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ORIGINAL CONTRIBUTION

Antihyperglycemic Activity of *Eclipta alba* Leaf on Alloxan-induced Diabetic Rats

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Eclipta alba, *an indigenous medicinal plant, has a folk (Siddha and Ayurvedha) reputation in rural southern India as a hypoglycemic agent. In order to confirm this claim, the present study was carried out to evaluate the antihyperglycemic effect of* E. alba *and to study the activities of liver hexokinase and gluconeogenic enzymes such as glucose-6-phosphatase and fructose 1,6-bisphosphatase in the liver of control and alloxan-diabetic rats. Oral administration of leaf suspension of* E. alba *(2 and 4 g/kg body weight) for 60 days resulted in significant reduction in blood glucose (from 372.0 ± 33.2 to 117.0 ± 22.8), glycosylated hemoglobin HbA_1c, a decrease in the activities of glucose-6 phosphatase and fructose1,6-bisphosphatase, and an increase in the activity of liver hexokinase.* E. alba *at dose of 2 g/kg body weight exhibited better sugar reduction than 4 g/kg body weight. Thus, the present study clearly shows that the oral administration of* E. alba *possess potent antihyperglycemic activity.*

INTRODUCTION

Diabetes mellitus is the most common disease associated with carbohydrate metabolism, affecting about 200 million people worldwide. Extracts of various plant materials capable of decreasing blood sugar have been tested in experimental animal models and their effects confirmed [1]. Many unknown and lesser-known plants are used in folk and tribal medicinal practices in India. The medicinal values of these plants are not much known to the scientific world. *E. alba* (Family Compositae) is one such medicinal plant popularly used for the inflammation, anthelmintic, astringent, deobstruent [2], and hepatoprotective effect [3]. *E. alba* is one of the ingredients of Trasina, the largest selling Ayurvedic antihyperglycemic drug (Dey's Medical, Calcutta) in India. [4]. It has been reported in a sonnet [5] that *E. alba* along with black cumin seeds (*Nigella sativa* L.) is capable of reducing sugar. Saint Ramalinga (Vallalar) in his songs advocates the intake of *E. alba* daily to strengthen body vitality [6]. Preliminary study in our laboratory is highly encouraging and revealed that significant blood glucose reduction was observed in alloxan-diabetic rats. This effect has never been experimentally demonstrated. Thus, we considered it

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interesting to check whether there is any scientific basis for the wide use in South India of *E. alba* as a hypoglycemic agent.

MATERIALS AND METHODS

Plant material

Eclipta alba L. Hassk. (syn. *E. prostrata* L., *Asteraceae*) popularly known as "Karichalankanni" in Tamil, "Bhangra" in English, and "Bringaraja" in Sanskrit, is an erect or prostrate, much branched plant, with white flower heads and is found in almost all parts of India, particularly in cool and moist places. *E. Alba* was collected in the area of Vallampadugai village, South Arcot district, Tamilnadu, India, in November. The herbarium of this plant was identified and authenticated [Herbarium No. 906460] by Botanical Survey of India, Coimbatore, Tamilnadu, India.

Preparation of plant material

Fresh leaves were collected and air-dried in shade at room temperature. The dried leaves were powdered mechanically and sieved using a fine muslin cloth. The fine powdered leaves were kept separately in airtight containers in a room temperature until the time of use. Two-percent gum acacia is used as vehicle solution.

Experimental animals

Male albino Wistar rats (150 to 200 g) bred in the Central Animal House, Rajah Muthiah Medical College, Annamalai University, were used in this study. The animals were fed on a pellet diet (Hindustan Lever, India) and water *ad libitum*. The animals were maintained in their respective groups for 60 days. All studies were conducted in accordance with the National Institute of Health's *Guide for the Care and Use of Laboratory Animals* [7], and the study was approved by the Institutional Ethical Committee of Rajah Muthih Medical College and Hospital, Annamalai University, Annamalai Nagar, Tamilnadu, India.

