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Therapeutic Evaluation of *Aloe vera* Leaf Gel Extract on Glycoprotein Components in Rats with Streptozotocin Diabetes

S. Rajasekaran and D. Sathishsekar
Department of Advanced Biochemistry, University of Madras
Guindy Campus, Chennai-600 025, India

Abstract: Generalized abnormalities in glycoprotein metabolism are reported in both naturally occurring and experimental diabetes. The effect of *Aloe vera*, a traditionally used plant for the treatment of diabetes mellitus, was examined in Streptozotocin (STZ)-induced diabetic rats on derangement in glycoprotein's levels. STZ injection (55 mg kg^{-1} body weight) caused massive alterations of glycoprotein components such as hexose, hexosamine and sialic acid in plasma and tissues (liver and kidney) of diabetic control and experimental groups of rats. Oral administration ethanolic extract of *Aloe vera* leaf gel extract (300 mg kg^{-1} body weight) for 21 days significantly restored the levels of hexose, hexosamine and sialic acid to near normalcy. These effects were compared with glibenclamide, a reference drug. Thus, the present study confirms that *Aloe vera* gel extract possesses a significant beneficial effect on glycoprotein components in STZ-induced diabetic rats, thereby preventing glycoprotein's mediated secondary diabetic complications.

Key words: *Aloe vera*, ethanolic extract, streptozotocin-induced diabetes, glycoprotein components

INTRODUCTION

Nature has been the sources of medicinal treatments for thousands of years and plants based system continue to play an essential role in the primary health care of 80% of the world's underdeveloped and developing countries (Valiathan, 1998). Because of perceived effectiveness, minimal side effects in clinical experience and relatively low cost, herbal drugs are widely prescribed even when their biologically active compounds are unknown (Pickup and Williams, 1991). Ethnobotanical information indicates that more than 800 plants are used as traditional remedies for the treatment of diabetes (Pushparaj *et al*., 2000), but only a few have received scientific scrutiny. Among these plants, *Aloe vera* has been used in herbal medicine for many cultures. Aloes are members of the *Liliaceae* family and are mostly succulent with whorl of elongated pointed leaves. Taxonomists now refer to *Aloe barbadensis* as *Aloe vera* (Klein and Penneys, 1988). The central bulk of the leaf contains the colorless mucilaginous pulp, made up of large thin-walled mesophyll cells containing the *Aloe vera* gel itself. Despite its wide use as a folk remedy over a long period of time, the biochemical details of its action on physiological/pathophysiological functions have not been systematically worked out. Our previous experimental results revealed the hypoglycaemic (Rajasekaran *et al*., 2004; 2005a), hypolipidaemic (Rajasekaran *et al*., 2006) and free radical scavenging properties (Rajasekaran *et al*., 2005b, c) of *Aloe vera* gel extract in Streptozotocin (STZ)-induced diabetic rats.

Diabetes mellitus is the name given to a group of disorders characterized by absolute absent or deficient insulin secretion or peripheral insulin resistance resulting in hyperglycemia. Impaired metabolism of a number of other biomolecules such as carbohydrates, lipids, proteins and

Corresponding Author: Dr. S. Rajasekaran, 5/65, Golden George Nagar, Chennai-600 107. Tamil Nadu, India
Tel: +91-44-26561030

glycoproteins has also been reported (Dhawan *et al*., 1996). Any abnormalities in the glycoprotein contents may play an important role in the pathophysiology of diabetes. Several hypotheses have been proposed to explain glycoprotein abnormalities and functions in diabetes and its secondary complications (Mittal *et al*., 1996).

In the view of the above report, the present study was undertaken to investigate the effect of *Aloe vera* leaf gel extract on plasma and tissue (liver and kidney) glycoprotein components in STZ-induced diabetic rats. The results were compared with glibenclamide, a known hypoglycemic drug.

MATERIALS AND METHODS

Animals Used

Male albino rats of Wistar strain weighing about 160-200 g were bred in the animal house of the Department of Advanced Biochemistry, University of Madras, Chennai City, India. They were housed in standard cages and fed rat chow, with a particular drinking fluid *ad libitum* and a 12 h light-dark cycle. The experiments were designed and conducted according to ethical norms approved by Ministry of Social Justices and Empowerment, Government of India and Institutional Animal Ethics Committee Guidelines (IAEC No. 01/034/04).

