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Protective effect of the whole plant extract of *Evolvulus alsinoides* on glycoprotein alterations in streptozotocin induced diabetic rats

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ABSTRACT

Objectives: To assess the effect of *Evolvulus alsinoides* (*E. alsinoides*) on glycoprotein levels in liver, kidney and pancreas of control and diabetes induced rats. **Methods:** Wistar albino rats were used for the present study. The diabetes was induced by a single intraperitoneal injection of streptozotocin at 45 mg/kg body weight. After the induction of diabetes the rats were treated with glibenclamiede and *E. alsinoides* for 45 d. At the end of the experimental period the glycoprotein levels were estimated by using standard protocols. **Results:** Significantly higher levels of glycoproteins were observed in the tissues of diabetic rats when compared with the control rats. After treated with ethanolic extract of *E. alsinoides* and standard drug resulted in the reduction of glycoproteins when compared with the diabetic control rats. **Conclusion:** The present study proved that that ethanolic extract of *E. alsinoides* owned a beneficial effect on glycoprotein components. Hence, it can be used in the prevention of glycoprotein medicated complications in diabetes mellitus.

1. Introduction

Diabetes mellitus is a chromic metabolic disorder associated with long term damages, dysfunctions, failure of organs especially the eyes, kidneys, nerves and cardiovascular system[1]. Glycoproteins are carbohydrate linked protein macromolecules found in the cell surface, which form the principal component of animal cells. Hexose, hexosamine, and sialic acid are the basic components of the glycoproteins. They play an important role in membrane transport, cell differentiation and recognition, the adhesion of macromolecules to the cell surface, and the secretion and absorption of macromolecules[2]. Under diabetic conditions, reactive oxygen species are produced mainly through glycation reaction, which occurs in various tissues and may play an important role in the development of diabetic complications. Advanced glycation end products (AGEs) modify galactose, fucose and sialic acid contents of specific cellular glycoproteins[3]. Impaired metabolism of glycoproteins plays a major role in the pathogenesis of diabetes mellitus[4,5]. In the present study, the pharmacological effect of ethanolic extract of *Evolvulus alsinoides* (*E. alsinoides*) on glycoprotein metabolism was investigated and the results were compared with glibenclamide standard drug.

2. Materials and methods

2.1. Plant material

The whole plant of *E. alsinoides* 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 (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.

2.2. Sample extraction

100 g of dried plant powder was extracted in 500 mL of ethanol in an orbitory shaker for 72 h. Repeated extraction was done with the same solvent till clear colorless solvent is obtained. Obtained extract was evaporated and stored at 0-4 ℃ in an air tight container.

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2.3. 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.

2.4. Induction of experimental diabetes

Rats were rendered diabetic by a single intraperitoneal injection of freshly prepared streptozotocin (45 mg/kg bw) in 0.1 M citrate buffer (pH 4.5) in a volume of 1 mL/kg body weight[6]. Diabetes was identified in rats by moderate polydypsia 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.

2.5. Experimental design

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 bw/d/rat); groups IV consisted of streptozotocin-induced diabetic rats treated ethanolic extract of *E. alsinoides* (150 mg/kg bw/d/rat) and group V were normal rats treated with ethanolic extract of *E. alsinoides* (150 mg/kg bw/d/rat)

2.6. Biochemical studies

After 45 d of treatment the animals were sacrificed under chloroform anesthesia. The tissues (liver and kidney) were quickly excised off, a portion of tissues washed with saline. Delipidised residues of tissues were prepared according to the method of Folch *et al*[7]. A known amount of delipidised residues of the tissues were hydrolysed with 2.0 mL of 4 N HCl at 100 ℃ for 4 h. The hydrolyzed material was neutralized and used for the estimation of hexose[8], hexosamine[9] and sialic acid[10].

2.7. Statistical analysis

The values were expressed as Mean±SD (n=6). 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.

3. Results

Hyperglycaemia in experimental diabetic rats leads to a decreased utilization of glucose by insulin dependent pathways, thereby enhancing the formation of glycoproteins[11]. At the cell surface or inside the cells, the glycocomponents such as fucose and sialic acid form specific structures, called glycanic chains covalently linked to lipids or proteins. An increase in the biosynthesis and or a decrease in the metabolism of glycoproteins could be related to the deposition of these materials in the basal membrane of pancreatic cells. In recent times, many traditionally important medicinal plants have been tested for their efficacy against impaired glycoprotein levels in diabetes[12]. The present study was undertaken to find out the therapeutic effect of *E. alsinoides* on glycoprotein metabolism in streptozotocin induced diabetic rats

Table 1
Effect of ethanolic extract of *E. alsinoides* on the hexose and hexosamine in the liver of control and experimental rats.

