellet diet (Hindustan Lever, Kolkata, India) and water ad libitum. All procedures described were reviewed and approved by the University Animals Ethical Committee.

Induction of Experimental Diabetes After 1 week of acclimatization, the rats were subjected to a 16-h fast. Diabetes was induced with a single injection of streptozotocin (STZ) 65 mg/kg body weight i.p. The STZ was freshly dissolved in citrate buffer (0.01 M, pH 4.5).8) The injection volume was prepared to contain 1.0 ml/kg.8) After 5 d, blood glucose levels were measured and animals with a concentration of greater than 225 mg/dl were used in the investiga-
**Experimental Design**  In the experiment, a total of 30 rats (6 normal; 24 STZ diabetic) were used. The rats were divided into five groups of 6 animals each: group I, normal, received normal saline solution (0.9% NaCl w/v, 5 ml/kg); group II, diabetic, received STZ 65 mg/kg once before treatment; group III, received MEHZ 150 mg/kg; group IV, received MEHZ 300 mg/kg; and group V, received tolbutamide 10 mg/kg as reference standard for 14 d.

The effects of MEHZ on STZ-induced diabetic rats were determined by measuring blood glucose levels, food and liquid intake, and changes in body weight. After 14 d of treatment, all the rats were decapitated after fasting for 16 h. The animals were dissected and a drop of blood from the heart was used for the estimation of blood glucose. Tissues (liver) were removed and cleared of blood. They were immediately transferred to ice-cold containers containing 0.9% w/v NaCl, homogenized in 0.1 N Tris–HCl buffer (pH 7.4), and used for the estimation of thiobarbituric acid-reactive substances (TBARS), reduced glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT) activity.

**Measurement of Blood Glucose Levels** At the beginning of the experiment and at 5-d intervals, body weight and blood glucose levels were measured. Blood samples were obtained by tail vein puncture of the normal and STZ-induced diabetic rats on day zero (0), day 5, day 10, and day 15. Blood glucose levels were determined using a glucometer (One Touch Ultra blood glucose monitoring system, LifeScan, Johnson and Johnson Company, Milpitas, CA, U.S.A.).

**Measurement of Total Cholesterol and Triglyceride** On day 15, blood samples (1 ml) were collected by tail vein puncture under mild ether anesthesia in Eppendorf’s tubes containing 50 μl of anticoagulant (10% trisodium citrate solution) from the normal and STZ-induced diabetic rats. Plasma was separated by centrifugation at 5000 rpm for 10 min and analyzed for total cholesterol and triglycerides using kits (Span Diagnostics Ltd., Surat, India).

**Determination of in Vivo Antioxidants**

**Determination of TBARS**  TBARS in tissues are estimated by the method of Fraga et al. To 0.5 ml of tissue homogenate, 0.5 ml of saline and 1.0 ml of 10% TCA were added, mixed well, and centrifuged at 3000 rpm for 20 min. To 1.0 ml of the protein-free supernatant, 0.25 ml of thiobarbituric acid (TBA) reagent was added; the contents were mixed well and boiled for 1 h at 95 °C. The tubes were then cooled to room temperature under running water and absorption measured at 532 nm.

**Determination of Reduced GSH**  GSH was determined using the method of Beutler and Kelly. Tissue homogenate 0.2 ml was mixed with EDTA 1.8 ml solution. Precipitating reagent 3.0 ml (metaphosphoric acid 1.67 g, EDTA disodium salt 0.2 g, sodium chloride 30 g in 1000 ml of distilled water) was added, mixed thoroughly and allowed to stand 5 min before centrifugation. To 2.0 ml of the filtrate, 4.0 ml of disodium hydrogen phosphate solution 0.3 M and 1.0 ml of DTNB reagent were added and absorbance was read at 412 nm.

**Assay of SOD**  The activity of SOD in tissue was assayed using the method of Kakkar et al. The assay mixture contained sodium pyrophosphate buffer 1.2 ml (pH 8.3, 0.025 mol/l), phenazine methosulphate 0.1 ml (186 mmol/l), NBT 0.3 ml (300 mmol/l), NADH 0.2 ml (780 mmol/l) and appropriately diluted enzyme preparation and water in a total volume of 3 ml. After incubation at 30 °C for 90 s, the reaction was terminated by the addition of 1.0 ml of glacial acetic acid. The reaction mixture was stirred vigorously and shaken with 4.0 ml of n-butanol. The color intensity of the chromogen in the butanol layer was measured at 560 nm against n-butanol.

**Assay of CAT**  CAT was assayed according to the method of Maehly and Chance. The estimation was done spectrophotometrically based on the decrease in absorbance at 230 nm. The tissue was homogenized in M/150 phosphate buffer (pH 7.0) at 1—4 °C and centrifuged at 5000 rpm. The reaction mixture contained phosphate buffer 0.01 M (pH 7.0), H₂O₂ 2 mM and the enzyme extract. The specific activity of CAT is expressed in terms of units/milligram protein. A unit is defined as the velocity constant per second.

**Statistical Analysis**  Data a expressed as mean±S.E.M. for 6 rats in each group. The biochemical parameters were analyzed statistically using one-way ANOVA, followed by Dunnett’s multiple-comparison test (DMRT). The minimum level of significance was fixed at p<0.05.

