Available online on 30.08.2019 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

Open Access Research Article

Beneficial effects of valencene on altered glycoprotein components in streptozotocin-nicotinamide induced diabetic rats

Ellappan Paari, Leelavinothan Pari*, Thangasamy Gunaseelan, Duraisamy Kannan

Department of Biochemistry & Biotechnology, Annamalai University, Annamalainagar-608002, Tamil Nadu, India.

ABSTRACT

Objective: To investigate the effect of valencene on dearrangement in glycoprotein levels in the streptozotocin(STZ)-nicotinamide(NA)induced diabetic rats. **Materials and Methods:** Diabetes was induced in experimental rats by a single intraperitoneal (i.p) injection of STZ (45 mg/kg b.w) dissolved in 0.1 M citrate buffer (pH 4.5) 15 minutes after the i.p injection of NA (110 mg/kg b.w). The levels of glycoproteins were altered in experimental diabetes mellitus. Valencene were administered to diabetic rats intragastrically at 100 & 200mg/kg bw for 30days. The effects of valencene on plasma glucose, insulin, plasma and tissue glycoproteins were studied. **Results:** Oral administration of valencene (200mg/kg b.w)for 30d, dose dependently improved the glycemic status in STZ-NA induced diabetic rats. The levels of plasma glucose were decreased with significant increase of plasma insulin level. The altered levels of plasma and tissue glycoprotein components were restored to near normal. **Conclusions:** The results of the present study show the potent beneficial effects of valencene in modifying the levels of glycoprotein components in plasma and tissues of diabetic rats.

Keywords: Diabetes, glycoprotein components, streptozotocin(STZ), nicotinamide(NA) valencene.

Article Info: Received 16 July 2019; Review Completed 22 August 2019; Accepted 25 August 2019; Available online 30 Aug 2019

Cite this article as:

Paari E, Pari L, Gunaseelan T, Kannan D, Beneficial effects of valencene on altered glycoprotein components in streptozotocin-nicotinamide induced diabetic rats, Journal of Drug Delivery and Therapeutics. 2019; 9(4-A):191-196 http://dx.doi.org/10.22270/jddt.v9i4-A.3393

***Address for Correspondence:**

Dr. Leelavinothan Pari, Professor, Faculty of science, Department of Biochemistry & Biotechnology, Annamalai University, Annamalainagar-608002, Tamil Nadu, India.

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder that causes many serious health problems and impaired quality of life for millions of people, worldwide. Globally, the prevalence of DM is on the increase. The international Diabetes Federation (IDF) estimates that on a global scale, the prevalence of diabetes will increase from 425 million people in 2017, to 629 million people by 2045[1]. DM is characterized by increased blood glucose level as a result of disrupted insulin-signalling, which leads to insulin deficiency resulting from autoimmune destruction of insulin-producing b-cells in the case of type-1DM, or insulin resistance and declining β-cell function in type-2 DM. The type-2 DM accounts for more than 90–95% of all cases of DM globally. It has been reported that prolonged exposure to uncontrolled chronic hyperglycemia in DM can lead to the impairment of the metabolism of glucose, lipids, proteins and glycoprotein components [2].

Glycoprotein is a conjugated protein covalently linked to one or more carbohydrate groups. They participate in various biological events such as cell–cell communication, protein stability, function, turnover, membrane transport, cell differentiation and recognition[3]. Thay are found on the surface of all cells and some are released into the bloodstream and body fluids, making blood and plasma more viscous. It is well-documented that the carbohydrate moieties of glycoprotein components, such as hexose, hexosamine, sialic acid and fucose are hydrophilic, make glycoproteins far more hydrophilic and allows the protein to fold into proper geometry and ensure stability[4]. The carbohydrate structure of glycoprotein components is altered in many pathological conditions, including DM [5]. Impaired metabolism of glycoprotein components may play a vital role in the pathogenesis of hepatic and renal diseases in DM. However, insulin deficiency during DM produces derangement of glycoprotein components metabolism, resulting in the thickening of the basal membrane of pancreatic cells. Further, an increase in the levels of glycoprotein components in diabetics indicates angiopathic complications [6].