Plant Material

Aloe vera (L.) plants were maintained and collected from our University Campus. The taxonomic identification of the *Aloe vera* plant was confirmed by a senior plant taxonomist, Prof. V. Kaviyarasan, Ph.D., at the Center for Advanced Studies in Botany, University of Madras and a voucher specimen (No. 1070) was deposited in the herbarium.

Preparation of *Aloe vera* Leaf Gel Extract

Mature, healthy and fresh leaves of *Aloe vera* having a length of approximately 75 to 90 cm were removed and washed with fresh water. The thick epidermis was selectively removed. The inner colorless mucilaginous pulp was homogenized and centrifuged to remove the fibers. The resultant supernatant was immediately lyophilized. The lyophilized sample was extracted with 95% ethanol. The filtrate was collected and evaporated to dryness under reduced pressure in a rotary evaporator. Known amount of solvent free extract was suspended in sterilized water freshly each time and administered intragastrically.

Induction of Experimental Diabetes

The rats were fasted for 16 h prior to induction of experimental diabetes by intraperitoneal injection (55 mg kg^{-1} body weight) of STZ (Sigma, St. Louis, MO, USA) freshly dissolved in 0.1 M cold sodium citrate buffer pH 4.5 (Rakieten *et al*., 1963). Control rats received equivalent amounts of buffer intraperitoneally. The animals were allowed to drink 5% glucose solution overnight to overcome the drug-induced hypoglycaemia. Hyperglycaemia was confirmed one week after induction via blood glucose level measurements after a 16 h fast. Animals with a fasting blood glucose level greater than 250 mg dL^{-1} were considered as diabetic and included in the present study. The food and water consumptions were measured daily and weekly, respectively.

Experimental Design

The rats were divided into four groups of six rats in each group as follows.

Group I: Control rats.
Group II: STZ-induced diabetic control rats.

Group III: Diabetic rats given *Aloe vera* leaf gel extract (300 mg kg^{-1} body weight rat^{-1} day^{-1}) inaqueous solution daily using an intra-gastric tube for 21 days.

Group IV: Diabetic rats given glibenclamide (600 μg kg^{-1} body weight 1 rat^{-1} 1 day^{-1}) in aqueous solution daily using an intragastric tube for 21 days.

Analytical Methods

On completion of 21 days of experimental period, the 16 h fasted rats were sacrificed by cervical decapitation. Blood samples were collected in a tube containing potassium oxalate and sodium fluoride for the estimation of blood glucose (Sasaki *et al*., 1972) and the plasma was separated for the estimation of glycoproteins. The plasma glycoproteins were precipitated with alcohol. Liver and kidney were dissected out, washed in ice-cold saline, patted dry and weighed. For the estimation of glycoproteins, the tissues were defatted by the method of Folch *et al*. (1957). Both the alcoholic precipitate and a known amount of defatted tissues were treated with 0.1N H_2SO_4 and hydrolyzed for 60-90 min at 90°C and aliquots were used for sialic acid estimation. To the remaining solution, 0.1N NaOH was added. The aliquots were used for hexose and hexosamine estimation. Hexose was estimated by the method of Niebes (1972). Hexosamine was determined by the method of Wagner (1979). Sialic acid was estimated by the method of Warren (1959).

Statistical Analysis

All the grouped data were statistically evaluated with SPSS/7.5 software. Hypothesis testing methods included one way analysis of variance (ANOVA) followed by Least Significant Difference (LSD) test. p values of less than 0.05 were considered to indicate statistical significance. All the results were expressed as Mean ± S.D. for six animals in each group.

RESULTS

A significant increase in the levels of blood glucose, food and water intake was observed in STZ-induced diabetic rats when compared with corresponding control rats. Administration of *Aloe vera* gel extract or glibenclamide to diabetic rats tends to bring these changes to near normalcy (Table 1).

