

EFFECT OF *EVOLVULUS ALSINOIDES* EXTRACT ON MEMBRANE BOUND ENZYME ACTIVITIES IN THE TISSUES OF STREPTOZOTOCIN INDUCED DIABETIC RATSDURASAMY GOMATHI¹, GANESAN RAVIKUMAR¹, MANOKARAN KALAISELVI², KANAKASABAPATHI DEVAKI¹ AND CHANDRASEKAR UMA^{3*}

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This paper is available online at www.jprhc.in**ABSTRACT**

The present study investigated the possible therapeutic effects of the whole plant ethanolic extract of *Evolvulus alsinoides* on oral glucose tolerance test and the membrane bound enzyme activity in streptozotocin induced diabetes rats. The antidiabetic effect of ethanolic extract of *Evolvulus alsinoides* were studied at different concentrations like 50 mg, 150 mg and 300 mg/kg body weight in streptozotocin induced diabetic rats for 14 days. In that 150 mg/kg dosage showed significant reduction in blood glucose. Hence further studies were carried out by using the dosage of 150 mg/kg. The effects of an ethanol extract of *Evolvulus alsinoides* on the activities of marker enzymes were also examined in the liver and kidney tissues. Decreased levels of Sodium potassium, magnesium ATPases were observed in streptozotocin induced diabetic rats whereas calcium ATPase was increased in diabetic rats. Administration of plant extract to diabetic rats for 45 days restored all membrane bound enzyme activities as to that of control rats and there is no significant difference were found between control and plant extract alone groups. It might be related in part to the modification of fatty acid content during diabetes. Thus the ethanolic extract of *Evolvulus alsinoides* may alleviate liver and renal damage associated with streptozotocin induced diabetes mellitus in rats.

Keywords: Streptozotocin, *Evolvulus alsinoides*, membrane bound enzymes, diabetes mellitus**INTRODUCTION**

Evolvulus alsinoides (L.) L. is a perennial herb belonging to the family Convolvulaceae. The plant is widely distributed in tropical and subtropical regions throughout the world. It grows commonly as a weed in open and grassy places throughout India. The previous reports showed that this plant is used in traditional medicine in East Asia, India, Africa and Philippines to cure fever, cough, cold, venereal diseases, azoospermia, adenitis and dementia. It has a known nootropic and anti-inflammatory activity. Mohammedan physicians used the plant as a general tonic to strengthen the brain and memory. It was used to treat bowel problems and to promote conception⁽¹⁾. However, it has some phytochemicals that are effective against the maladies for which people use them. The isolation of evolvin, kaempferol-3-O- β -D glucopyranoside, coumarin etc., from *Evolvulus alsinoides* was previously reported^(2, 3). The aim of the present study was to identify the effect of *Evolvulus alsinoides* on glucose loaded diabetic rats and on membrane bound enzyme activity in tissues.

MATERIALS AND METHODS**Plant material**

The whole plant of *Evolvulus alsinoides* (L.) L. used for the investigation was obtained from Coimbatore District, Tamilnadu, India. The plant was authenticated by Dr. P.Satyanarayana, Botanical Survey of India, TNAU Campus, Coimbatore, India. The voucher number is BSI/SRC/5/23/2011-12/Tech.-514. Fresh plant material was washed under running tap water, air dried and powdered.

Sample extraction

The extraction procedure was conducted by using 100g of dried plant powder and 500ml of ethanol in an orbital shaker for 72hrs. Repeated extraction was done with the same solvent till clear colorless solvent is obtained. Obtained extract was evaporated and stored at 0-4°C in an air tight container

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. The study was approved by Institutional animal ethical committee constituted for the purpose of CPCSEA.

Induction of experimental diabetes

Rats were rendered diabetic by a single intraperitoneal injection of freshly prepared streptozotocin (45 mg/kg body weight) in 0.1M citrate buffer (pH 4.5) in a volume of 1 ml/kg body weight⁽⁴⁾. Diabetes was identified in rats by moderate polydipsia and marked polyuria. After 48 h of streptozotocin administration, blood glucose levels were estimated and rats with a blood glucose ranging between 200–400 mg/dl were considered diabetic and used for the experiments.