Several modern drugs are effective in preventing DM, but their prolonged use may lead to adverse effects. Hence, considerable attention has been shifted to the use of dietary

constituents and natural products as an alternative or complimentary treatment for diabetic medication to reduce the adverse effects caused by the synthetic drug. In recent years, terpenoids are extensively studied upon various diseases including diabetes. It has been documented to posses pharmacological effects against various diseases. valencene((3R,4aS,5R)-4a,5-Dimethyl-3-(prop-1-en-2-yl) 1,2,3,4,4a,5,6,7octahydronaphthalene) (Figure.1), is a sesquiterpene, is found in the essential oils of citrus fruits, grape, and citrus derivatives, etc [7]. It possesses pharmacological activities such as antimicrobial [8], anti-oxidant [9], anticancer [10] and anti-inflammatory properties [11].

To date, no such information exists to explain the effect of valencene on the components of glycoprotein in diabetic rats. Therefore, the present study was designed to assess the role of valencene on the levels of plasma and tissue glycoprotein components in streptozotocin (STZ)-nicotinamide (NA)-induced diabetic rats. Recently, we reported that valencene at 200 mg/kg b.w. exhibited significant antidiabetic effect (Paari et al 2019),Therefore, the same dose was fixed in the present study.

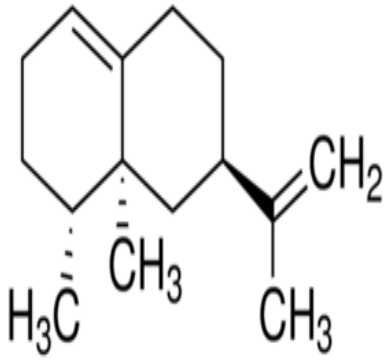

Figure.1. Chemical structure of Valencene ((3R,4aS,5R)-4a,5-Dimethyl-3-(prop-1-en-2-yl) 1,2,3,4,4a,5,6,7-octahydronaphthalene)

2. MATERIAL AND METHODS

2.1. Animals

Adult male albino Wistar rats (180–200 g) were obtained from the Central Animal House, Department of Experimental Medicine, Rajah Muthiah Medical College and Hospital, Annamalai University and maintained at a constant temperature (25±1°C) on a 12 h light/12 h dark cycle with feeds (National Institute for Nutrition, Hyderabad, India) and water was provided *ad libitum*. All animals used in this study were cared for according to the Care and Use of Laboratory Animals Guidelines by Ministry of Social Justices and Empowerment, Government of India. The experimental protocol was approved by the animal ethics committee of Rajah Muthiah Medical College and Hospital (Reg. No. 160/1999/CPCSEA, Proposal number:1176, 2017), Annamalai University, Annamalainagar.

2.2. Induction of diabetes

Diabetes was induced in overnight-fasted experimental animals by a single intraperitoneal (i.p) injection of STZ (45 mg/kg b.w.), dissolved in citrate buffer (0.1M, pH 4.5), 15 min after the i.p administration of NA (110 mg/kg b.w.) [12] Hyperglycemia was confirmed by measuring plasma glucose levels 72 hours after STZ injection. Animals with plasma glucose levels greater than 250 mg/dL were used in the present study.

A total of 36 rats (24 diabetic rats and 12 normal rats) were used and they were divided into six groups with six rats in each group as follows:

Group I: Normal control (vehicle treated)

Group II:Normal rats received valencene (200 mg/kg b.w) intragastrically suspended in 1 mL corn oil for 30 days

Group III:Diabetic control

Group IV:Diabetic rats received valencene (100 mg/kg b.w) intragastrically suspended in 1 mL corn oil for 30 days

Group V:Diabetic rats received valencene (200 mg/kg b.w) intragastrically suspended in 1 mL corn oil for 30 days

Group VI:Diabetic rats received glibenclamide (600 µg/kg b.w) intragastrically in aqueous solution for 30 days.