Experimental induction of diabetes

Diabetes was induced in male Wistar albino rats by intraperitoneal administration of alloxan monohydrate (150 mg/kg body weight) dissolved in normal saline. Since alloxan is capable of producing fatal hypoglycemia as a result of massive pancreatic insulin release, rats were treated with 30 percent glucose solution orally at different time intervals after six hours of alloxan induction, and 5 percent glucose solution was kept in bottles in their cages for the next 24 hr to prevent hypoglycemia. After 10 days, rats with diabetes mellitus having glycosuria (indicated by Benedict's test) and hyperglycemia with blood glucose range of 250 to 375 mg/dl were used for this experiment.

Experimental design

Animals were divided into six groups of six rats each. Feed and water were provided *ad libitum* to the animals:

- Group 1, Control vehicle only (2 percent gum acacia).
- Group 2, Diabetic control.
- Group 3, Control + *E. Alba* suspension (2 g/kg body wt).
- Group 4, Diabetic + *E. Alba* suspension (2 g/kg body wt).
- Group 5, Diabetic + *E. Alba* suspension (4 g/kg body wt.).
- Group 6, Diabetic + glibenclamide (600 mg/kg body wt).

The leaf suspension was given by oral intragastric tube. After 60 days of treatment, the rats were fasted overnight and sacrificed by cervical decapitation. The blood glucose [8] and glycosylated hemoglobin [9] were estimated. The liver was dissected out and washed with ice-cold

saline immediately. A portion of the tissue was homogenized using a Potter-Elvejham homogenizer, and the extract was used for the estimations of hexokinase [10], glucose 6-phosphatase [11], fructose 1,6-bisphosphatase [12], protein [13], hemoglobin [14], inorganic phosphorus [15], and blood urea [16] using a semi-autoanalyzer.

Statistical analysis

Values were represented as mean ± SD. Data were analyzed using analysis of variance and group means were compared with Duncan's multiple range test.

RESULTS

Changes in blood glucose and body weight in diabetic and on treatment of diabetic rats with *E. Alba*, glibenclamide are presented in Table 1. A significant increase in blood glucose and significant reduction in body weight are observed in diabetic rats when compared with control rats. Oral administration of *E. Alba* (2 and 4 g/kg body weight) for 60 days shows significant reduction in blood glucose and a remarkable improvement in body weight in diabetic rats when compared with untreated diabetic rats. Table 2 shows the effects of *E. Alba* and glibenclamide on hemoglobin, glycosylated hemoglobin, serum protein, and urea in control and alloxan-diabetic rats. There is a significant reduction in hemoglobin and serum protein while glycosylated hemoglobin and blood urea significantly increased in diabetic rats when compared with control rats. Oral administration of *E. Alba* (2 and 4 g/kg body weight) significantly brings the value to near normal. Effects on the administration of *E. Alba* and glibenclamide on hepatic hexokinase and glucose-6-phosphatase, fructose-1, 6-bisphosphatase of liver are presented in Table 3. The activity of hepatic hexokinase is significantly decreased while glucose-6-phosphatase and fructose-1, 6-bisphosphatase are significantly elevated in allaxon treated diabetic rats as compared to normal rats. Administration of *E. Alba* (2 and 4 g/kg body wt) and glibenclamide increases the activity of hexokinase and decrease the activities of glucose 6-phosphatase and fructose-1,6-

Table 1. Changes in blood glucose and body weight in control and alloxan diabetic rats treated with *E. alba* and glibenclamide.