Experimental protocol

The animals were divided into five groups of six animals each. Group I served as a control; group II consisted of streptozotocin-induced diabetic rats; group III consisted of streptozotocin-induced diabetic rats treated with glibenclamide (1.25 mg/kg body weight/day/rat); groups IV consisted of streptozotocin-induced diabetic rats treated with ethanolic extract of *Evolvulus alsinoides* (150 mg/kg body weight/day/rat) and group V were normal rats treated with ethanolic extract of *Evolvulus alsinoides* (150 mg/kg body weight/day /rat)

Oral glucose tolerance test

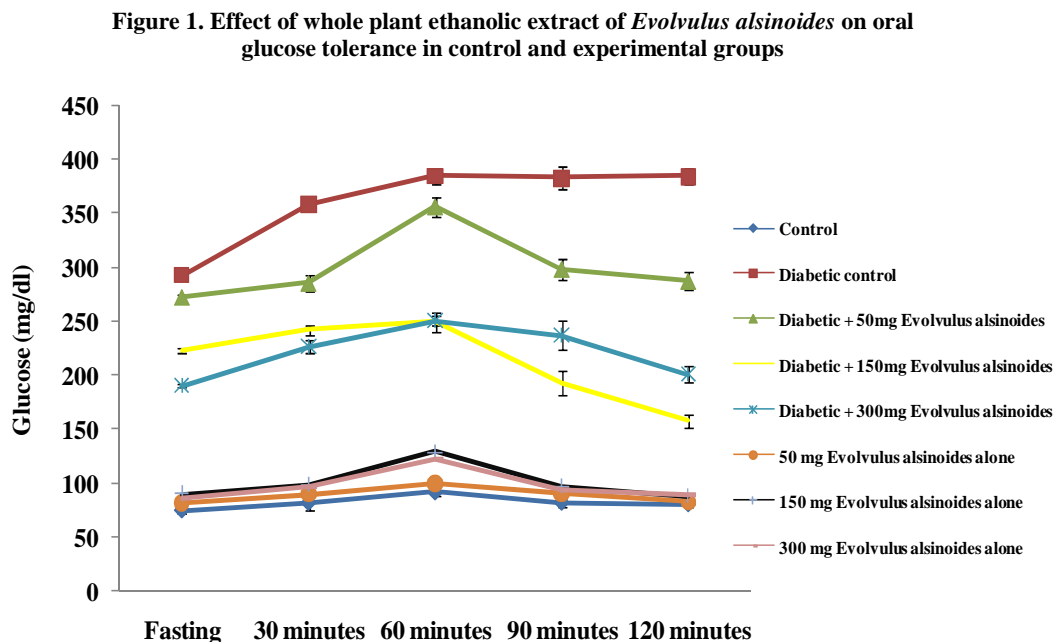
After the treatment period on the 14th day, fasting blood sample was collected from the tail vein of the rats. Four more blood samples were collected at 30, 60, 90 and 120 minute intervals after administration of glucose at a concentration of 2 g/kg bw⁽⁵⁾. The blood samples were collected for the estimation of glucose.

Biochemical studies

After 45 days of treatment the animals were sacrificed under chloroform anesthesia. Liver and kidneys were quickly excised off, a portion of tissues washed with saline and homogenates were prepared using 0.1 M Tris-HCl buffer, pH 7.4. The homogenate was centrifuged and the supernatant was used for the determination of membrane bound enzymes like Na⁺ K⁺ ATPase by the method of Bonting, 1970⁽⁶⁾, Ca²⁺ ATPase and Mg²⁺ ATPase by the method of Ohnishi *et al.*, 1982⁽⁷⁾.

RESULTS

In the present study the effect of *Evolvulus alsinoides* ethanol extract were studied on the activity of membrane bound enzymes in the tissues of streptozotocin induced diabetic rats. Figure 1 shows the antihyperglycemic effect of ethanolic extract of *Evolvulus alsinoides* at various concentrations like 50mg, 150mg and 300mg per kg body weight. Among the concentrations 150mg/kg body weight of *Evolvulus alsinoides* showed significant reduction in blood glucose levels when compared with 50 and 250mg/kg body weight ethanolic extract of *Evolvulus alsinoides*. In diabetic rats, the peak increase in blood glucose concentration was observed after 60 min and it was remained upto 120 min but *Evolvulus alsinoides* treated (150mg/kg) diabetic rats showed significant decrease in blood glucose concentration at 60 min and at 120 min interval. There is no significant difference between control and



Values are expressed as Mean \pm SD for six animals in each group.