At the end of the experimental period, all the animals were fasted overnight, anaesthetized and sacrificed by cervical dislocation. Blood samples were collected in tubes containing potassium oxalate and sodium fluoride (3:1) mixturefor the estimation of fasting plasma glucose. The liver and kidney were dissected out, washed in ice-cold saline and stored at -80°C until used. The liver and kidney were weighed and 10% tissue homogenate was prepared with 0.1 M Tris–HCl buffer, pH 7.4. After centrifugation, the clear supernatant was obtained and used for biochemical assays.

Biochemical estimations

Extraction of glycoprotein components

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 glycoprotein components. This was used for the estimation of hexose, hexosamine, fucose and sialic acid in plasma. For the extraction of glycoprotein components from the tissues (liver or kidney), a known weight of the tissue was homogenized in 7.0 mL of methanol. The contents were filtered and homogenized with 14 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 was decanted. A weighed amount of defatted tissue was suspended in 3.0 mL of 2 N HCl and heated at 90C for 4 h. The sample was cooled and neutralized with 3.0 mL of 2 N NaOH. Samples from this were used for the estimation of hexose, hexosamine, sialic acid and fucose in tissues [13].

Determination of glycoprotein components levels

Hexose was estimated by the method of Niebes (1972)[14]. The reaction mixture contained 0.5 mL of tissue homogenate/plasma, 0.5 mL of 5%phenol and 2.5 mL of conc. H2SO4 and boiled for 20 min, and absorbance was read at 490 nm.

Hexosamine was estimated by the method of Elson and Morgan (1933) [15] with slight modifications by Niebes (1972). Briefly, the reaction mixture contained 0.5 mL plasma/1.0 mL tissue homogenate and 2.5 mL of 3 N HCl. It was boiled for 6 h and neutralized with 6 N NaOH. To 0.8 mL of the neutralized sample,0.6 mL of acetyl acetone reagent was added and boiled for

30 min. The mixture was treated with 2.0 mL of Ehrlich's reagent. The colour developed was read at 540 nm colorimetrically.

Sialic acid was determined by the method of Warren (1959) [16].In brief, 0.5 mL of tissue homogenate/plasma was treated with 0.5 mL of de-ionized water and 0.25 mL of periodic acid and incubated at 37C for 30 min. 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 min. 5.0 mL of acidified butanol was then added and the absorbance was read at 540 nm.

Fucose was estimated by the method of Dische and Shettles (1948) [17] 0.5 mL of tissue homogenate/plasma was treated with 4.5 mL of H_2SO_4 and boiled for 3 min. Cysteine hydrochloride reagent (0.1 mL) was then added. After 75 min in the dark, the absorbance was read at 393 and 430 nm. The glycoprotein levels were expressed as mg/100 g for defatted tissue and mg/dL for plasma

Statistical analysis

The experimental results were expressed as means ± SD and subjected to one-way analysis of variance (ANOVA), using a computer software package (SPSS version 11.5; SPSS Inc., Chicago,IL) and the comparisons of significant differences among the groups were performed using Duncan's post that has multiple range test (DMRT), $p < 0.05$ was considered as significantly different between means.

RESULTS

Effect of valencene on plasma glucose and plasma insulin levels

Table.1 reveals the levels of plasma glucose and insulin in normal control and experimental rats. The level of fasting plasma glucose was significantly ($p<0.05$) increased and the level of plasma insulin was significantly ($p<0.05$) decreased in STZ-NA induced diabetic control rats as compared to normal control rats. Treatment with valencene (200 mg/kg body weight) daily for a period of 30 days to diabetic rats showed a significant ($p<0.05$) decrease in the level of plasma glucose and a significant ($p<0.05$) increase in the level of plasma insulin when compared to STZ-NA induced diabetic control rats. Normal control and valencene treated group did not show any significant changes in plasma glucose and insulin levels.

