International Journal of Diabetes and Endocrinology
2017; 2(2): 30-35
http://www.sciencepublishinggroup.com/j/ijde
doi: 10.11648/j.ijde.20170202.13

Preventive Effect of Phloretin on Components of Glycoprotein Changes in Streptozotocin Induced Diabetic Rats

Tamilarasan Nithiya, Rajangam Udayakumar

Department of Biochemistry, Government Arts College (Autonomous), Kumbakonam, Tamilnadu, India

Email address:
udayabiochem@yahoo.co.in (R. Udayakumar)

To cite this article:
Tamilarasan Nithiya, Rajangam Udayakumar. Preventive Effect of Phloretin on Components of Glycoprotein Changes in Streptozotocin Induced Diabetic Rats. *International Journal of Diabetes and Endocrinology*. Vol. 2, No. 2, 2017, pp. 30-35.
doi: 10.11648/j.ijde.20170202.13

Received: February 28, 2017; **Accepted**: April 28, 2017; **Published**: July 3, 2017

Abstract: The present study was aimed to evaluate the protective effect of phloretin on glycoprotein components in serum and tissues of streptozotocin induced diabetic rats. Diabetes was induced in male Wistar rats by a single intraperitoneal injection of STZ (60 mg/kg b.w). Phloretin (25mg and 50mg/kg b.w) was administered orally to diabetic rats for 45 days. The effect of phloretin on serum glucose, glycated hemoglobin, serum and tissue glycoprotein components were studied. Phloretin administration to diabetic rats decreased the level of glucose, glycated hemoglobin and glycoprotein components in serum. There was observed a significant decrease in the level of sialic acid and significantly elevated levels of hexose, hexosamine and fucose in liver and kidney of diabetic rats. The altered levels of serum and tissue glycoprotein components were restored to near normal in diabetic rats treated with phloretin at 50mg/kg b.w. The present findings suggest that phloretin can potentially ameliorates the hyperglycemia and changes in glycoprotein components abnormalities in streptozotocin induced diabetic rats. So, the phloretin may be used as an effective therapeutic agent for diabetes mellitus in future.

Keywords: Streptozotocin, Diabetes Mellitus, Phloretin, Glycoprotein

1. Introduction

Diabetes mellitus is a serious, complex metabolic disorder of multiple etiologies, characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion (β-cell dysfunction), insulin action (insulin resistance) or both [1]. Hyperglycemia is affecting the cellular antioxidant defense system and damages the cells. Free radicals are formed disproportionately in diabetes by glucose oxidation, non-enzymatic glycation of proteins, and the subsequent oxidative degradation of glycated proteins, which occurs in various tissues [2]. This process leads to long-term damage, dysfunction and failure of various organs, especially the eyes, kidney, nerves, heart and blood vessels and creates a huge economic burden related to the management of diabetic complications [3].

Glycoproteins are carbohydrate linked protein macromolecules found in the cell surface, which form the principal component of animal cells. Hexose, hexosamine, fucose 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. Impaired metabolism of glycoproteins plays a major role in the pathogenesis of diabetes mellitus [4]. In recent times, many traditionally important medicinal plants and their phytochemicals have been tested for their efficacy against impaired glycoprotein levels in diabetes [5].

Plants constitute an important source of active natural products. They have a remarkable role in the traditional medicine in different countries. Recently, plants and their active components have been widely used in the treatment of diabetes. Plant derived phenolic compounds have a high potential for lowering blood glucose level. Flavonoids are widely distributed group of plant phenolics, which are abundant in foods. Phloretin is a natural flavanoid, present in apple and strawberries. Phloretin has been reported to possess various pharmacological activities like anti-

inflammatory activity by antagonizing prostaglandins [6], protect the skin from UV light induced photodamage and also it possess antioxidant [7], antitumour [8] and hepatoprotective [9] activities. To our knowledge, there is no detailed report on protective role of phloretin on glycoprotein components in diabetic rats. Hence, this study was aimed to investigate the effect of phloretin on serum and tissues glycoprotein components in STZ-induced diabetic rats.