The hexose, hexosamine and sialic acid contents obtained from plasma and tissue homogenates of control and experimental groups of rats are shown in Table 2, Fig. 1 and 2, respectively. A marked

Table 1: Effect of *Aloe vera* extract on blood glucose level, food and water intake in control and experimental groups of rats

Groups	Blood glucose (mg dL^{-1})	Food intake (g rat^{-1} day^{-1})	Water intake (mL rat^{-1} day^{-1})
Control	83.75±5.19	15.2±1.21	27.8±2.24
Diabetic control	312.45±23.74[a]	61.0±5.97[a]	95.3±8.57[a]
Diabetic + *Aloe vera*	90.18±5.60[b]	22.1±1.90[b]	33.2±2.82[b]
Diabetic + Glibenclamide	119.24±7.75[b]	28.5±2.62[b]	39.8±3.06[b]

Values are given as mean±SD for groups of six animals in each group. [a]$p<0.05$ when compared with control rats. [b]$p<0.05$ when compared with diabetic control rats

Table 2: Effect of *Aloe vera* extract on glycoprotein components in plasma of control and experimental groups of rats

Groups	Hexose (mg dL^{-1})	Hexosamine (mg dL^{-1})	Sialic acid (mg dL^{-1})
Control	106.3±7.97	40.1±2.20	43.3±2.77
Diabetic control	147.1±12.50[a]	77.8±4.97[a]	62.7±4.64[a]
Diabetic + *Aloe vera*	112.7±9.24[b]	43.4±2.47[b]	44.8±3.20[b]
Diabetic + Glibenclamide	115.9±10.19[b]	45.2±2.80[b]	47.1±3.76[b]

Values are given as mean±SD for groups of six animals in each group. [a]$p<0.05$ when compared with control rats. [b]$p<0.05$ when compared with diabetic control rats

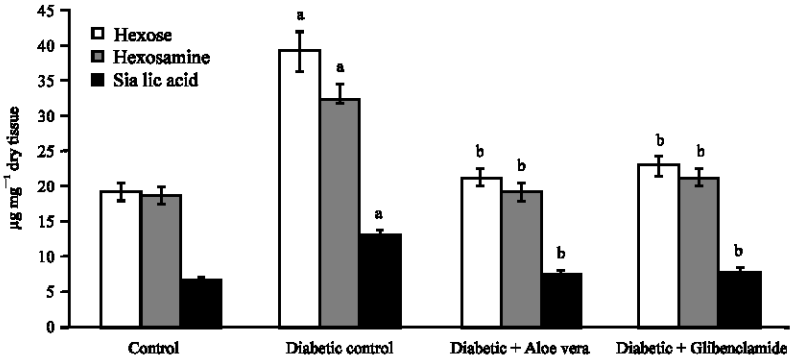

Fig. 1: Effect of *Aloe vera* extract on glycoprotein components in liver of control and experimental groups of rats. Values are given as mean ± SD for groups of six animals in each group. [a]p<0.05 when compared with control rats. [b]p<0.05 when compared with diabetic control rats

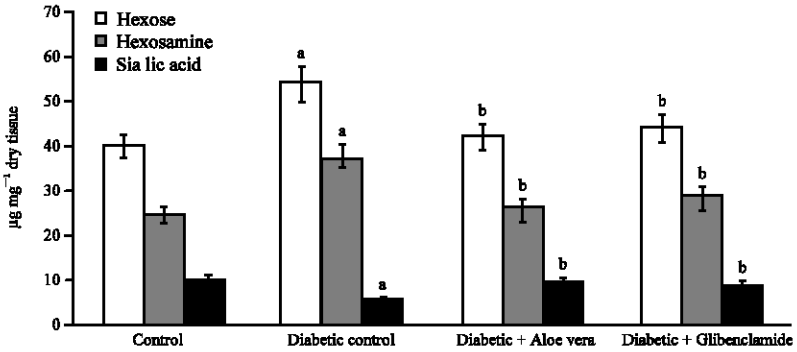

Fig. 2: Effect of *Aloe vera* extract on glycoprotein components in kidney of control and experimental groups of rats. Values are given as mean±SD for groups of six animals in each group. [a]p<0.05 when compared with control rats. [b]p<0.05 when compared with diabetic control rats

rise in the levels of hexose and hexosamine was noticed in plasma, liver and kidney of diabetic rats when compared to those of control rats. A marked elevation in the level of sialic acid was seen in plasma and liver, whereas a significant decrease was seen in kidney of diabetic rats in comparison with control rats. Subsequently, *Aloe vera* extract and glibenclamide treatment resulted in the normalization of the altered glycoprotein components to near normalcy.