Table 1, 2 and 3 shows the activities of membrane bound enzymes in liver and kidney of control and experimental groups. The decreased activity of Na⁺ K⁺ ATPase, Mg²⁺ ATPase and increased activity of calcium ATPase were observed in streptozotocin induced diabetic rats when compared with control rats. Whereas the enzyme activities were found to near normal after treatment with ethanolic extract of *Evolvulus alsinoides* and glibenclamide treated rats when compared with diabetic rats.

Table 1
Effect of ethanolic extract of *Evolvulus alsinoides* on the activity of sodium potassium ATPase and calcium ATPase in the liver of control and experimental groups

Groups	Sodium potassium ATPase	Calcium ATPase
Control	1.89 \pm 0.11 ^d	1.73 \pm 0.05 ^a
Diabetic control	0.83 \pm 0.12 ^a	4.74 \pm 0.12 ^d
Diabetic + Glibenclamide	1.64 \pm 0.13 ^c	2.01 \pm 0.15 ^b
Diabetic + <i>Evolvulus alsinoides</i>	1.37 \pm 0.20 ^b	2.28 \pm 0.05 ^c
<i>Evolvulus alsinoides</i> alone	1.93 \pm 0.13 ^d	1.73 \pm 0.02 ^a

Units

ATPase - μ moles of phosphorus liberated /mg protein

Values are expressed as mean \pm SD for six animals in each group. Values not sharing common superscript letters (a-d) differ significantly at p<0.05 (DMRT).

Table 2
Effect of ethanolic extract of *Evolvulus alsinoides* on the activity of sodium potassium ATPase and calcium ATPase in the kidney of control and experimental groups

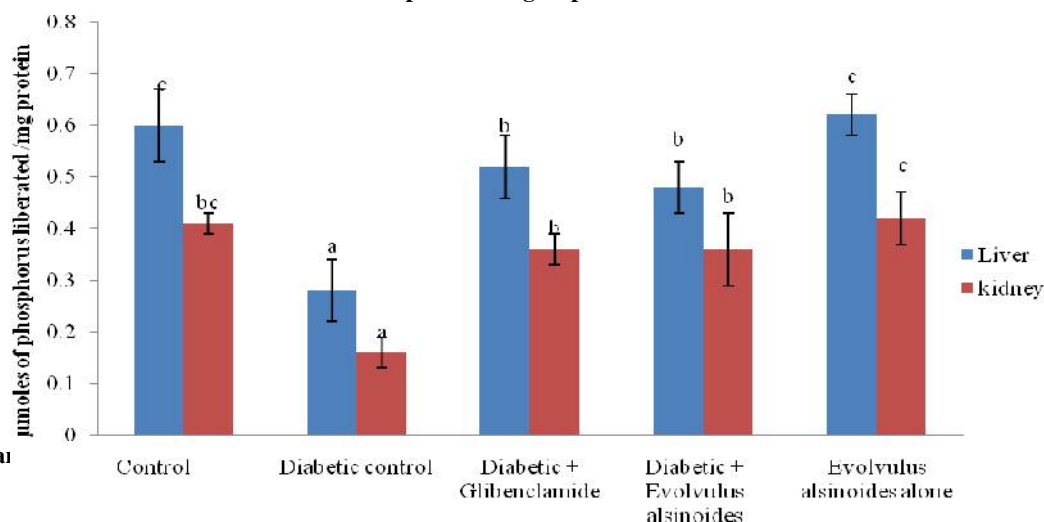
Groups	Sodium potassium ATPase	Calcium ATPase
Control	2.13 \pm 0.10 ^d	1.53 \pm 0.04 ^a
Diabetic control	0.88 \pm 0.14 ^a	2.81 \pm 0.14 ^d
Diabetic + Glibenclamide	1.95 \pm 0.10 ^c	1.76 \pm 0.08 ^b
Diabetic + <i>Evolvulus alsinoides</i>	1.60 \pm 0.10 ^b	1.96 \pm 0.17 ^c
<i>Evolvulus alsinoides</i> alone	2.17 \pm 0.11 ^d	1.51 \pm 0.06 ^a

Units

ATPase - μ moles of phosphorus liberated /mg protein

Values are expressed as mean \pm SD for six animals in each group. Values not sharing common superscript letters (a-d) differ significantly at p<0.05 (DMRT).