Table:1. Effect of Valencene on the levels of plasma glucose and insulin in control and experimental rats.

Groups	Plasma glucose (mg/dL)	Plasma insulin (µU/mL)
Normal control	86.72 ± 6.60[a]	15.45 ± 1.17[a]
Normal + Valencene (200 mg/kg b.w.)	83.99 ± 6.43[a]	15.58 ± 1.19[a]
Diabetic control	285.76 ± 21.75[b]	8.41 ± 0.64[b]
Diabetic + Valencene (200 mg/kg b.w.)	138.24 ± 10.58[c]	12.43 ± 0.95[c]
Diabetic + glinbenclamide (600µg/kg b.w.)	112.97± 8.64 [d]	14.62± 1.12[a]

Each value is mean ± SD for six rats in each group. In each group, means with different superscript letter (a–e) differ significantly at $p < 0.05$ (DMRT).

Effect of valencene on plasma glycoprotein components

Figure:2 shows the changes in the levels of plasma hexose, hexosamine, sialic acid, and fucose in normal control and experimental rats. There was a significant ($p<0.05$) increase in the levels of plasma hexose, hexosamine, sialic acid, and fucose in STZ-NA induced diabetic control rats compared to normal control rats. Valencene and glibenclamide treatment to STZ-NA induced diabetic rats revealed a significant ($p<0.05$) reduction of hexose, hexosamine, sialic acid and fucose in the plasma when compared to STZ-NA induced diabetic control rats. Normal control and valencene treated group did not show any significant changes in plasma glycoprotein components.

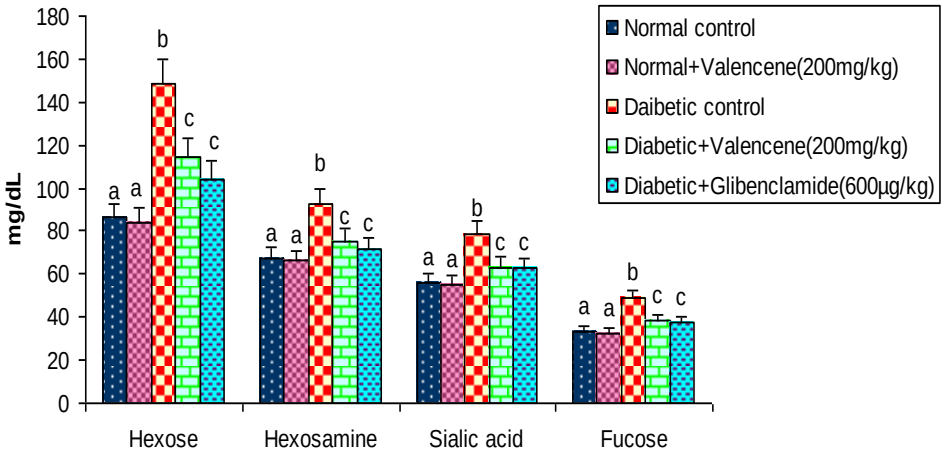

Figure 2: Changes in the levels of plasma glycoprotein components.

Each column is mean ± SD for six rats in each group. Values not sharing a common superscript letter differ significantly at P<0.05 (DMRT).

Effect of valencene on tissue glycoprotein components

The concentrations of hexose, hexosamine and fucose were significantly (p<0.05) increased in the liver and kidney whereas the concentration of sialic acid was significantly (p<0.05) decreased in the liver and kidney of STZ-induced diabetic control rats compared to normal control rats. Valencene and glibenclamide treatment significantly (p<0.05) decreased the concentration of hexose, hexosamine, fucose and significantly (p<0.05) increased the concentration of sialic acid in the liver and kidney of STZ-NA induced diabetic rats when compared to STZ-NA induced diabetic control rats (Figures:3 and Figures: 4). Normal control and valencene treated group did not show any significant changes in tissue glycoprotein components .