DISCUSSION

Generalized abnormalities in glycoproteins metabolism are reported in both naturally occurring and experimental diabetes. This study was therefore undertaken to assess the effect of ethanolic extract of *Aloe vera* leaf gel on glycoprotein components in STZ-induced diabetic rats. Intraperitoneal injection of STZ produced various cardinal symptoms of diabetes such as, hyperglycemia, polyphagia and polydipsia. These findings are consistent with earlier findings (Ananthan *et al*., 2004). Increased food consumption observed in diabetic control rats in comparison to normal rats indicates polyphagic condition due to excessive break down of tissue proteins and this may be attributed to the non-availability of glucose to the cells due to insulin deficiency (Chatterjee and Shinde, 2002). Treatment

with *Aloe vera* decreased food and water consumption to some extent, which clearly indicating control over polyphagia and polydipsia and this may be resulted form antihyperglycaemic effect of the gel extract (Rajasekaran *et al*., 2004).

In diabetes, the plasma concentration of glycoprotein components was found to increase significantly, especially in poorly controlled cases (Sonmez *et al*., 1997). The increases in plasma glycoprotein components have been reported to be associated with severity and duration of diabetes mellitus. Glycoproteins found in a variety of tissues including the arterial wall and are very similar in structure and composition to those in plasma (Radhakrishna (n) moorthy and Berenson, 1973). Therefore, vascular complications that involve complex protein-carbohydrate molecules contribute to an increase in plasma glycoproteins.

Glycoprotein levels in the tissues are mainly determined by the balance between their biosynthetic rate and their degradation by glycohydrolases. Any abnormalities in the glycoprotein content may play an important role in the pathophysiology of diabetes. Several hypotheses have been proposed to explain tissue glycoprotein abnormalities and functions in diabetes and its secondary complications (Mittal *et al*., 1996). The liver is primarily responsible for producing the large amount of glycoproteins in blood. Synthesis of glucosamine from glucose is an insulin-dependent pathway, in hyperglycemia and uncontrolled insulinopenic state, glucose is redirected to an insulin-independent pathway. This could lead to the accumulation of high levels of glycoproteins in the liver (Robinson *et al*., 1995).

Diabetes mellitus affects the kidney and is the leading cause of diabetic nephropathy. In addition to prominent roles played by factors such as oxidative stress, abnormal lipid metabolism and renal accumulation of lipids and others, abnormal glycoprotein metabolism have also been proposed to play a pivotal role in the pathogenesis of diabetic nephropathy (Kimmelsteil and Wilson, 1936). The increased availability of glucose in the hyperglycemic state accelerates the synthesis of basement membrane components i.e., glycoproteins (Spiro and Spiro, 1971). This is due to depressed utilization of glucose by insulin-dependent pathway, there by enhancing the formation of hexose and hexosamine for the accumulation of glycoproteins (Patti *et al*., 1999). Rasch *et al*. (1995) showed that this elevated levels of glycoproteins manifest themselves as the basement membrane thickening observed in diabetic nephropathy. The decrease in the content of sialic acid in diabetic kidney may be due to the increased utilization for the synthesis of fibronectin, which contains sialic acid residues in the core structure. The synthesis of fibronectin was also reported to increase significantly in diabetic patients and animals (Schiller and Dorfman, 1957). Administration of *Aloe vera* extract significantly restored all these changes to near normal level.

In conclusion, the altered glycoprotein components in plasma, liver and kidney of diabetic rats can be assigned as a direct result of hyperglycemia. The presently observed control over the levels of glycoprotein components following *Aloe vera* extract therapy may have been due to the normalization of glucose homeostasis, which may help in the restoration of glycoprotein components in diabetic rats. Preliminary phytochemical investigations in our laboratory revealed the presence of phenols, sterols, triterpenoids, carotenoids, anthroquinones and glycosides as biologically active constituents in the gel extract (Rajasekaran *et al*., 2005b). Hence the observed hypoglycemic property of *Aloe vera* may be due to the presence of these active principles in the gel extract. There is an on going research to isolate and characterize the bioactive compound (s) responsible for the antidiabetic action in these crude extract and to use the (se) compound (s) in a bioassay-directed experiment.

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