2. Materials and Methods

2.1. Chemicals

Phloretin and streptozotocin were purchased from Sigma Chemicals Company, St. Louis, Mo. USA. All other chemicals and reagents were of analytical grade and purchased from Himedia Laboratories Pvt. Ltd, Mumbai, India.

2.2. Animals

Male Albino rats (*Rattus norvegicus*) weight ranges between 180-200g were used in the present investigation. The animals were housed in standard polypropylene cages and maintained under controlled room temperature and humidity with 12-h light and 12-h dark cycle and were fed standard diet of known composition and water *ad libitum*. The animals were acclimatized to the laboratory condition for 2-week prior to the start of experiment. The study was approved by the institutional Animal Ethical Committee (BDU/IAEC/2015/NE/42/Dt.17.03.2015), Bharathidasan University,Tiruchirappalli, Tamilnadu, India.

2.3. Induction of Diabetes Mellitus

Diabetes was induced in overnight fasted experimental rats by a single intraperitoneal injection of STZ (60mg/kg b.w) dissolved in freshly prepared citrate buffer (0.1 M, pH 4.5). After five days, blood glucose was analysed and determined the rats with fasting blood glucose greater than 250mg/dl were used in the present study.

2.4. Experimental Design

The animals were randomly divided into five groups of six animals in each group (24 diabetic surviving and 6 normal). Group I - normal control; Group II - diabetic control; Group III - diabetic rats treated with phloretin (25 mg/kg b.w.); Group IV - diabetic rats treated with phloretin (50 mg/kg b.w.) and Group V - diabetic rats treated with glibenclamide (600µg/kg b.w.). Phloretin and glibenclamide were given orally to diabetic rats using intragastric tube. No death of the diabetic animals was observed till the end of the study. The initial and final body weight of the rats in each group was recorded. At the end of experimental period, the animals were deprived of food overnight and sacrificed by cervical decapitation. Blood was collected for the estimation of glucose, glycated hemoglobin and glycoprotein components. Liver and kidney tissues were dissected out, washed in ice-cold saline and stored at 4 $^{\circ}$C for the measurement of glycoprotein components like hexose, hexosamine, fucose and sialic acid.

2.5. Estimation of Glucose and Glycated hemoglobin

Serum glucose was estimated using a commercial kit (Sigma Diagnostics Pvt, Ltd., Baroda, India) by the method of Trinder [10]. Glycated hemoglobin was estimated by the method of Nayak and Pattabiraman [11].

2.6. Determination of Glycoprotein Components

For the estimation of glycoprotein components, the liver and kidney tissues were defatted by the method of Folch *et al*. [12]. Protein-bound hexoses were estimated by the method of Niebes [13], hexosamine was estimated by the method of Wagner [14], sialic acid was determined by the method of Warren [15] and fucose was estimated by the method of Dische [16].

2.7. Statistical Analysis

Data presented as means ± SD and subjected to statistical significance were evaluated by one way Analysis of Variance (ANOVA) and the individual comparisons were obtained by Duncan's Multiple Range Test (DMRT). Values are considered statistically significant at $P \leq 0.05$.

3. Results

The blood glucose and glycated hemoglobin (HbA1c) were determined in normal and experimental animals and the results were presented in Table 1. Blood glucose and glycated haemoglobin levels were significantly ($P \leq 0.05$) increased in diabetic rats when compared with normal control rats. Diabetic rats were treated with phloretin at two different doses like 25mg and 50mg/kg b.w and glibenclamide at 600µg/kg b.w observed significantly decreased levels of serum glucose and glycated haemoglobin. Phloretin at a dose of 50mg/kg b.w showed a highly significant effect than 25mg/kg b.w. Based on these data, the effective dose was fixed as 50mg/kg b.w and it was used for further analysis.

Table 2 showed the changes in the levels of hexose, hexosamine, fucose and sialic acid in serum of streptozotozin induced diabetic rats treated with phloretin at 50mg/kg b.w. Significantly($P \leq 0.05$) increased levels of hexose, hexosamine, fucose and sialic acid were observed in serum of diabetic rats when comparison with normal rats. Treatment of diabetic rats with phloretin at 50mg/kg b.w and glibenclamide at 600µg/kg b.w showed significant reduction in the levels of hexose, hexosamine, fucose and sialic acid.