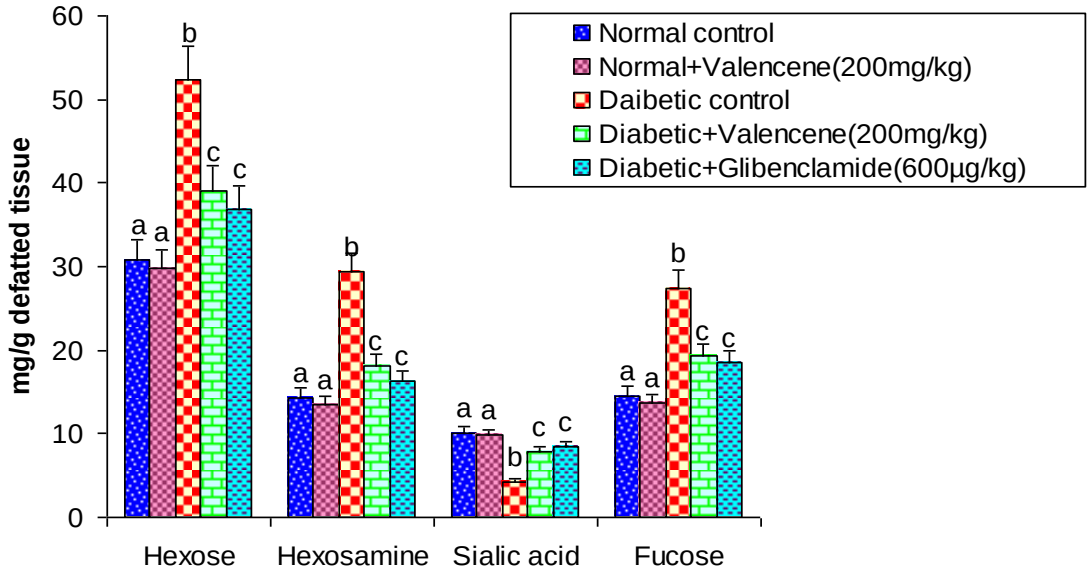

Figure 3: Changes in the levels of liver glycoprotein components.

Each column is mean ± SD for six rats in each group. Values not sharing a common superscript letter differ significantly at P<0.05 (DMRT).

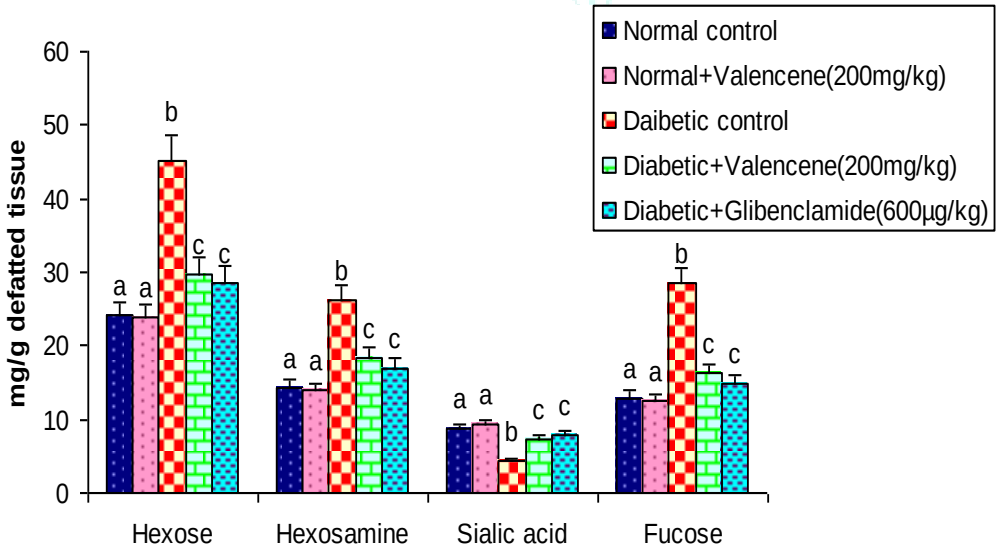

Figure 4: Changes in the levels of kidney glycoprotein components.

