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ORIGINAL ARTICLE / RESEARCH

Influence of *Helicteres isora* Bark Extracts on Plasma and Tissue Glycoprotein Components in Streptozotocin Diabetic Rats

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ABSTRACT

Background: The present study investigated the effect of aqueous bark extracts of Helicteres isora on dearrangement in glycoprotein levels in the streptozotocin-induced diabetic rats.

Materials and Methods: The bark extracts of H. isora (100, 200 mg/kg) was administered orally for 30 days to normal and diabetic rats. The effect of bark extracts on glucose, insulin, and plasma and tissue glycoproteins were studied. The effect of bark extract was compared with tolbutamide, a reference drug.

Result: The levels of glucose, glycosylated haemoglobin and plasma glycoproteins containing hexose, hexosamine and fucose were increased significantly, whereas the level of plasma insulin and haemoglobin were decreased significantly in diabetic 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 streptozotocin-diabetic rats. Administration of H. isora (100, 200 mg/kg) to diabetic rats was followed by a decreased level of glucose, glyosylated haemoglobin and plasma glycoproteins. The levels of plasma insulin, haemoglobin and tissue sialic acid were increased, whereas the levels of tissue hexose, hexosamine and fucose were near normal.

Conclusion: The present study indicates that the bark extract of H. isora possesses a significantly beneficial effect on the glycoprotein moiety in addition to its anti-diabetic effect.

Key words: Helicteres isora, streptozotocin, glycoprotein components, anti-diabetic effect

Introduction

Type 2 diabetes mellitus typically involves an abnormal β-cell function that results in relative insulin deficiency, insulin resistance accompanied by decreased glucose transport into muscle and fat cells, and increased hepatic glucose output. All of these contribute to hyperglycaemia, resulting in the impairment of the metabolism of glucose, lipids, proteins and glycoproteins [1]. The level of different types of serum glycoproteins are maintained within a narrow range in health [2] but are elevated in many pathological conditions, cardiovascular disease [3] and diabetes mellitus [4]. Defects in insulin secretion and insulin action are universally present in type 1 diabetes, and also in type 2 diabetes, in both human and animal models.

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Glycoproteins are carbohydrate-linked protein macromolecules found in the cell surface, which is the principal component of animal cells. Abnormal levels of glycoproteins are important in the pathogenesis of liver and kidney diseases in diabetes. Glycoproteins are rich in extracellular matrix, and they contribute a major source to the structure of the matrix [5]. It is well documented that the oligosaccharide moieties of glycoproteins, hexose, hexosamine, fucose and sialic acid, have an important role in protein stability, function and turnover [6]. In the diabetic state, glucose is utilised by the insulin-independent pathways leading to the synthesis of glycoproteins, and even a mild deficiency of insulin influences the thickening of the basement membrane [7]. The raised levels of glycoproteins in diabetics may also be a predictor of angiopathic complications [7]. The therapy of non-insulin-dependent diabetes mellitus presently relies upon compounds from a number of chemical classes: sulfonylureas, non-sulfonylureas, biguanides, etc. A wide variety of structurally distinct molecules stimulate insulin secretion from pancreatic β-cells by different mechanisms of action.

The bark of *Helicteres isora* Linn. (Sterculiaceae) has been used in the indigenous systems of medicine in India for the treatment of diabetes mellitus since time immemorial. The plant is a shrub or small tree available in forests throughout the Central and Western India. The roots and the bark are expectorant, demulcent and are useful in colic, scabies, gastropathy, diabetes, diarrhoea and dysentery [8]. The fruits are astringent, refrigerant, stomachic, vermifugal, vulnerary and useful in griping of bowels, flatulence of children [9] and antispasmodic effect [10]. The aqueous extract of the bark showed significant hypoglycaemic [11], lowering effect of hepatic enzymes [12] and antiperoxidative effect [13].

In non-insulin-dependent or type 2 diabetes mellitus, oral hypoglycaemic agents are used to stimulate the pancreatic β-cells to secrete insulin and/or increase the sensitivity of peripheral insulin receptors to the action of endogenous insulin [14],[15],[16]. The last few years have witnessed the introduction of a number of new oral agents for the treatment of type 2 diabetes, with the hope of achieving better glycaemic control. A clinically used tolbutamide (a sulphonylurea drug) is known to lower the blood glucose level by stimulating β-cells to release insulin [17]. Tolbutamide enhances the sensitivity of both hepatic and peripheral tissues to insulin. The drug also inhibits gluconeogenesis in the liver.