**RESULTS**

Table 1 summarizes the bodyweight, and food and liquid intake in normal and experimental animals. There was a significant decrease (p<0.01) in the body weight of the diabetic controls (group II) compared with the normal controls (group I). Administration of MEHZ to diabetic rats (groups IV, V) after attaining normal glycemic control increased body weight significantly, comparable to the increase in the body weight of normal rats. Diabetic controls (group II) had a high intake of food and liquids, while the food and liquid intake was decreased in the MEHZ-treated groups in comparison to that in the diabetic controls (group II).

Table 2 shows the level of blood glucose in normal and
STZ-induced diabetic rats. There was a significant increase in blood glucose level \((p<0.01)\) in diabetic rats when compared with normal controls. Administration of MEHZ 150 and 300 mg/kg body weight, and tolbutamide 10 mg/kg significantly decreased blood glucose in diabetic rats (groups III—V).

The effects of MEHZ 150 and 300 mg/kg on plasma total cholesterol and triglyceride levels as shown in the Table 2. A significant increase in the cholesterol \((p<0.01)\) and triglyceride \((p<0.01)\) levels was observed in the diabetic group. MEHZ treatment significantly reduced cholesterol and triglyceride levels.

The concentrations of TBARS and GSH in tissues in experimental diabetic rats are shown in Figs. 1 and 2. There was a significant elevation of TBARS in STZ-diabetic controls when compared with normal rats. Administration of MEHZ and tolbutamide significantly decreased the levels of TBARS in the liver when compared with diabetic controls (group II). There was a significant \((p<0.01)\) decrease in the concentration of GSH in the STZ-diabetic control group when compared with the normal controls. Administration of MEHZ 150 and 300 mg/kg body weight reduced the levels of GSH in the liver \((p<0.05)\) during diabetes.

SOD and CAT in experimental animal tissues are summarized in Figs. 3 and 4. There was a significant \((p<0.05)\) reduction in the activity of SOD in the liver during diabetes. Administration of MEHZ 150 and 300 mg/kg body weight and tolbutamide 10 mg/kg body weight increased the activity of SOD in the liver to near normal.

**DISCUSSION**

Recent studies have clearly demonstrated the importance of medicinal plants in the treatment of experimental diabetes, where oxidative stress induced apoptosis or \(\beta\)-cell death.\(^{17,18}\) As an important process involved in many pathologic diseases including diabetes, a number of medicinal plants have been used to control blood glucose and to inhibit or trigger
the fundamental cellular process making oxidative stress and apoptosis amenable to pharmacologic intervention.

Oral administration of MEHZ showed significant hypoglycemic effects against STZ-induced diabetes in rats. The extract significantly lowered the levels of blood glucose, and TBARS and significantly increased the levels of GSH, SOD, and CAT.

To our knowledge, this is the first study in which a follow-up was carried out on the effects of MEHZ on the levels of glucose, lipid peroxides, and nonenzymatic antioxidants in diabetic animals. In our study, we observed elevated levels of blood glucose in STZ-induced diabetic rats. Administration of MEHZ significantly decreases the levels of blood glucose in diabetic rats.

Lipid peroxide-mediated tissue damage has been observed in the development of both type I and II diabetes mellitus. It has been observed that insulin secretion is closely associated with lipoxygenase derived peroxides. Increased lipid peroxidation under diabetic conditions can be due to increased oxidative stress in cells as a result of depletion of antioxidant scavenger systems. Alterations in both nonenzymatic and enzymatic antioxidants we observed in the present study. In MEHZ MEHZ 150 mg/kg body weight and 300 mg/kg body weight and tolbutamide 10 mg/kg body weight treated diabetic rats, the TBARS levels were low which may be due to the free radical-scavenging action of active ingredients in MEHZ.

We observed a decrease in GSH in the liver during diabetes. The decrease in liver GSH levels represents increased utilization due to oxidative stress. A significant decrease in the levels of GSH was observed in MEHZ-treated rats when compared with STZ-induced diabetic controls.

SOD protects tissues against oxygen free radicals by catalyzing the removal of superoxide radical (O$_2^-$), which damages the membrane and biological structures. CAT has been shown to be responsible for the detoxification of significant amounts of H$_2$O$_2$. SOD and CAT are the two major scavenging enzymes that remove the toxic free radicals in vivo. Reduced activities of SOD and CAT in the liver and kidney have been observed during diabetes and this may result in a number of deleterious effects due to the accumulation of superoxide radicals and hydrogen peroxide. MEHZ 150 mg/kg body weight and 300 mg/kg body weight and tolbutamide 10 mg/kg body weight treated rats showed decreased lipid peroxidation that is associated with increased activity of SOD and CAT.

Hypercholesterolemia and hypertriglyceridemia have been reported to occur in streptozotocin diabetic rats. The significant increases observed in our experiment are in accordance with those reports. Under normal circumstances, insulin activates enzyme lipoprotein lipase and hydrolyses triglycerides. However, in insulin-deficient individuals, it fails to activate the enzyme and causes hypertriglyceridemia. MEHZ lowers triglyceride levels by activation of enzyme lipoprotein lipase. In addition, treatment of animals with MEHZ caused a decrease in total cholesterol levels, although this was less marked than the decrease in triglycerides. Repeated administration of MEHZ thus had a beneficial effect on the hyperlipidemia associated with hyperglycemia.

The results of the present investigation of MEHZ showed significant antidiabetic activity. It also corrected altered metabolic functions. However, further comprehensive chemical and pharmacologic investigations need to be carried out to elucidate the exact mechanism of action.

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REFERENCES