Each column is mean ± SD for six rats in each group. Values not sharing a common superscript letter differ significantly at P<0.05 (DMRT).

DISCUSSION

Diabetes is a progressive disease whose biochemical basis for progression and complications are poorly understood. In the diabetic state, glucose is redirected through insulin-independent pathways resulting in enhanced production of carbohydrate moieties of glycoproteins. Therefore, there is an elevation in the levels of protein-bound hexose, hexosamine, fucose, and sialic acid in plasma of diabetic animals.

Glycoproteins are carbohydrate linked protein macromolecules found in the cell surface, which form the principle components of animal cells. They play an imporatant role in membrane transport, cell differentiation and recognition, the adhesion of macromolecules to cell surface and the excretion and absorption of macromolecules [18]. Prolonged elevation of blood glucose in diabetes may result in structural and functional alteration of both circulating and membrane bound proteins [19]. Alterations in the diabetic state of the composition of the carbohydrate components of glycoproteins, especially serum glyloproteins and glycoproteins of the capillary basement membrane have been reported [20].

In diabetic state, abnormalities of glycoprotein metabolism are commonly observed [21]. In ours study, we have noted the elevated levels of hexose, hexosamine, fucose and sialic acid in the plasma and tissues (liver and kidney) of streptozotocin induced diabetic rats. The secretion or shedding from cell membrane glycoconjugates into the circulation leads to the elevation of plasma glycoprotein components. Also, insulin deficiency and high levels of plasma glucose in diabetic condition may result in an increased synthesis of glycoprotein components [22]. Valencene and glibenclamide treatment to STZ-induced diabetic rats significantly decreased plasma glycoprotein components to near normal levels, by virtue of its antihyperglycaemic effects.

Hexosamine a nitrogenous sugar in which an amino group replaces a hydroxyl group. The level of hexosamine increased significantly in the plasma and tissues of diabetic rats, which may be due to insulin deficiency. Further, the concomitant oxidative stress increases the expression of GFAT (Glutamine: Fructose 6-phosphate amino transferase), the rate-limiting enzyme of this pathway leading to higher hexosamine levels [23]. Protein-bound hexose in the cell membrane provides hydrophobic nature. In this study, we observed increased levels of hexose in the plasma and tissues of diabetic rats, which may be due to depressed utilization of glucose by insulin-dependent pathway,thereby enhancing the formation of hexose and hexosamine for the accumulation of glycoproteins [24].Treatment with valencene and glibenclamide significantly lowered hexose and hexosamine levels, which might be due to its antihyperglycaemic effects.

Sialic acid (SA) can be used as a measurement of the acute phase response because many of these glycoproteins have sialic acid as the terminal, non-reducing positions of carbohydrate chains on the outer and inner membrane surfaces [25]. SA is an acetylated derivative of neuraminic acid and is an essential component of glycoproteins and glycolipids. Vascular endothelium carries a high concentration of sialic acid where it governs permeability. It is necessary for the cell surface residency of platelet and promotes endothelial barrier integrity. It also acts as a co-factor of many cell receptors and is positively associated with most of the serum acute phase reactants. In diabetic state, extensive micro vascular damage sheds sialic acid into circulation. Several studies have highlighted that sialic acid metabolism is drastically altered in diabetic condition. Such an elevation of sialic acid level in the plasma leads to complications like retinopathy, nephropathy and neuropathy. A recent study of Prajna et al [26],states that increased SA is a potential risk factor for development of nephropathy in diabetic patients. Similarly raised levels of serum SA is implicated in cardiovascular diseases. However,decreased level of sialic acid is observed in the tissues of diabetic rats which may be related to increased synthesis of fibronectin, which contains sialic acid in its core structure. Further, the decreased tissue sialic acid level is associated with oxidative stress-induced desialylation of glycoproteins [27]. In our study, a significant elevation in plasma sialic acid with a fall in hepatic and renal tissues was observed in diabetic control rats. Oral administration of valencene to diabetic treated rats significantly restored the levels of SA in plasma, hepatic and renal tissues to near normal which is comparable with glibenclamide.

