ABSTRACT

The present investigation was carried out to evaluate the protective role of Helicteres isora plant extract on glycoprotein metabolism in streptozotocin (STZ) induced type 2 diabetic rats. Helicteres isora plant extract was administered orally (200 mg/kg body weight) for 45 days to normal and diabetic rats. The effects of Helicteres isora plant extract on plasma and tissue glycoproteins (hexose, hexosamine, sialic acid and fucose) were determined. The levels of plasma glycoproteins containing hexose, hexosamine, sialic acid and fucose were significantly increased in diabetic rats when compared with normal control rats. There was a significant decrease in the level of sialic acid and elevated levels of hexose, hexosamine and fucose in the liver and kidney of STZ induced diabetic rats. On oral administration of Helicteres isora plant extract to diabetic rats showed decreased levels of plasma glycoproteins. The level of tissue sialic acid was increased whereas the levels of tissue hexose, hexosamine and fucose were reversed to near normal. The present study indicates that the Helicteres isora plant extract possesses a significant protective effect on glycoprotein metabolism in addition to its anti-diabetic effect.

Keywords: Helicteres isora, Diabetes, Plasma Glycoproteins, Streptozotocin.

INTRODUCTION

Medicinal plants played an important role in Indian culture since Rig Veda (5600 BC) where about 67 medicinal plants were recorded. It is estimated that 80% of population rely on traditional medicines due to high cost of modern medicines, lack of availability of required medicines and personal preferences. It is identified that about 20,000 plants have good medicinal value and 7500 species are used by traditional communities[1]. Diabetes is becoming the third killer of mankind, after cancer and cardiovascular diseases, because of its high prevalence, morbidity and mortality[2]. Diabetes is widely recognized as one of the leading causes of death in the world[3]. People suffering from diabetes are not able to produce or properly use insulin in the body, so they have a high level of blood glucose. Diabetes is a chronic disease caused by inherited and/or acquired deficiency in production of insulin by the pancreas, or by the ineffectiveness of the insulin produced, thus results in decreased glucose transport into muscle and fat cells, and increased hepatic glucose output. These defects contribute to hyperglycemia, resulting in the impairment of the metabolism of glucose, lipids, proteins and glycoproteins[4]. In the diabetic state, glucose is used by the insulin independent pathways, leading to the synthesis of oligosaccharide moieties of glycoprotein; hexose, hexosamine, fucose, and sialic acid have an important role in protein stability, function, and turnover[5]. Glycoproteins are carbohydrate - lipid linked protein macromolecules found in the cell surface, which is the principle component of animal cell. The oligosaccharide moieties of glycoproteins, hexose, hexosamine, fucose and sialic acid have an important role in protein stability, function and turnover[6], membrane transport, cell differentiation and recognition, secretion and absorption of macromolecules and the adhesion of...
macromolecules to the cell surface[7]. Glycoproteins play a major role in the pathogenesis of diabetes mellitus due to impaired metabolism[8]. Insulin deficiency and high levels of plasma glucose in diabetic condition may result in an increased synthesis of glycoproteins[9].

The level of glycoproteins has been associated with severity and duration of diabetes. At the cell surface or inside the cells, fucose and sialic acid form specific structures, called glycanic chains covalently linked to lipids or proteins. An increase in the biosynthesis and or decrease in the metabolism of glycoproteins could be related to deposition of these materials in the basal membrane of pancreatic cells.

**MATERIALS AND METHODS**

**Animals**

Male albino Wistar strain rats (weighing 180–200 g b.w.) were procured from the Central Animal House, Rajah Muthiah Medical College (RMMC), Annamalai University. They were acclimatized to animal house conditions, and fed with standard pellet diet (Hindustan Lever Limited, Mumbai, India) and water ad libitum. The rats used in the present study were maintained in accordance with the guidelines of the National Institute of Nutrition, Indian Council of Medical Research Hyderabad, India and the study approved by the ethical committee (Vide. No: 845), Annamalai University.

**Drugs and Chemicals**

All the chemicals used in this experiment were obtained from Sigma Chemical Company (St Louis, MO, USA), Hi Media (Mumbai, India), and SD-Fine Chemicals (Mumbai, India). All chemicals used were of analytical grade.