To our knowledge, no other biochemical investigations had been carried out on the effect of bark extracts of *H. isora* in streptozotocin-diabetic rats on glycoproteins status, so the present investigation was carried out to study the effect of bark extracts of *H. isora* on plasma and tissue glycoproteins in streptozotocin-induced diabetic rats.

Materials and Methods

Chemicals

All the drugs and biochemicals used in this experiment were purchased from Sigma Chemical Company Inc., St Louis, MO, USA. The chemicals were of analytical grade.

Collection and processing of plant material

The bark of *H. isora* L. was collected from Solakkadu, Kollimalai, Namakkal District, Tamil Nadu, India, and authenticated by Fr. K.M. Matthew, Director, Rapinat Herbarium, St. Joseph's College, Tiruchirapalli. Voucher Herbarium specimens have been deposited in the (collection number 23644, 27406) Herbarium for future references.

The dried bark of *H. isora* L. was ground into fine powder with auto-mix blender. Then the fine powder was suspended in equal amount of water and stirred intermittently and left overnight. The macerated pulp was then filtered through a coarse sieve and the filtrate was dried at reduced temperature. This dry mass (yield 185 g/kg of powdered bark) served as aqueous extract of *H isora* L. for experimentation.

Animals

Male Wistar albino rats (weighing 160–200 g) were procured from the Animal House, Bharathidasan University, Tiruchirapalli, under standard environmental conditions (12 h light/dark cycles at 25–28°C, 60–80% relative humidity). They were fed with a standard diet (Hindustan Lever, India) and water ad libitum and allowed to acclimatise for 14 days before the procedure. All studies were conducted in

accordance with the National Institute of Health guide [18].

Experimental induction of type 2 diabetes in rats

Rats were made diabetic by single intraperitoneal administration of streptozotocin (60 mg/kg) dissolved in 0.1 M citrate buffer, pH 4.5 [19]. Forty-eight hours later, blood samples were collected and glucose levels were determined to confirm the development of diabetes. Only those animals that showed hyperglycaemia (blood glucose levels >240 mg/dl) were used in the experiment [20],[21].

Experimental procedure

In the experiment, a total of 42 rats (36 surviving diabetic rats and six control rats) were used. The rats were divided into seven groups of six rats each.

Group I were control rats (vehicle treated). Group II and III were normal rats administered orally with bark extracts 100, 200 mg/kg bw for 30 days. Group IV were diabetic control rats, and Group V and VI were diabetic rats administered orally with bark extracts 100, 200 mg/kg bw for 30 days. Group VII were diabetic rats given orally with tolbutamide 250 mg/kg bw for 30 days. At the end of the experimental period, the rats were deprived of food overnight and blood was collected in a tube containing potassium oxalate and sodium fluoride for the estimation of plasma glucose, haemoglobin, and glycosylated haemoglobin. Plasma was separated for the assay of insulin. Liver and kidney were dissected out, washed in ice-cold saline, patted dry and weighed.

Analytical methods

Determination of plasma glucose and insulin

Plasma glucose was estimated colorimetrically using commercial diagnostic kits (Sigma Diagnostics Pvt Ltd., Baroda, India) [22]. Plasma insulin was assayed using an enzyme-linked immunosorbent assay (ELISA) kit (Roche Diagnostics, Germany).

Determination of haemoglobin and glycosylated haemoglobin levels

The level of haemoglobin was estimated by using the cyanmethaemoglobin method described by Drabkin and Austin [23]. The glycosylated haemoglobin level was estimated according to the method of Sudhakar Nayak and Pattabiraman [24] with modifications according to Bannon [25].