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[28]. Arise in fucose levels could be due to increased glycosylation in the diabetic state. In diabetes, three proteins (haptoglobin, a1acid glycoprotein and a1-antitrypsin) synthesized in the liver are mainly responsible for the increase in fucose levels. The metabolism and synthesis of these proteins may be altered in diabetes leading to changes in plasma in the hyperglycemic state accelerates the synthesis of glycoproteins. Due to increased glycosylation in the diabetic state the fucose levels could be increased. Oral administration of valencene treatment to STZ-NA induced diabetic rats significantly decreased fucose concentration, which may be due to the regulation of the fucosylated protein levels, by its antihyperglycaemic effects.

CONCLUSION

From the above findings, we conclude that valencene ameliorated glycoproteins components in STZ-NA induced diabetic rats. Valencene through its insulinotropic effect on remnant pancreatic β-cells reversed the altered glycoprotein levels in plasma, hepatic and renal tissues of diabetic rats and thus serves as a promising agent in the management of diabetes mellitus.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgments

The authors thank the Indian Council of Medical Research Grant No.3/1/2(17) Endo-Online/2018-NCD-II, New Delhi, India for providing financial support in the form of a Senior Research Fellowship to **Mr Paari. E.**

REFERENCES

1. International Diabetes Federation, IDF Diabetes ATLAS, 8th ed., (2017).
2. Dhawan D, Bandhu HK, Singh B, Ajaib S, Nagpal JP, Effect of D-400 (a herbal formulation) on the regulation of glucose metabolism in diabetic rats. Indian J Pharmacol, 1996;28:224–226.
3. Wiese TJ, Dunlap JA, Yorek MA, Effect of l-fucose and d-glucose concentration on l-fucoprotein metabolism in human Hep G2 cells and changes in fucosyltransferase and alfa l-fucosidase activity in liver of diabetic rats. Biochim Biophys Acta, 1997; 1335:61–72.
4. Peppa M, Stavroulakis P, Raptis SA, Advanced glycoxidation products and impaired diabetic wound healing. Wound Repair Regen, 2009; 17: 461-472.