**Experimental induction of type 2 diabetes in rats**

Non-insulin dependent diabetes mellitus was induced by the method of Masiello et al., (1998)[10], in overnight fasted rats by a single intraperitoneal injection of 45 mg/kg body weight STZ, STZ was dissolved in citrate buffer (0.1M, pH 4.5) and nicotinamide was dissolved in normal saline. Hyperglycemia was confirmed by the elevated glucose levels in plasma, determined at 72 h and then on day 7 after injection. The animals with plasma glucose concentration more than 250 mg/dl were used for the study.

**Experimental procedure**

In the experiment, a total of 24 rats (12 diabetic surviving rats and 12 normal rats) were used. The rats were divided six in each group. *Helicteres isora* was dissolved in vehicle solution (corn oil) and administered orally using an intragastric tube for a period of 45 days.

**Group 1: Normal control rats (vehicle treated)**

**Group 2: Normal rats + *Helicteres isora* (200 mg/kg b.w)**

**Group 3: Diabetic rats**

**Group 4: Diabetic rats + *Helicteres isora* (200 mg/kg b.w)**

At the end of the experimental period, the rats were deprived of food overnight and sacrificed by decapitation. Blood sample was collected in a tube containing potassium oxalate and sodium fluoride (3:1) for the estimation of plasma glycoproteins. Liver and kidney were dissected out, washed in ice-cold saline, patted dry and weighed.

**Biochemical assays**

**Extraction of glycoproteins**

To 0.1 ml of plasma, 5.0 ml of methanol was added, mixed well and centrifuged for 10 min at 3000×g. The supernatant was decanted and the precipitate was again washed with 5.0 ml of 95% ethanol, recentrifuged and the supernatant was decanted to obtain the precipitate of glycoproteins. This was used for the estimation of hexose and hexosamine.

For extraction of glycoproteins from the tissues, a known weight of the tissue was homogenized in 7.0 ml of methanol. The contents were filtered and homogenized with 14.0 ml of chloroform. This was filtered and the residue was successively homogenized in chloroform-methanol (2:1v/v) and each time the extract was filtered. The residue (defatted tissues) was obtained and the filtrate decanted. A weighed amount of defatted tissue was suspended in 3.0 ml of 2N HCl and heated at 90°C for 4h. The sample was cooled and neutralized with 3.0 ml of 2N NaOH. Aliquots from this were used for estimation of fucose, hexose, hexosamine and sialic acid.

**Determination of glycoproteins**

Plasma and tissue hexose and hexosamine were estimated by the method of Dubois and Gilles, 1933[11] with slight modifications by Niebes, 1972[12] respectively. Sialic acid
and fucose were estimated by the method of Dische and Shettle, 1948[13] respectively.

**Statistical analysis**

The data for various biochemical parameters were analyzed using analysis of variance (ANOVA) and the group means were compared by Duncan’s Multiple Range Test (DMRT) using a statistically software package (SPSS for Windows, V.13.0, Chicago, USA). Results were presented as mean ± S.D. p<0.05 were considered as statistically significant.

**RESULTS**

In the present study we have reported that plant extract 230mg/kg body weight showed better effects. Therefore 230mg/kg body weight was used in the present study.

**Effect of H.isora extract on plasma and tissue glycoproteins**

Figure 1 shows the changes in the levels of plasma glycoproteins of control and diabetic rats. Significantly higher levels of glycoproteins were observed in the plasma of the diabetic rats when compared with normal control rats. Treatment with *H.isora* extract to diabetic rats resulted in a significant reduction of glycoproteins in the plasma when compared with diabetic control rats.

The levels of liver and kidney glycoprotein of control and experimental rats were shown in Figures 2–5. The levels of glycoproteins, hexose, hexosamine and fucose were significantly increased whereas the level of sialic acid was significantly decreased in diabetic rats. Oral administration of *H.isora* extract significantly reversed these changes in the liver and kidney of diabetic rats.

![Figure 1: Changes in the levels of plasma glycoproteins in control and experimental rats. NC-Normal control, N+Ex- normal + extract, DC-diabetic control, D+Ex-diabetic + extract; Values are given as mean ±S.D. for 6 rats in each group. Values not sharing a common superscript letter differ significantly at p<0.05(DMRT).](image)

![Figure 2: Changes in the levels of tissue hexose in control and experimental rats. NC-Normal control, N+Ex- normal + extract, DC-diabetic control, D+Ex-diabetic + extract; Values are given as mean ±S.D. for 6 rats in each group. Values not sharing a common superscript letter differ significantly at p<0.05(DMRT).](image)
Figure 3: Changes in the levels of tissue hexosamine in control and experimental rats. NC-Normal control, N+Ex-normal + extract, DC-diabetic control, D+Ex-diabetic + extract; Values are given as mean ± S.D. for 6 rats in each group. Values not sharing a common superscript letter differ significantly at p<0.05 (DMRT).