Groups	Glycoproteins (mg/g of tissue)	
	Hexose	Hexosamine
Control	22.10±0.79^{a}	6.64±0.15^{a}
Diabetic control	53.57±2.75^{d}	9.37±0.71^{b}
Diabetic + Glibenclamide	27.37±0.72^{b}	6.97±0.20^{a}
Diabetic + *E. alsinoides*	30.51±1.54^{c}	7.03±0.27^{a}
E. alsinoides alone	22.36±0.76^{a}	6.71±0.16^{a}

Values are expressed as Mean±SD for six animals. Values not sharing common superscript letters (a–f) differ significantly at P<0.05 (DMRT).

Table 2
Effect of ethanolic extract of *E. alsinoides* on the hexose and hexosamine in the kidney of control and experimental rats.

Groups	Glycoproteins (mg/g of defatted tissue)	
	Hexose	Hexosamine
Control	21.39±0.93^{a}	6.01±0.20^{a}
Diabetic control	56.45±3.46^{c}	7.75±1.07^{b}
Diabetic + Glibenclamide	23.98±1.17^{ab}	6.36±0.57^{a}
Diabetic + *E. alsinoides*	26.30±2.19^{b}	6.41±0.53^{a}
E. alsinoides alone	21.79±0.94^{a}	6.07±0.30^{a}

Values are expressed as Mean±SD for six animals. Values not sharing common superscript letters (a–f) differ significantly at P<0.05 (DMRT).

Glycoprotein levels in the tissues were determined by the balance between their biosynthetic rate and their degradation of glycohydrolases. Abnormalities in the glycoprotein content may plan an important role in the pathophysiology of diabetes mellitus. Several hypotheses have been proposed to explain tissue glycoprotein abnormalities, functions and its secondary complications in diabetes[2].

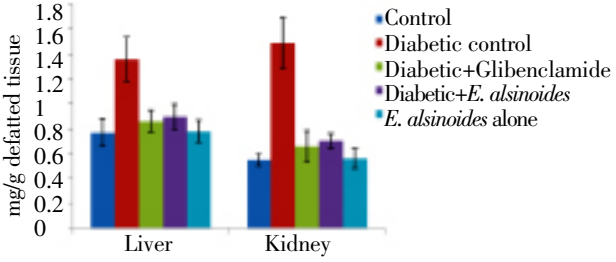

Figure 1. Effect of ethanolic extract of *E. alsinoides* on sialic acid in the liver and kidney of control and experimental rats.
Values are expressed as Mean±SD for six animals. Values not sharing common superscript letters (a–f) differ significantly at P< 0.05 (DMRT).

In this study, the levels of glycoproteins like hexose, hexosamine and sialic acid in the liver and kidney of normal and diabetic rats were studied and the results were depicted in Table 1, 2 and Figure 1. The high levels of all glycoproteins were observed in the tissues of the streptozotocin induced rats when compared with the normal control rats. Treatment with ethanolic extract of *E. alsinoides* for 45 d resulted in a significant

reduction of glycoproteins in the tissues of diabetic rats and there was no significant difference were observed between control and *E. alsinoides* alone group rats.

Increased glycosylation of various proteins in diabetic patients has been reported[13]. In this study, we have observed increased levels of hexose, hexosamine and sialic acid in the liver and kidney of streptozotocin induced diabetic rats. The increase in plasma glycoprotein components has been associated with the severity and duration of diabetes. In hyperglycaemia, free amino groups of proteins react slowly with the carbonyl groups of reducing sugars such as glucose, to yield a Schiff's-base intermediate (Maillard reaction). These Schiff-base intermediates undergo Amadori rearrangement to a stable ketoamine derivative (fructosamine)[14].

Sialic acid is a terminal component of the non-reducing end of the carbohydrate chains of glycoproteins and glycolipids which are essential constituents of many hormones and enzymes present in serum and tissues. Serum sialic acid is almost completely bound to glycoproteins and lipids. Total sialic acid in the serum has received considerable attention as a possible marker for cardiovascular disease and mortality[15]. In diabetes mellitus, the sialic acid concentrations were found to increase in serum and diabetic tissues[5]. In our study, administration of *E. alsinoides* extract decreased the content of sialic acid in the liver and kidney of streptozotocin induced diabetic rats (Figure 1).

4. Discussion

In the diabetic state, a deficiency in insulin secretion causes derangement of glycoprotein metabolism, which results in basal membrane thickening. Excess availability of glucose in the hyperglycaemic state accelerates the synthesis of glucose basement membrane components *i.e.* glycoproteins[16]. *E. alsinoides* administration for 45 d to diabetic rats restored the levels of glycoproteins in liver and kidney tissues. The decreased hyperglycaemic state with increased levels of plasma insulin observed in *E. alsinoides* treated diabetic rats might have been responsible for the decrease of glycoproteins levels in liver, kidney and pancreas. Our results are in agreement with the previous reports of Ramkumar *et al*[12]and Senthilkumar and Subramanian[5].

From the present study it can be concluded that the treatment with ethanolic extract of *E. alsinoides* restored the levels of glycoprotein components in diabetic rats may have been due to the normalization of glucose homeostasis. Thus it can be used in the protection of glycation of membrane proteins during diabetes mellitus

Conflict of interest

We declare that we have no conflict of interest

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