Determination of glycoproteins levels

For the estimation of glycoproteins, the tissues were defatted by the method of Folch et al. [26] and the defatted tissues were treated with 0.1 N H_2SO_4 and hydrolysed at 80°C, and aliquot was used for sialic acid estimation. To the remaining solution, 0.1 N NaOH was added. The aliquots were used for fucose, hexose and hexosamine estimation. Hexose was estimated by the method described by Niebes [27]. The reaction mixture contained 0.5 ml of aliquot/plasma, 0.5 ml of 5% phenol and 2.5 ml of conc. H_2SO_4 and was boiled for 20 min, and absorbance was read at 490 nm. Hexosamine was estimated by the method of Elson and Morgan [28], with slight modifications by Niebes [27]. Briefly, the reaction mixture contained 0.5 ml plasma/1.0 ml aliquot and 2.5 ml of 3 N HCl. It was boiled over 6 hours and neutralised with 6 N NaOH. To 0.8 ml of the neutralised sample was added 0.6 ml of acetyl acetone reagent, and it was boiled for 30 minutes. The mixture was treated with 2.0 ml of Ehrlich's reagent. The colour developed was read at 540 nm colorimetrically. Sialic acid and fucose were determined by the method of Warren [29] and Dische and Shettles [30], respectively. Briefly, 0.5 ml of aliquot/plasma was treated with 0.5 ml of water and 0.25 ml of periodic acid and was incubated at 37°C for 30 minutes; 0.2 ml of sodium meta-arsenate and 2.0 ml of thiobarbituric acid were added to the reaction mixture, which was heated for 6 minutes; 5.0 ml of acidified butanol was then added. The absorbance was read at 540 nm. For fucose estimation, 0.5 ml of aliquot/ plasma were treated with 4.5 ml of H_2SO_4 and boiled for 3 minutes; 0.1 ml of cysteine hydrochloride reagent was then added. After 75 minutes in the dark, the absorbance was read at 393 and 430 nm.

Statistical analysis

The data for various biochemical parameters were analysed using analysis of variance (ANOVA) and the group means were compared by Duncan's multiple range test (DMRT). Values were considered statistically significant when $P < 0.05$ [31].

Results

Plasma glucose and insulin levels

[Table/Fig 1] demonstrates the levels of plasma glucose and insulin in control and experimental animals. In diabetic rats, the level of plasma glucose was significantly increased, whereas the plasma insulin was significantly decreased. The administration of bark extracts (100, 200 mg/kg) significantly reversed the changes in a dose-dependent manner. The bark extract at a dose of 200 mg/kg bw showed a highly significant effect compared to 100 mg/kg bw. Administration of bark extracts were compared with tolbutamide (250 mg/kg bw), a reference drug.

Table/Fig 1: Changes in the levels of plasma glucose, insulin, haemoglobin and glycosylated haemoglobin in control and experimental animals

Groups	Plasma glucose (mg/dl)	Insulin (µU/ml)	Haemoglobin (g/dl)	Glycosylated Hb (mg/g Hb)
Normal control	85.5 ± 4.4^{a}	225 ± 10.9^{a}	11.5 ± 1.5^{a}	4.4 ± 0.5^{a}
Normal + HI (100 mg/kg)	75 ± 3.6^{a}	235 ± 10.3^{b}	12.6 ± 1.4^{a}	3.9 ± 0.9^{a}
Normal + HI (200 mg/kg)	70 ± 4.2^{a}	247 ± 11.5^{b}	13.2 ± 1.7^{a}	3.5 ± 0.4^{a}
Diabetic control	260 ± 10.5^{b}	110 ± 6.4^{c}	7.6 ± 0.8^{b}	13.3 ± 2.5^{b}
Diabetic + HI (100 mg/kg)	135 ± 9.3^{d}	185 ± 7.5^{d}	12.5 ± 1.5^{c}	7.9 ± 2.2^{c}
Diabetic + HI (200 mg/kg)	120 ± 8.8^{e}	210 ± 9.5^{e}	11.4 ± 1.9^{d}	6.9 ± 1.0^{d}
Diabetic + tolbutamide (250 mg/kg)	115 ± 7.7^{e}	200 ± 8.9^{e}	10.9 ± 1.4^{d}	7.3 ± 1.5^{d}

Values are given as mean ± ▫SD for six rats in each group. Values not sharing a common superscript letter differ significantly at $p < 0.05$ (DMRT).