Figure 4: Changes in the levels of tissue sialic acid in control and experimental rats. NC-Normal control, N+Ex-normal + extract, DC-diabetic control, D+Ex-diabetic + extract; Values are given as mean ± S.D. for 6 rats in each group. Values not sharing a common superscript letter differ significantly at p<0.05 (DMRT).

Figure 5: Changes in the levels of tissue fucose in control and experimental rats. NC-Normal control, N+Ex-normal + extract, DC-diabetic control, D+Ex-diabetic + extract; Values are given as mean ± S.D. for 6 rats in each group. Values not sharing a common superscript letter differ significantly at p<0.05 (DMRT).
DISCUSSION

The use of traditional medicine and medicinal plants in most developing countries, as a normative basis for the maintenance of good health, has been widely observed[14]. Furthermore, an increasing reliance on the use of medicinal plants in the society has been traced to the extraction and development of several drugs and chemotherapeutics from these plants as well as from traditionally used rural herbal remedy[14].

Diabetes mellitus is a chronic metabolic disease with the highest rates of prevalence and mortality worldwide that is caused by an absolute or relative lack of insulin and or reduced insulin activity, which results in hyperglycemic and abnormalities in carbohydrate, protein and fat metabolism[15]. In diabetes, synthesis of glycoproteins was decreased because of reduced incorporation of glucose caused by insulin deficiency. Several studies have emphasized the multiplicity of disturbances affecting the metabolism of carbohydrates, proteins and lipids in diabetes[16,17].

In this study, we have observed altered levels of hexose, hexosamine, fucose and sialic acid in plasma and tissues of STZ induced diabetic rats. Glycation is a nonenzymatic reaction of glucose and the saccharide derivatives with proteins, nucleotides and lipids[18]. In hyperglycemia, the reactions occur between reducing sugars and amino groups of proteins to yield a Schiff’s base intermediate. These schiff’s base intermediate undergoes rearrangement to form a relatively stable Amadori product. The Amadori product further undergoes a series of reactions through dicarbonyl intermediates to form AGE (advanced glycation endproducts). Glycation occurs inside and outside the cells. Glycation of cellular proteins produces changes in structure and loss of enzymatic activity. These effects are countered by protein degradation and renewal.

In extracellular matrix the glycation produces changes in macromolecular structure affecting matrix-matrix and matrix cell interactions associated with decreased elasticity and increased fluid filtration across the arterial wall and endothelial cell adhesion[19].

When the concentration of AGES increased above a critical level, cell surface AGE receptors become activated. Abnormalities in the metabolism of glycoproteins are observed in both naturally occurring and experimental diabetes[20]. The increases in plasma glycoprotein components have been reported to be associated with the severity and duration of diabetes.

Fucose is member of a group of essential sugars that the body requires for functioning of cell to cell communication and its metabolism appear to be altered in various disease conditions such as diabetes mellitus[21]. Due to increased glycosylation in the diabetic state the fucose levels could be increased. Experiments conducted in our laboratory showed elevated levels of fucose in diabetic animals[22]. Our results suggest that the increased fucosylated proteins in diabetic rats could be due to increase in the synthesis and/or decrease in degradation of these proteins. 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. Sialic acid is an important constituent for the characteristic changes of transformed cells; the liver is the major site involved in the synthesis of sialic acid and other glycoproteins[9].

The synthesized glycoproteins are made to circulate in blood. There is a pronounced increase in serum rather than in other organs. The decrease in the content of sialic acid in tissues may be due to the utilization for the synthesis of fibronectin, which contains sialic acid residues in the core structure[23].

Agents with antioxidant or free radical scavenging property may inhibit oxidative reactions associated with glycation. In this context, previous studies have shown that decrease in hyperglycemia could lead to a decrease in glycoprotein levels[24]. Administration of Helicteres isora plant extract to diabetic rats resulted in a significant reversal of all these changes to near normal.

CONCLUSION

In conclusion, oral administration of Helicteres isora plant extract exhibits a protective effect on the carbohydrate moieties of glycoproteins in STZ induced diabetic rats.

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REFERENCES