The changes in the levels of hexose, hexosamine, fucose and sialic acid in liver of normal control and experimental rats were observed and presented in Table 3. The levels of hexose, hexosamine and fucose were significantly increased and the level of sialic acid was significantly decreased in liver tissues of diabetic rats. Oral administration of phloretin and glibenclamide to diabetic rats for a period of 45 days

significantly ($P \leq 0.05$) reversed these changes in the levels of glycoprotein components to near normal.

Table 4 showed that the effect of phloretin and glibenclamide on the levels of glycoprotein components in kidney of STZ-induced diabetic rats. Diabetes induced with STZ in rats showed a significantly increased level of hexose, hexosamine and fucose and significantly decreased level of sialic acid in kidney. Diabetic rats treated with phloretin (50mg/kg b.w) and glibenclamide (600µg/kg b.w) showed significantly decreased levels of hexose, hexosamine and fucose and increased level of sialic acid in kidney when compared with diabetic untreated rats.

Table 1. *Effect of phloretin on blood glucose and glycated hemoglobin.*

Groups	Glucose (mg/dl)	Glycated hemoglobin (mg/g Hb)
Group I Normal control	85.23±5.96^{b}	3.24±0.19^{c}
Group II Diabetic control	284±22.68^{a}	6.75±0.32^{a}
Group III Diabetic+Phloretin(25mg/kg b.w)	98±20.06^{b}	3.85±0.21^{b}
Group IV Diabetic+Phloretin(50mg/kg b.w)	90±7.28^{b}	3.20±0.21^{c}
Group V Diabetic+Glibenclamide(600µg/kg b.w)	97±20.77^{b}	3.37±0.19^{c}

Values are expressed as mean ± SD of six rats from each group. Values not sharing a common superscript letter differ significantly at 5% level ($p \leq 0.05$) using Duncan's Multiple Range Test (DMRT).

Table 2. *Effect of phloretin on hexose, hexosamine, fucose and sialic acid in serum.*

Groups	Hexose (mg/dl)	Hexosamine (mg/dl)	Fucose (mg/dl)	Sialic acid (mg/dl)
Group I Normal control	90.23±6.31^{c}	80.24±5.61^{c}	27.62±2.14^{c}	54.62±5.43^{c}
Group II Diabetic control	124.41±8.70^{a}	100.02±6.94^{a}	42.48±3.82^{a}	76.24±6.98^{a}
Group III Diabetic + Phloretin(50mg/kg b.w)	88.62±6.20^{c}	82.47±3.72^{c}	28.82±3.02^{c}	60.42±4.25^{c}
Group IV Diabetic + Glibenclamide(600µg/kg b.w)	102.13±7.19^{b}	91.98±6.53^{b}	30.96±2.48^{c}	63.04±4.46^{c}

Values are expressed as mean ± SD of six rats from each group.
Values not sharing a common superscript letter differ significantly at 5% level ($p \leq 0.05$) using Duncan's Multiple Range Test (DMRT).

Table 3. *Effect of phloretin on hexose, hexosamine, fucose and sialic acid in liver.*

Groups	Hexose (mg/g)	Hexosamine (mg/g)	Fucose (mg/g)	Sialic acid (mg/g)
Group I Normal control	26.78±2.46^{c}	10.04±0.82^{c}	15.18±1.44^{c}	9.88±0.62^{c}
Group II Diabetic control	42.04±3.42^{a}	18.92±1.62^{a}	28.06±2.14^{a}	4.92±0.28^{a}
Group III Diabetic + Phloretin(50mg/kg b.w)	28.06±2.14^{c}	11.54±0.88^{c}	16.76±0.99^{c}	8.12±0.53^{c}
Group IV Diabetic + Glibenclamide(600µg/kg b.w)	32.16±2.28^{c}	12.05±0.82^{c}	16.22±1.13^{c}	8.16±0.57^{c}

Values are expressed as mean ± SD of six rats from each group.
Values not sharing a common superscript letter differ significantly at 5% level ($p \leq 0.05$) using Duncan's Multiple Range Test (DMRT).