Haemoglobin and glycosylated haemoglobin levels

[Table/Fig 2] shows the levels of haemoglobin and glycosylated haemoglobin in the blood of control and experimental rats. The diabetic rats showed a significant decrease in the level of total haemoglobin and a significant increase in the level of glycosylated haemoglobin. The administration of bark extracts (100, 200 mg/kg bw) and tolbutamide (250 mg/kg bw) to diabetic rats reversed the changes in total haemoglobin and glycosylated haemoglobin.

Effect of bark extracts on plasma and tissue glycoproteins

[Table/Fig 3] shows the changes in the level of plasma glycoproteins of control and experimental rats. There was a significant increase of plasma glycoproteins in diabetic rats. Administration of bark extracts and tolbutamide significantly decreased the level of plasma glycoproteins. The levels of liver and kidney glycoprotein of control and experimental rats are shown in [Table/Fig 4]. The levels of glycoproteins containing hexose, hexosamine and fucose were significantly increased, whereas the level of sialic acid was significantly decreased in diabetic rats. Administration of *H. isora* bark extracts (100, 200 mg/kg bw) and tolbutamide (250 mg/kg bw) significantly reversed these changes in the glycoproteins levels in the liver and kidney of diabetic rats. The effect of bark extracts were compared with tolbutamide.

Discussion

Diabetes mellitus is a heterogeneous endocrine disorder in which hyperglycaemia is the unifying feature, and, as knowledge of the heterogeneity of this disorder increases, more appropriate therapies are required [32]. The esters of selected carboxylic metabolites, which are mediating the Krebs cycle, or their precursors such as pyruvic acid, succinic acid and glutamic acid, are currently under

investigation as potent insulinotropic tools in the treatment of non-insulin-dependent diabetes [33]

Table/Fig 2: Changes in the levels of blood haemoglobin and glycosylated haemoglobin in control and experimental animals

Groups	Haemoglobin (g/dl)	Glycosylated Hb (mg/g haemoglobin)
Normal control	11.8 ± 2.2[a]	4.2 ± 0.8[a]
Normal + HI (100 mg/kg)	12.8 ± 2.7[a]	4.0 ± 1.1[a]
Normal + HI (200 mg/kg)	13.6 ± 2.7[a]	3.8 ± 0.6[a]
Diabetic control	8.2 ± 1.7[b]	13.5 ± 2.7[b]
Diabetic+ HI (100 mg/kg)	11.4 ± 1.6[c]	5.8 ± 1.6[d]
Diabetic + HI (200 mg/kg)	12.4 ± 2.3[d]	6.4 ± 1.9[d]
Diabetic + tolbutamide (250 mg/kg)	12.2 ± 2.4[d]	6.2 ± 1.5[d]

Values are given as mean ± SD for six rats in each group. Values not sharing a common superscript letter differ significantly at $p < 0.05$ (DMRT).

In the present investigation, treatment with bark extracts of *H. isora* showed significant antihyperglycaemic activity. The administration of bark extracts and tolbutamide to decrease the increased blood glucose concentration to normal glycaemic concentration is an essential trigger for the liver to revert to its normal homeostasis during experimental diabetes. It is well documented that bark extracts trigger a proinsulin synthesis and insulin release, similar to glucose-induced insulin synthesis and release [34].

Hyperglycaemia is the clinical hallmark of poorly controlled diabetes, which is known to cause protein glycation, also known as non-enzymatic glycosylation [35]. It has been reported that various proteins, including haemoglobin, albumin, collagen, and low-density lipoprotein, a crystalline of lens and fibronectin, undergo non-enzymatic glycation in diabetes [36],[37]. In long-term diabetes, the glycosylated form of Hb has an altered affinity for oxygen, and this may be a factor in tissue anoxia [38],[39]. Glycosylated haemoglobin is found to be significantly increased in diabetic animals, and the amount of this increase is directly proportional to the fasting blood glucose level [40],[41]. The level of total haemoglobin is found to be decreased in the diabetic group, and this may be due to the increased formation of glycosylated haemoglobin. This was well correlated with earlier studies, which reported that there was a decrease in the level of haemoglobin in experimental diabetic rats [42]. The increase in the level of haemoglobin in animals given bark extract may be due to the decreased level of blood glucose.