5. Pari L, Murugan P, Changes in glycoprotein components in streptozotocin-nicotinamide induced type 2 diabetes: influence of tetrahydrocurcumin fromCurcuma longa. Plant Foods Hum Nutr, 2007; 62:25–29.
6. Neerati P, Barla R, Bedada S, Influence of diabetes mellitus on P-glycoprotein function in rat intestine. Pharmacologia,2011; 2(10): 293-298.
7. Beekwilder J, van Houwelingen A, Cankar K, et al, Valencene synthase from the heartwood of Nootka cypress (Callitropsis nootkatensis) for biotechnological production of valencene. *Plant Biotechnol. J* , 2014; 12: 174–182.
8. Ebrahimabadi AH, Ebrahimabadi EH, Djafari-Bidgoli Z, et al. Composition and antioxidant and antimicrobial activity of the essential oil and extracts of Stachys inflata Benth from Iran. *Food Chem*,2010; 119: 452–458.
9. Prakash B, Shukla R, Singh P, et al. Efficacy of chemically characterized Piper betle L. essential oil against fungal and aflatoxin contamination of some edible commodities and its antioxidant activity. Int J Food Microbiol 2010; 142: 114–119.
10. Bartikova H, Hanusova V, Skalova L, et al. Antioxidant, pro-oxidant and other biological activities of sesquiterpenes. Curr Top Med Chem 2014; 14 : 2478- 2494.
11. Abreu RMV, Ferreira CFRI and Queiroz MJRP. QSAR model for predicting radical scavenging activity of di(hetero)arylamines derivatives of benzo[b]thiophenes.European. Journal of Medicinal Chemistry, 2009; 44: 1952–1958.
12. Indumathi D, Sujithra K, Srinivasan S, Vinothkumar V, Betanin exhibits significant potential as an antihyperglycemic and attenuating the glycoprotein components in streptozotocin–nicotinamide-induced experimental rats. Toxicology Mechanisms and Methods, 2018; 28(7), 547–554.
13. Sundaram R, Naresha R, Shanthi P, Sachdanandam P, Antihyperglycemic effect of iridoid glucoside, isolated from the leaves of Vitex negundoin streptozotocin-induced diabetic rats with special reference to glycoprotein components. Phytomedicine, 2012; 19:211–216.
14. Niebes P, Determination of enzymes and degradation products of glycosaminoglycan metabolism in the serum of healthy and varicose subjects. Clin Chim Acta, 1972; 42:399–408.
15. Elson LA, Morgan WTJ, A colorimetric method for the determination of glucosamine and chondrosamine. Biochem J, 1933; 27:1824–1828.
16. Warren L, The thiobarbituric acid assay of sialic acids. J Biol Chem, 1959; 234:1971–1975.
17. Dische Z, Shettles LB, A specific color reaction of methylpentoses and a spectrophotometric micromethed for their determination. J Biol Chem,1948;175:596–603.
18. Parillo F, Arias MP, Supplizi AV. Glycoprofile of the different cell types present in the mucosa of the horse guttural pouches.Tissue Cell, 2009; 41: 257-265.
19. Ciftci G, Yarim GF. Evaluation of IGF-I levels and serum protein profiles of diabetic cats and dogs. J Vet Sci,2011; 12: 325-331.
20. Buse MG. Hexosamines, insulin resistance, and the complications of diabetes: current status. Am J Physiol Endocrinol Metab, 2006; 290:1-8.
21. Anil Kumar P, Haseeb A, Suryanarayana P, Ehtesham NZ, Reddy GB, Elevated expression ofa-A- and a-B-crystallins in streptozotocin-induced diabetic rat. Arch Biochem Biophys, 2005; 444:77–83.
22. Berenson GS, Radhakrishnamurthy Dalferes ER. Connective tissue macromolecular changes in rats with experimentally induced diabetes and hyperinsulinism. Diabetes 1972; 21: 733–743.
23. Mcclain DA, Hazel M, Parker G, Adipocytes with increased hexosamine flux exhibit insulin resistance, increased glucose uptake, and increased synthesis and storage of lipid. Am J Physiol Endocrinol Metab, 2005; 288: 973–979.
24. Rajamani U, Joseph D, Roux S, Essop MF. The hexosamine biosynthetic pathway can mediate myocardial apoptosis in a rat model of diet-induced insulin resistance. Acta Physiol, 2011; 202: 151-157.
25. Pickup JC, Inflammation and activated innate immunity in the pathogenesis of type 2 diabetes. Diabetes Care, 2004; 27:813–823.
26. Prajna K, Kumar AJ, Rai S, Shetty SK, Rai T. Shrinidhi, Begum M, shashikala. Predictive value of serum sialic acid in type-2 diabetes mellitus and its complication (Nephropathy). J Clin Diag Res,2013;7:2435-2437.
27. Goswami K, Koner BC, Level of sialic acid residues in platelet proteins in diabetes, aging, and Hodgkins lymphoma: a potential role of free radicals in desialylation. Biochem Biophys Res Commun, 2002;297:502–505.
28. Mondoa EI, Kitei M, The new healing science of glyconutrients In: Sugars that heal. New York: 2001;Ballantine Publishing.