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Antidiabetic Activity of Ethanolic Extract of *Zaleya decandra* in Alloxan-Induced Diabetic Rats

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Abstract Diabetes mellitus is a complex disorder that disturbs the metabolism of carbohydrates, fats, and proteins. Medicinal plants play an important role in the management of diabetes mellitus. The present study was aimed to evaluate the antidiabetic potential of *Zaleya decandra* roots on alloxan-induced diabetes in rats. Oral administration of ethanolic extract of the root (200 mg/kg body weight/day) for 15 days restored the levels of glucose, cholesterol, triglycerides, total proteins, urea, creatinine, lipid peroxidation level, and antioxidant enzymes significantly in diabetic rats. Histopathological studies showed significant changes like necrosis and degeneration in the liver and pancreas of alloxan-induced diabetic rats. Also these histopathological abnormalities were found to be normalized after treatment with *Z. decandra* extract. The efficacy of the root extract was found to be equivalent when compared to the standard hypoglycemic drug glibenclamide (1.25 mg/kg body weight/day, orally) in diabetic rats.

Keywords *Zaleya decandra* · Antidiabetic activity · Alloxan-induced diabetes · Ethanolic extract

Introduction

Diabetes mellitus is a major illness of the human race implicated with numerous clinical manifestations. It is a clinical syndrome characterized by hyperglycemia due to absolute or relative deficiency of insulin. According to World Health Organization projections, the diabetes population is likely to increase to 300 million or more by the year 2025 [1, 2]. Currently available therapies for diabetes include insulin and various oral antidiabetic agents such as sulfonylureas, biguanides, α-glucosidase inhibitors, and glinides, which are used as monotherapy or in combination to achieve better glycemic regulation [3]. Many of

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these oral antidiabetic agents have a number of serious adverse effects [4]. Thus, the management of diabetes without any side effects is still a challenge.

Approximately 80% of the world population is almost entirely dependent on traditional medicines [5]. The ethnobotanical details and the antidiabetic potential of several plants are known at present [6], but there is very little information about plants which possess both hypoglycemic and antioxidant properties; such a plant would be very useful as an antidiabetic agent.

Zaleya decandra is a prostrate weed belonging to the family Aizoaceae. It is distributed in the tropical and subtropical regions of the world. It is called Gdabani in Hindi and Vellai shaaranai in Tamil. The root is used for the treatment of hepatitis, asthma, and orchitis and also the decoction of the root's bark is credited with properties of aperients [7]. The juice of the leaves is dropped into the nostrils to relieve partial headache [8]. The aqueous extract of *Trianthema decandra* (=*Z. decandra*) root possesses hepatoprotective activity against carbon-tetrachloride-induced liver damage in rats [9]. The ethanol extract of *T. decandra* exhibited liver protective effect against carbon-tetrachloride-induced hepatotoxicity and also possessed antioxidant activities [10]. The present study has been undertaken to identify the antidiabetic effect of *Z. decandra* in diabetic rats.

Materials and Methods

Plant Material

The roots of *Z. decandra* were collected from Pollachi, TamilNadu, India. The plant was identified and authenticated by Dr. G.V.S. Murthy from The Botanical Survey of India, Coimbatore, Tamil Nadu, India. A voucher specimen was deposited to the Botanical Survey of India (No.BSI/SC/5/23/08-09/Tech.1231). The roots of *Z. decandra* were washed with distilled water, shade dried, powdered, and stored in an air-tight container for further use.

Preparation of Root Extract

The powdered roots of *Z. decandra* (100 g) was extracted with 500 ml of 95% ethanol. The extract was concentrated under vaccum and the yield of the extract was 1.64%.

Preliminary Phytochemical Screening

The phytochemical screening of *Z. decandra* was performed as per procedure [11, 12].

Animals

Wistar albino rats weighing about 150–180 g were procured from Karpagam University Animal House, Coimbatore, India. The animals were under standard conditions and fed with rodent diet and water ad libitum. The study was approved by Institutional Animal Ethical Committee constituted for the purpose of CPCSEA.

Experimental Induction of Diabetes

Diabetes mellitus (NIDDM) was induced in adult Wistar albino rats by a single intraperitoneal injection of alloxan monohydrate at the dose of 120 mg/kg body weight.

The severity of diabetes was checked in the alloxan-treated rats by Benedict's qualitative test in the urine sample. Alloxanic rats were used when the elevated glucose level in plasma was effective and permanent.