Glycation is a non-enzymatic reaction of glucose and other saccharide derivatives with proteins, nucleotides and lipids [43]. Non-enzymatic glycation (Maillard reaction) is a complex series of reactions between reducing sugars and amino groups of proteins, which leads to browning, fluorescence and cross-linking of the proteins. The reaction is initiated by the reversible formation of a Schiff base, which undergoes a 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 end-products). Formation of some AGEs combines both the glycation and the oxidative steps in a process termed glycoxidation [44]. Glycation occurs inside and outside cells. Glycation of cellular proteins produces changes in structure and loss of enzymatic activity. These effects are countered by protein degradation and renewal.

Table/Fig 3 Changes in the levels of plasma glycoproteins in control and experimental animals

Groups	Hexose (mg/dl)	Hexosamine (mg/dl)	Sialic acid (mg/dl)	Fucose (mg/dl)
Normal control	92.7 ± 7.8	78.9 ± 6.9[a]	57.8 ± 5.5[a]	28.7 ± 2.7
Normal + HI (100 mg/kg)	91.9 ± 7.5[a]	78.4 ± 7.9[a]	56.5 ± 5.1[a]	24.2 ± 3.6
Normal + HI (200 mg/kg)	92.5 ± 6.8[a]	77.5 ± 6.3[a]	55.6 ± 6.8[a]	22.3 ± 3.3
Diabetic control	120.2 ± 8.9[b]	95.5 ± 7.3[b]	72.3 ± 6.4[b]	40.2 ± 4.7[c]
Diabetic + HI (100 mg/kg)	115.5 ± 7.9[c]	87.6 ± 6.6[c]	65.2 ± 5.9[c]	35.3 ± 3.3[d]
Diabetic + HI (200 mg/kg)	100.9 ± 7.6[c]	80.7 ± 6.9[c]	60.2 ± 6.8[c]	30.3 ± 7.6[d]
Diabetic + tolbutamide (250 mg/kg)	110.5 ± 6.6[c]	85.8 ± 7.8[c]	60.7 ± 6.9[c]	30.7 ± 6.2[d]

Values are given as mean ±SD for six rats in each group. Values not sharing a common superscript letter differ significantly at $p < 0.05$ (DMRT).

Table/Fig 4: Changes in the levels of tissue glycoproteins component in control and experimental animals.

Groups	Hexose (mg/g defatted tissue)		Hexosamine (mg/g defatted tissue)		Sialic acid (mg/g defatted tissue)		Fucose (mg/g defatted tissue)	
	Liver	Kidney	Liver	Kidney	Liver	Kidney	Liver	Kidney
Normal control	28.2 ± 1.9[a]	22.3 ± 1.7[a]	10.2 ± 2.0[a]	14.7 ± 2.6[a]	8.7 ± 2.1[a]	7.9 ± 1.6[a]	15.2 ± 3.4[a]	13.5 ± 2.3[a]
Normal + HI (100 mg/kg)	27.1 ± 1.7[a]	21.3 ± 1.1[a]	9.2 ± 0.9[b]	12.8 ± 2.7[b]	9.1 ± 1.9[b]	8.3 ± 1.3[b]	14.5 ± 2.6[a]	13.2 ± 1.9[a]
Normal + HI (200 mg/kg)	26 ± 1.5[a]	20.7 ± 1.9[a]	8.5 ± 1.2[b]	11.5 ± 1.3[b]	9.5 ± 1.1[b]	8.7 ± 0.9[b]	14.5 ± 1.4[a]	12.5 ± 1.5[a]
Diabetic control	44.3 ± 2.2[b]	40.2 ± 2.3[b]	20.2 ± 1.9[c]	27.5 ± 1.7[c]	4.3 ± 0.9[c]	3.7 ± 0.4[c]	26.8 ± 3.9[b]	25 ± 2.6[b]
Diabetic + HI (100 mg/kg)	37.2 ± 2.9[c]	34.3 ± 2.2[c]	15.7 ± 1.4[d]	24.5 ± 2.2[d]	5.9 ± 1.5[d]	4.8 ± 0.9[d]	19.7 ± 1.7[c]	20.4 ± 1.3[c]
Diabetic + HI (200 mg/kg)	32.3 ± 2.3[c]	28.4 ± 1.7[c]	12.5 ± 1.1[d]	20.5 ± 1.4[d]	6.8 ± 0.9[d]	5.8 ± 0.2[d]	17.4 ± 1.3[c]	16.5 ± 1.4[c]
Diabetic + tolbutamide (250 mg/kg)	35.8 ± 2.9[d]	30.3 ± 1.9[d]	14.4 ± 1.3[e]	22.2 ± 1.2[e]	7.2 ± 1.3[d]	4.4 ± 1.5[e]	18.6 ± 1.9[d]	19.3 ± 1.7[d]

Values are given as mean ± SD for six rats in each group. Values not sharing a common superscript letter differ significantly at $p < 0.05$ (DMRT).