Figure 1
Effect of ethanolic extract of *Evolvulus alsinoides* on the activity of Magnesium ATPase in liver and kidney of control and experimental group rats



Values are expressed as mean \pm SD for six animals in each group. Values not sharing common superscript letters (a-d) differ significantly at $p < 0.05$ (DMRT).

DISCUSSION

Streptozotocin-induced hyperglycaemia has been described as a useful experimental model to study the activity of antidiabetic agents⁽⁸⁾. Streptozotocin selectively destroyed the pancreatic insulin secreting cells, leaving less active cell resulting in a diabetic state⁽⁹⁾. The test samples might possess metformin like effect on peripheral tissues either by promoting glucose uptake and metabolism or by inhibiting hepatic gluconeogenesis. The phytochemical studies revealed the presence of tannins, carbohydrate, terpenes, saponins and flavonoids in the ethanolic extract of plant material. Flavonoid and terpenes possess antidiabetic action⁽¹⁰⁾. Effect of the flavonoids on pancreatic β -cells leading to their proliferation and secretion of more insulin has been proposed by Mahesh and Menon, 2004⁽¹¹⁾ and Balasubashini, *et al.*, 2004⁽¹²⁾ as the mechanism by which they reduced hyperglycaemia caused by streptozotocin in diabetic rats. These secondary metabolites present in our sample may also be acting similarly thereby decreasing the high blood glucose levels of streptozotocin-diabetic rats.

$\text{Na}^+ \text{K}^+$ -ATPase has been implicated in the development of complications and adaptive changes in diabetes. In our study the decreased activity of $\text{Na}^+ \text{K}^+$ ATPase and increased activity of calcium ATPase were observed in streptozotocin induced diabetic rats when compared with normal rats. This is in agreement with the earlier published data of Mayanil *et al.*, 1982⁽¹³⁾. In experimental diabetes, changes in $\text{Na}^+ \text{K}^+$ -ATPase activity have been reported in the heart, peripheral nerve, kidney and intestine⁽¹⁴⁾. The magnitude and direction of the changes depend on the duration of diabetes and the organ involved. The physiological significance of these changes is not clear, although experimental diabetes is known to result in adaptive changes in the liver. A defect in liver microsomal fatty acid desaturation has been reported and these changes may have some effect on microsomal membrane-bound enzymes^(15, 16).

Mg^{2+} ATPase activities were significantly decreased ($p < 0.05$) and Ca^{2+} ATPase activity was increased in streptozotocin induced diabetic rats when compared with control rats whereas the enzyme activity was found to be increased in plant and standard drug treated group rats. There is no significant difference were observed in plant alone treated group rats and control rats which was supported by Mayanil *et al.*, 1982⁽¹³⁾. An increase in Ca^{2+} levels can activate endogenous enzyme such as phospholipases, proteases and endonucleases whose activities depend on micromolar concentration of Ca^{2+} and can impair cell function and structure⁽¹⁷⁾. During diabetes, Sodium Potassium ATPase activity has been demonstrated to be particularly sensitive to the membrane fatty acid environment^(18, 19). A defect in fatty acid desaturation is one of the complications associated with experimental diabetes^(20, 21).

CONCLUSION

The present study revealed the significant decrease in blood glucose level after treatment with *Evolvulus alsinoides* in glucose loaded diabetic rats and also it normalizes the activities of membrane bound enzyme levels in experimental rats. This investigation indicates the protective effects of the whole plant ethanolic extract of *Evolvulus alsinoides* in the tissues (liver and kidney) of streptozotocin induced diabetic rats.

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CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest

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