Experimental Protocol

The animals were divided into five groups of six animals each. Group I served as a control; group II consisted of alloxan-induced diabetic rats; group III consisted of alloxan-induced diabetic rats treated with *Z. decandra* root extract (200 mg/kg body weight/day by oral administration for 15 days); group IV were normal rats treated with *Z. decandra* root extract (200 mg/kg body weight/day by oral administration for 15 days); and group V served as positive control treated with glibenclamide (1.25 mg/kg body weight/day by oral administration for 15 days).

Biochemical Studies

After 15 days of treatment the animals were sacrificed under chloroform anesthesia. The blood was collected and serum was separated and used for biochemical estimations. Liver was quickly excised off, a portion of liver washed with saline and liver homogenate was prepared using 0.1 M phosphate buffer, pH 7.4. The liver homogenate was centrifuged and the supernatant was used for the determination of lipid peroxidation and for antioxidant study. The levels of glucose, cholesterol, triglycerides, total proteins, urea, and creatinine in serum were estimated using standard kits of Ranbaxy Laboratories, New Delhi. In the hepatic tissue samples lipid peroxidation [13] was determined. Malondialdehyde, formed as an end product of the peroxidation of lipids served as an index of oxidative stress. The liver homogenate was also used for assay of the antioxidant enzymes such as superoxide dismutase [14], catalase [15], and glutathione peroxidase [16].

Histological Observation

One portion of liver and pancreas of all the experimental groups were fixed in 10% formalin for histological observation.

Statistical Analysis

The values were expressed as mean±SD. The statistical analysis was carried out by one-way analysis of variance using SPSS (version 10) statistical analysis program. Statistical significance was considered at $p<0.05$.

Results

The ethanolic root extract of *Z. decandra* contained carbohydrates, flavonoids, alkaloids, steroids, cardioglycosides, terpenoids, tannins, and phenolic groups. Table 1 show the levels of glucose, cholesterol, triglycerides, total protein, urea, and creatinine in serum of normal, diabetic, diabetic+*Z. decandra*, *Z. decandra* and positive control groups. The levels of glucose, cholesterol, triglycerides, urea, and creatinine in serum were found to be significantly increased in alloxan-induced diabetic group when compared to normal control group. The levels of the above parameters were significantly reversed on treatment with *Z.*

Table 1 Effect of ethanolic extract of *Z. decandra* root on biochemical parameter.

Parameter	Group I	Group II	Group III	Group IV	Group V
Glucose (mmol/L)	5.83±0.48	11.55±0.68*	6.27±0.57*	6.49±0.53*	6.05±0.46*
Cholesterol (mmol/L)	3.25±0.23	6.24±0.27*	3.12±0.23*	3.14±0.27^{NS}	3.06±0.23*
Triglycerides (mmol/L)	0.77±0.07	2.50±0.19**	0.80±0.06*	0.78±0.07^{NS}	0.86±0.06*
Total protein (g/L)	81±6.2	43±3.5*	81±6.7*	83±6.9^{NS}	80±6.6*
Urea (mmol/L)	0.31±0.02	0.69±0.05**	0.39±0.03*	0.33±0.254^{NS}	0.38±0.02*
Creatinine (μmol/L)	53.04±3.53	106.08±7.95*	55.69±3.53*	54.80±3.53 ^{NS}	51.27±3.53*

Values are mean±SD, n=6. Group II compared with group I, group III and V compared with group II, group IV compared with group I

NS not significant

*p<0.05, **p<0.01

decandra as observed in that of group V treated with glibenclamide. The level of total protein in serum was decreased in alloxan-induced diabetic group when compared to normal control group and it was significantly reversed on treatment with *Z. decandra* as observed in that of group V treated with glibenclamide.

Table 2 show the effect of *Z. decandra* on lipid peroxidation, superoxide dismutase, catalase, and glutathione peroxidase in liver of control and experimental groups. In alloxan-induced diabetic group, lipid peroxidation was increased significantly; whereas superoxide dismutase, catalase, and glutathione peroxidase were found to be decreased when compared to control group. The administration of *Z. decandra* to alloxan-induced diabetic animals altered the above changes as observed in that of group V treated with glibenclamide by regulating the lipid peroxidation level and antioxidant enzymes to nearly that of normal levels.