Table 4. *Effect of phloretin on hexose, hexosamine, fucose and sialic acid in kidney.*

Groups	Hexose (mg/g)	Hexosamine (mg/g)	Fucose (mg/g)	Sialic acid (mg/g)
Group I Normal control	26.83±1.87^{c}	12.42±0.84^{c}	11.04±0.77^{c}	9.56±0.66^{c}
Group II Diabetic control	43.04±3.42^{a}	27.14±1.24^{a}	30.12±2.10^{a}	3.45±0.24^{a}
Group III Diabetic + Phloretin(50mg/kg b.w)	29.46±2.03^{c}	15.92±1.14^{c}	12.03±0.84^{c}	8.92±0.82^{c}
Group IV Diabetic + Glibenclamide(600µg/kg b.w)	31.15±2.30^{c}	18.79±1.36^{c}	16.44±1.15^{b}	8.14±0.56^{c}

Values are expressed as mean ± SD of six rats from each group.Values not sharing a common superscript letter differ significantly at 5% level ($p \leq 0.05$) using Duncan's Multiple Range Test (DMRT).

4. Discussion

Streptozotocin is the most commonly used agent for the induction of diabetes in experimental animal model. It enters the pancreatic β cells through glucose transporter 2 channels in the plasma membrane which causes cellular toxicity that leads to hypoinsulinemia and hyperglycemia. STZ-induced diabetes mellitus is associated with the generation of reactive oxygen species causing oxidative damage [17].

In this study, the reduction in blood glucose level after the administration of phloretin to diabetic rats may be due to phloretin exert insulin like effect on peripheral tissues by enhancing glucose uptake and inhibiting hepatic gluconeogenesis or inhibiting the absorption of glucose in intestine. These results are in agreement with Ambika *et al*. [18], who reported that administration of p-hydroxy cinnamic acid, a phenolic compound to diabetic rats significantly decreased the glucose level by enhanced release of insulin from β-cells.

Diabetes mellitus is associated with the development of vascular degenerative complications affecting both large and small vessels. Several studies explained the disturbance of carbohydrate, protein and lipid metabolism in diabetes mellitus. Glycation is a non-enzymatic reaction of glucose and other saccharide derivatives with protein, nucleotides and lipids. Glycation of cellular proteins produces changes in

structure and activity of enzymes. The increased level of serum glycoprotein in diabetic rats could be a consequence of abnormal carbohydrate metabolism. Insulin deficiency and increased serum glucose level may result in an increased synthesis of glycoprotein [19]. The increased serum glycoprotein components have been associated with the severity of diabetes. The elevation in the levels of serum glycoprotein components might be due to the secretion from cell membrane glyco conjugates in the circulation [20]. In the present study, we observed that the increased levels of hexose, hexosamine, fucose and sialic acid in serum of STZ induced diabetic rats. Administration of phloretin to STZ induced diabetic rats showed reduced levels of serum glycoprotein components to near normal. Our results are in agreement with Muthukumaran et al. [21], who reported that syringic acid improved glycoprotein levels in diabetic rats.

Prolonged elevation of blood glucose and insulin deficiency in diabetes mellitus as results in the thickening of basal membrane of pancreatic beta cells and also changes in structural and functional alterations in circulating and membrane bound proteins [22]. In the diabetic state, alterations in the composition of the carbohydrate components of glycoproteins, especially serum glycoproteins and glycoproteins of the capillary basement membrane have been reported [23]. The increased level of hexoses in diabetic rats may be linked with disturbances in carbohydrate metabolism. Treatment of diabetic rats with phloretin and glibenclamide showed significantly decreased level of hexose due to improved glycemic control.

Liver plays an important role for producing a large amount of glycoproteins present in the blood. The elevated levels of serum glycoproteins in diabetic condition could be a consequence of abnormal carbohydrate metabolism [24]. Numerous molecular mechanisms are concerned with hyperglycemia induced metabolic disturbances in diabetes. Among these hexosamine biosynthetic pathway represents a minor metabolic route of glucose at fructose 6-phosphate reactions step of glycolysis. This pathway is considered as a sensor of nutrients and an increase in this pathway is regarded as a key factor in the metabolic complications of diabetes [25]. Prolonged hyperglycemia due to insulin deficiency associated with oxidative stress increases the expression of GFAT gene (Glutamine: Fructose 6-phosphate amino transferase), which is the rate-limiting enzyme of this pathway leading to an increase in the levels of hexosamine [26].