Glycation of the extracellular matrix 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 [45]. When the concentration of AGEs increased above a critical level, cell surface AGE receptors become activated. This is associated with increased expression of extracellular matrix proteins, vascular adhesion molecules, cytokines and growth factor. Depending on the cell type and concurrent signalling, this can be associated with chemotaxis, angiogenesis, oxidative stress, and cell proliferation or apoptosis [45]. These processes are thought to contribute to disease

mechanisms associated with the development of diabetic complications [46].
The biochemical markers hexose, hexosamine, sialic acid and fucose have been measured in the liver and serum because liver is responsible for the synthesis of all major proteins, which are then secreted into the blood. The present experiment attempts the protective role of bark extracts on glycoprotein levels in control and experimental rats. Glycoproteins are observed in both naturally occurring and experimental diabetes [47],[48]. Berenson and Radhakrishnamurthy Dalferes [49] reported that streptozotocin diabetic rats exhibited a significant modification in the connective tissue macromolecule. The requirement of insulin for the biosynthesis of the carbohydrate moiety of mucoproteins from glucose is thus evident. Decreased incorporation of the carbohydrate moieties in diabetic rats may be due to insulin deficiency. The increases in plasma glycoprotein components have been reported to be associated with the severity and duration of diabetes.

Glycoproteins found in a variety of tissues including the arterial wall are very similar in structure and composition to those in circulation [50]. Therefore, vascular complications that involve complex protein carbohydrate molecules could contribute to an increase in plasma glycoproteins. The biosynthesis of the carbohydrate moieties of glycoprotein forms the insulin-independent pathways for the utilisation of glucose-6-phosphate. But the deficiency of insulin during diabetes produces a dearrangement of glycoprotein metabolism, resulting in the thickening of basal membranes. The increased availability of glucose in the hyperglycaemic state accelerates the synthesis of basement membrane components, i.e., glycoproteins [51].

This is due to the depressed utilisation of glucose by insulin-dependent pathways, thereby enhancing the formation of hexose, hexosamine and fucose for the accumulation of glycoproteins [52]. An earlier study has shown that in normal circulation, fucose concentration increase as much as eight-fold in diabetes [53]. In diabetes, three serum proteins (haptoglobin, α-1 acid glycoprotein and α-1-antitrypsin) synthesised in the liver are mainly responsible for the increase in bound fucose levels [6],[54]. The metabolism and synthesis of these proteins may be altered in diabetes leading to changes in serum fucose content. Yorek et al. [55] found that fucose was significantly increased in serum from a more severely diabetic set of rats, compared with the more moderately diabetic rats.

Recent reports have also indicated that hepatic and serum fucosidase activities are increased in streptozotocin-induced diabetic rats. Our results suggest that the increased fucosylated proteins in diabetic rats could be due to an increase in the synthesis and/or decrease in degradation of these proteins. Sialic acid is an acylated derivative of neuraminic acid and exists as a terminal component of the non-reducing end of carbohydrate chains of glycoprotein in mammals. Their implications in a variety of surface-related vital cell function in numerous tissues are well documented [56]. The sialic acid moiety of carbohydrate epitope is important for biological interactions, including cell adhesion to selectin and lectins [57]. Thus, 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. The synthesised glycoproteins are made to circulate in blood [56]. Hence, 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 utilisation for the synthesis of fibronectin, which contains sialic acid residues in the core structure [58]. The synthesis of fibronectin was also reported to increase significantly in various tissues of diabetic patients and animals. The bark extracts of *H. isora* and tolbutamide treatment of diabetic rats resulted in a significant reversal of all these changes to near normal.

Conflict of Interest

None.

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