Groups	Blood glucose (mg/dl)		Body weight (g)		Changes body wt. (g)
	Initial	Final	Initial	Final	
Control (2% gum acacia)	81 ± 5.6	85.6 ± 4.8[a]	170 ± 5.8	211 ± 8.7	+41
Diabetic control	353 ± 18.5	393 ± 13.6[b]	195 ± 4.1	125 ± 4.1	-70
Control + *E. alba* (2 g/kg body wt)	89.3 ± 6.8	90.2 ± 5.4[a,c]	166 ± 11.8	172.6 ± 12.4	+6.6
Diabetic + *E. alba* (2 g/kg body wt)	372 ± 33.1	117 ± 22.8[d]	193 ± 4.7	166 ± 16.9	-27
Diabetic + *E. alba* (4 g/kg body wt)	364 ± 36	159 ± 38[c,d]	182 ± 17.5	147 ± 17.5	-35
Diabetic + glibenclamide (600 µg/kg body wt)	267 ± 29	187 ± 12.5[a,d]	182 ± 17.5	147 ± 17.5	-35

Values are mean ± SD for six animals each. Values not sharing a common superscript differ significantly at $p < 0.05$. DMRT, Duncan's multiple range test.

Table 2. Effect of *E. alba* on hemoglobin, glycolylated hemoglobin (HbA_1c), protein, and urea in control and alloxan diabetic rats.

Groups	Hemoglobin (g %)	Glycolylated hemoglobin (mg/g of Hb)	Protein (g/dl)	Urea (mg/dl)
Control (2% gum acacia)	14.05 ± 0.9	0.203 ± 0.04[a]	5.82 ± 0.18[a]	22.0 ± 2.0[a]
Diabetic control	9.06 ± 1.7	0.569 ± 0.08[b]	4.20 ± 0.37[b]	38.6 ± 6.8[b]
Control + *E. alba* (2 g/kg body wt)	14.4 ± 0.4	0.205 ± 0.03[a]	5.83 ± 0.27[a]	22.0 ± 2.0[a]
Diabetic + *E. alba* (2 g/kg body wt)	13.53 ± 1.5	0.286 ± 0.02[c]	5.06 ± 0.08[c]	24 ± 3.3[a,d]
Diabetic + *E. alba* (4 g/kg body wt)	14.6 ± .7	0.268 ± 0.05[a,c]	5.42 ± 0.64[a,c]	33.0 ± 6.0[d]
Diabetic + glibenclamide (600 µg/kg body wt)	12.23 ± 0.6	0.239 ± 0.02[a,c]	5.43 ± 0.44[a,c]	22.0 ± 2.0[a]

Values are mean ± SD for six animals each. Values not sharing a common superscript differ significantly at $p < .05$. DMRT, Duncan's multiple range test.

bisphosphatase as compared to diabetic rats. Control animals administered with *E. Alba* 2 g/kg body weight do not show any significant changes in the any of the parameters studied.

DISCUSSION

Blood sugar level increased as expected in alloxan-injected animals, since alloxan causes a massive reduction in insulin release, by the destruction of the beta-cells of the islets of Langerhans and inducing hyperglycemia [17]. Oral administration of *E. Alba* (2 and 4 g/kg body wt.) resulted in a significant reduction in the blood glucose and improvement in body weight. The decrease in body weight in diabetic rats clearly shows a loss or degradation of structural proteins due to diabetes. The structural proteins are known to contribute for the body weight [18]. Protein synthesis is decreased in all tissues due to absolute or relative deficiency of insulin (an anabolic hormone) in alloxan-induced diabetic rats. The ability of the *E. Alba* to protect from maximum body weight loss seems to be due to its ability to reduce hyperglycemia. The present study also indicates that

E. Alba can inhibit alloxan renal toxicity as seen from the blood urea level. The glycosylated hemoglobin gives an idea about patient's overall glucose levels in the preceding six to eight weeks. Glycosylated hemoglobin comprises about 3.4 to 5.8 percent total hemoglobin in normal human red cells, but it is increased in patients of overt diabetes [19]. It is found to increase in diabetic patients up to 16 percent and the amount of this increase is directly proportional to the long lasting fasting blood sugar level. In our study, the glycosylated hemoglobin level was high showing that the diabetic animals had high blood glucose level. The values decreased very much in *E. Alba*-administered animals showing the influence of the leaf suspension on sugar reduction.