Hexosamine functions are as physiologic glucose sensors that serve as an adaptor in redirecting excess calories just before storage as fat [27]. The results of the present study are in harmony with previous study in that the diabetic rats showed elevated level of hexosamine, which could be due to increased expression of GFAT gene and increased serum glucose [28]. In this study, STZ induced diabetic rats had elevated level of hexosamine in serum and tissues when compared with normal control rats. The significant elevation of hexosamine in serum, liver and kidney of diabetic rats was observed and it may be due to insulin deficiency. Diabetic rats treated with phloretin and glibenclamide showed significant decrease in the level of hexosamine in serum and tissues when compared with diabetic untreated rats, which could be due to increased secretion of insulin.

Fucose (6-deoxy-L-galactose) is a group of essential sugars that the body utilized for the functioning of cell to cell communication and its metabolism appears to be altered in various disease conditions such as diabetes mellitus [29]. There are three serum proteins like haptoglobin, α1 acid glycoprotein and α 1-antitrypsin were synthesized in liver of diabetic condition. In hyperglycemic state, the metabolism and synthesis of these three proteins may be altered leading to changes in the serum accelerates the synthesis of glycoproteins. The fucose level could be increased due to increased glycosylation in diabetic rats. In the present study, a rise in fucose levels could be due to increased glycosylation in diabetic condition. Oral administration of phloretin and glibenclamide has restored the level of fucose to near normal which could be due to improved glycemic control. The results of the present study are in agreement with Udaiyar Muruganathan [30], who reported that the elevated levels of fucose were lowered by insulin secretion in carvone treated diabetic rats.

Sialic acid is an acute phase protein synthesized from neuraminicacid, which forms terminal sugar in carbohydrates taking part in glycoprotein structure. Sialic acid plays an important role in biological systems like cell-cell recognition, protein targeting, conformational stabilization, adhesion and intracellular signaling [31]. Hyperglycemia mediated oxidative stress and inflammation in diabetic condition bring about the damages to cellular membrane may contribute to increase in serum sialic acid level. In addition to this, vascular endothelium is rich in sialic acid moieties where it regulates permeability. In our study, a significant increase in the level of sialic acid in serum of diabetic rats was observed when compared to the normal control rats. Insulin deficiency and the resulting hyperglycemia are associated with impairment in endothelium leading to release of sialic acid into circulation [32]. In diabetes, the tissue sialic acid level was decreased significantly. This may be due to utilization of sialic acid for the synthesis of fibronectin which contain sialic acid residue in the structure. Administration of phloretin and glibenclamide to diabetic rats increased the sialic acid content in tissues and decreased the level of sialic acid in serum. Similarly the *trans*-anethole treated diabetic animals showed the same effect in STZ induced diabetic animal model [33].

5. Conclusion

From the above observations, we concluded that the oral administration of phloretin to diabetic rats reversed the changes in the level of glucose and glycated hemoglobin in blood and glycoprotein components in serum, liver and kidney. Phloretin may have beneficial effect by the enhancement of insulin action in diabetic rats as evident by the decreased level of serum glucose in phloretin treated diabetic rats. So, the phloretin may be useful in the

preparation of drug ingredient for the prevention of diabetes mellitus.

References

[1] Kardeşler L, Buduneli N, Bıyıkoglu B, Çetinkalp S, Kutukçuler N. (2008). "Gingival crevicular fluid PGE2, IL-1ß, t-PA, PAI-2 levels in type 2 diabetes and relationship with periodontal disease".Clin. Biochem., vol. 41(10-11): pp. 863-868.

[2] Kuma G, Banu S, Murugesan A.G. (2009). "Influence of *Helicteresisora* administration for diabetes mellitus: Its effect on erythrocyte membrane and antioxidant status". Food ChemToxicol., vol. 47: pp.1803-9.

[3] Schuster D.P., Duvuuri V. (2002). "Diabetes mellitus".Clin Podiatr Med and Surg., vol.19: pp.79-107.