Histological examination of liver sections of the alloxan-induced rats shows degeneration in liver and pancreas. The liver and pancreatic sections of rats treated with ethanolic extract of *Z. decandra* show absence of necrosis and degeneration (Figs. 1 and 2).

Discussion

The present study was aimed to assess the hypoglycemic activity of ethanolic extract of roots of *Z. decandra*. Different mechanisms of action to reduce blood glucose levels with

Table 2 Effect of ethanolic extract of *Z. decandra* root on antioxidant enzymes and lipid peroxidation level.

Parameter	Group I	Group II	Group III	Group IV	Group V
Lipid peroxidation	20.17±1.55	48.23±3.71*	21.41±1.6*	21.2±1.7^{NS}	22.21±1.7*
Catalase	0.23±0.017	0.13±0.010*	0.26±0.02*	0.25±0.02^{NS}	0.29±0.022*
SOD	25.18±1.93	12.22±0.94*	23.42±1.57*	22.31±1.71^{NS}	25.09±1.40*
GPX	0.56±0.043	0.32±0.024*	0.54±0.041*	0.51±0.042^{NS}	0.56±0.04*

Values are mean±SD, n=6, group II compared with group I, group III and V compared with group II, group IV compared with group I. The values are expressed as units per milligram of protein

NS not significant

*p<0.05

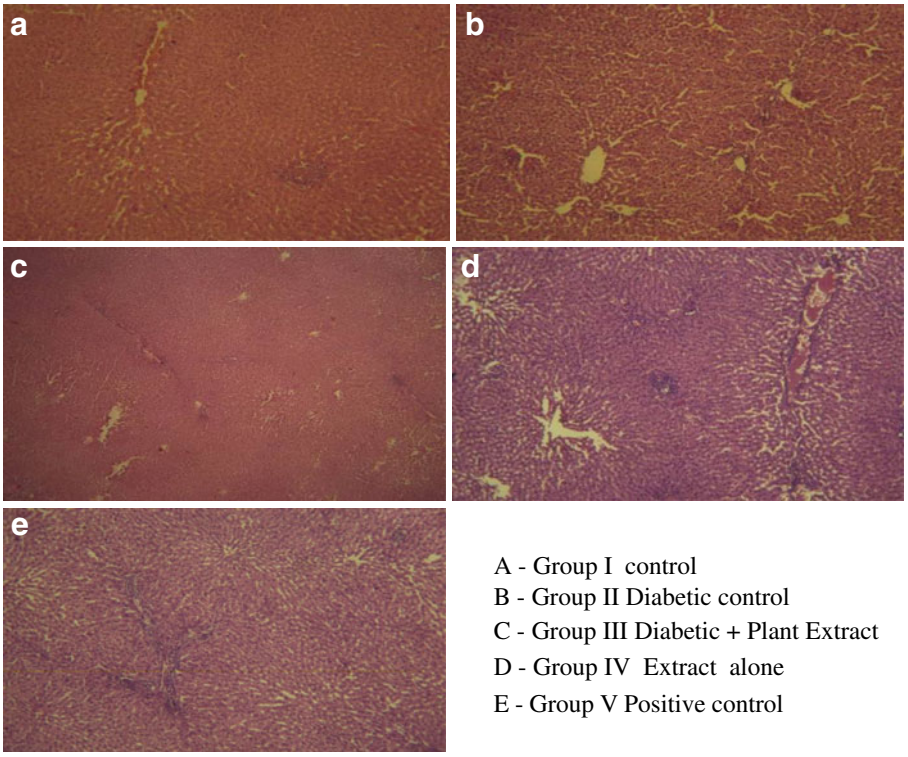

Fig. 1 Histopathology of liver

the help of plant extracts already exist. From the results it is assumed that the root extract could be responsible for stimulation of insulin release and the observed restoration of metabolic activities. A number of other plants have also been shown to exert hypoglycemic activity through stimulation of insulin release [17, 18]. Some plants exhibit properties similar to the well-known sulfonylurea drugs like glibenclamide; they reduce blood glucose in normoglycemic animals [19, 20]. Glibenclamide is reported to enhance the activity of β cells of pancreas resulting in secretion of larger amounts of insulin which in turn brings down blood glucose level [21]. Elevated blood lipids especially cholesterol and triglycerides as well as reduction of protein level are other indicators of diabetic condition [22]. As seen in the present study the level of serum lipids was raised in diabetes and such an elevation represents a risk factor for coronary heart disease [23]. This abnormal high level of serum lipids was mainly due to decrease in the action of lypolytic hormones on the fat depots mainly due to the action of insulin. Under normal circumstances, insulin activates the enzyme lipoprotein lipase, which hydrolyses triglycerides. However, in diabetic state lipoprotein lipase is not activated due to insulin deficiency resulting in hypertriglyceridemia [24] and insulin deficiency is also associated with hypercholesterolemia due to metabolic abnormalities [25]. Administration of *Z. decandra* root extract improved considerably, serum lipids of diabetic rats. The results in Table 1 showed significant increase in the level of urea and creatinine which are markers of renal dysfunction [26] in the diabetic groups compared to control level. After treatment of alloxan diabetic rats with *Z. decandra*, the