Insulin influences the intracellular utilization of glucose in a number of ways. Insulin increases hepatic glycolysis by increasing the activity and amount of several key enzymes including glucokinase, phosphofructokinase, and pyruvate kinase. Hexokinase is universally present in cells

Table 3. Effect of *E. alba* on the activities of hepatic enzymes in control and experimental animals.

Groups	Glucokinase (Unit[a]/mg protein)	Glucose-6-phosphatese (Unit[b]/mg protein)	Fructose 1,6-biphosphatese (Unit[c]/mg protein)
Control (2% gum acacia)	0.241 ± 0.035[a]	0.186 ± 0.005[a]	0.419 ± 0.020[a]
Diabetic control	0.082 + .005[b]	0.412 ± 0.021[b]	0.681 ± 0.075[b]
Control + *E. alba* (2 g/kg body wt)	0.240 + 0.015[a]	0.180 ± 0.030[a]	0.422 ± 0.022[a]
Diabetic + *E. alba* (2 g/kg body wt)	0.234 ± 0.016[a]	0.287 ± 0.007[c]	0.480 ± 0.008[c]
Diabetic + *E. alba* (4 g/kg body wt)	0.215 ± 0.043[a]	0.255 ± 0.036[a,c]	0.490 ± 0.020[c]
Diabetic + glibenclamide (600 µg/kg body wt)	0.238 ± 0.053[a]	0.223 ± 0.026[a,c]	0.469 ± 0.023[a,c]

Values are mean ± SD for six animals each. Values not sharing a common superscript differ significantly at $p < 0.05$. DMRT, Duncan's multiple range test. a, µmoles of glucose phosphorylated/h; b, µmoles of pi liberated/min; c, µmoles of pi liberated/min.

of all types. Hepatocytes also contain a form of hexokinase called hexokinase D or glucokinase, which is more specific for glucose and differ from other forms of hexokinase in kinetic and regulatory properties [20]. Glucokinase (also hexokinase IV) catalyzes the conversion of glucose to glucose-6-phosphate and play a central role in the maintenance of glucose homeostasis.

In the liver, the enzyme is an important regulator of glucose storage and disposal [21]. In our study, the hexokinase activity was decreased in alloxan-diabetic rats which may be due to insulin deficiency (insulin stimulates and activates glucokinase). Treatment with *E. Alba* or glibenclamide, elevated the activity of glucokinase in liver. *E. Alba*, like glibenclamide, may stimulate insulin secretion, which may activate glucokinase, thereby increasing utilization of glucose and thus increased utilization leads to decreased blood sugar level.

Insulin decreases gluconeogenesis by decreasing the activities of key enzymes such as glucose-6-phosphatase, fructose 1,6, bisphosphatase, phosphoenolpyruvate carboxykinase, and pyruvate carboxylase [22]. In our study, increased activities of glucose-6-phosphatase and fructose-1,6 bisphosphatase were observed in the liver of alloxan-diabetic rats. Glucose 6-phosphatase, one of the key enzymes in the homeostatic regulation of blood glucose level, catalyzes the terminal step in both gluconeogenesis and glycogenolysis [23, 24] and fructose 1,6-bisphosphatase, catalyzes one of the irreversible step in gluconeogeneis, and serves as a site for the regulation of process [25]. Increased activities of these two gluconeogenic enzymes (glucose-6-phosphatase and fructose 1,6 bisphosphatase) in the liver may be due to activation or increased synthesis of the enzymes contributing to the elevated glucose production in diabetes. Animals treated with *E. Alba* may primarily be modulating and regulating the activities of these two gluconeogenic enzymes either through regulation of cAMP or inhibition of gluconeogenesis [26].

In conclusion, we have demonstrated that the folk medicinal plant *E. Alba* possesses a hypoglycemic effect. Further, active research is underway in our labora-

tory to elucidate the mechanisms of action of this medicinally important plant.

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