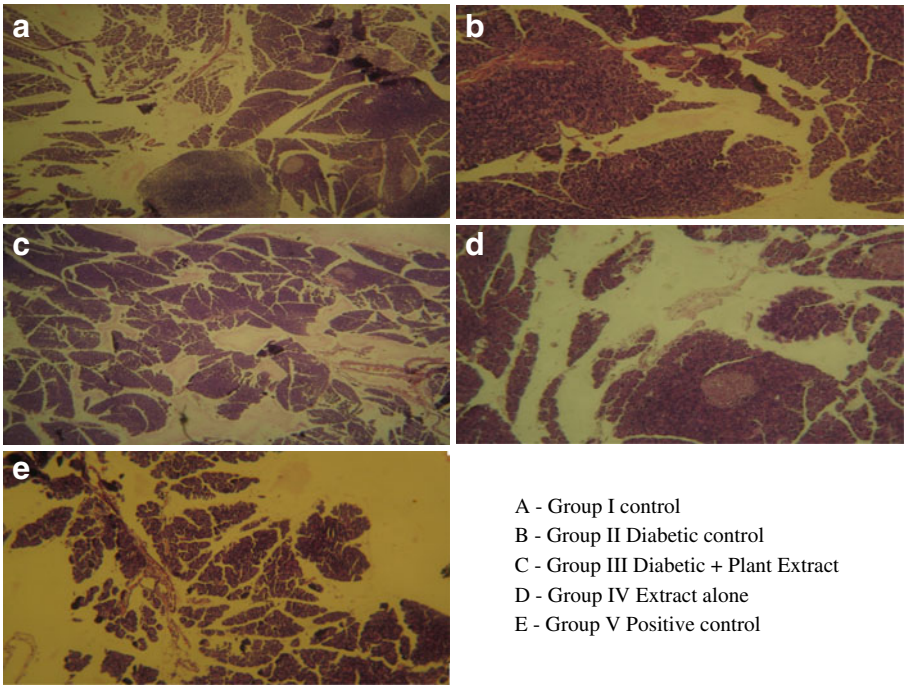

Fig. 2 Histopathology of pancreas

level of urea and creatinine were significantly decreased compared to those in untreated diabetic group.

Several studies have demonstrated the involvement of free radicals in the genesis of diabetes mellitus and their role in the induction of lipid peroxidation during diabetes [27, 28]. It has been reported that in diabetes mellitus oxygen free radicals are generated by stimulating H_2O_2 in vitro as well as in vivo in pancreatic β-cells [29]. In this study, the increase in lipid peroxidation indicates increased oxidative stress, generating more free radicals. Administration of *Z. decandra* decreased the lipid peroxidation. This significant reduction in lipid peroxidation can be attributed to the antioxidant activity of various phytochemicals present in the ethanolic extract of the roots of *Z. decandra*. Oxidative stress plays a role in the causation of diabetes and antioxidants have been shown to have a role in the alleviation of diabetes [30]. The decreased activities of superoxide dismutase, catalase, and glutathione peroxidase in the liver during diabetes may be due to production of reactive oxygen free radicals. Treatment with *Z. decandra* increased the activity of these enzymes and thus may help to counteract the damage by the free radicals generated during diabetes.

The histological examination of liver and pancreas revealed that the normal architecture was disturbed by alloxan induction. In the liver and pancreas of rats treated with *Z. decandra*, the normal cellular architecture was retained as observed with glibenclamide, thereby confirming the antidiabetic effect of the ethanolic extract of *Z. decandra*. Longer duration studies of *Z. decandra* roots for characterization and purification of the individual component in this plant is suggested in formulating the strategy of treatment.

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