[4] Prakasam A, Sethupathy S, Pugalendi K.V. (2005). "Influence of *Casearia esculenta* root extract on glycoprotein components in streptozotocin diabetic rats".Pharmazie, vol. 60: pp. 229-232.

[5] Pari L, Murugan P. (2007). "Changes in glycoprotein components in streptozotocin - nicotinamide induced type 2 diabetes: influence of tetrahydrocurcumin from *Curcuma longa*". Plant Foods Hum Nutr., vol. 62: pp. 25-29.

[6] Blazso G, Gabor M. (1995). "Effects of prostaglandin antagonist phloretin derivatives on mouse ear edema induced with different skin irritants". Prostaglandins, vol. 50: pp. 161-168.

[7] Oresajo C. (2008). "Protective effects of a topical antioxidant mixture containing vitamin C, ferulic acid and phloretin against ultraviolet induced photodamage in human skin". J Cosmet Dermatol., vol. 7: pp. 290-297.

[8] Chih-Hsiung Wu, Yuan-Soon Ho, Chia-Yi Tsai, Ying-Jan Wang, How Tseng, Po-Li Wei, Chia-Hwa Lee, Ren- Shyan Liu, Shyr-Yi Lin. (2009). "*In vitro* and *in vivo* study of phloretin-induced apoptosis in human liver cancer cells involving inhibition of type II glucose transporter". Int J Cancer, vol. 124: pp. 2210-2219.

[9] Ali Reza EbadollahiNatanzia, ShimaMahmoudian, BagherMinaeie, OmidSabzevari. (2011). "Hepatoprotective activity of phloretin and hydroxychalcones against acetaminophen induced hepatotoxicity in mice". Iran J Pharm Sci., 7(2): 89-97.

[10] Trinder P. (1969). "Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor". Ann Clin Biochem., vol. 6: pp. 24.

[11] Nayak S.S., Pattabiraman T.N. (1981). "A new colorimetric method for the estimation of glycosylated haemoglobin". Clin Chim Acta, 109:267- 274.

[12] Folch J, Lees M, Solane S. G. H. (1957). "A simple method for isolation and purification of total lipids from animal tissues". J Biol Chem., vol.26: pp.497-509.

[13] Niebes P. (1972). "Determination of enzymes and degradation products of glycosaminoglycan metabolism in the serum of healthy and varicose subjects". Clin Chim Acta, vol. 42: pp. 399-408.

[14] Wagner W. D. (1979). "A more sensitive assay discriminating galactosamine and glucosamine in mixtures". Analytical Biochemistry, vol. 94: pp. 394-396.

[15] Warren L. (1959). "The thiobarbituric acid assay of sialic acids". J Biol Chem., vol. 234: pp. 1971-1975.

[16] Dische Z, Shettles L. B. (1948). "A specific color reaction of ethylpentoses and a spectrophotometric micromethod for their determination". J Biol Chem., vol. 175: pp. 595-603.

[17] Szkudelski T. (2001). "The mechanism of alloxan and streptozotocin action in β cells of the rat pancreas". Physiol Res., vol. 50: pp. 536-546.

[18] Ambika S, Saravanan R, Thirumavalavan K. (2013). "Antidiabetic and antihyperlipidemic effect of p-hydroxycinnamic acid on streptozotocin induced diabetic Wistar rats". Biomedicine and Aging Pathology, 3(4): 253-257.

[19] Brownlee M. (2001). "Biochemistry and the molecular cell biology of diabetic complications". Nature, vol. 414:pp. 813-820,

[20] Pari L, Srinivasan S. (2010). "Preventive effect of diosmin, a bioflavonoid, on glycoprotein changes in streptozotocin nicotinamide induced type 2 diabetic rats". In J Pharm Sci Res., vol.10: pp. 89-95.

[21] Muthukumaran J, Srinivasan S, Venkatesan R. S., Ramachandran V, Muruganathan U. (2013)."Syringic acid, a novel natural phenolic acid, normalizes hyperglycemia with special reference to glycoprotein components in experimental diabetic rats". J Acu Dis., pp. 304-309.

[22] Ciftci G, Yarim G. F. (2011). "Evaluation of IGF-I levels and serum protein profiles of diabetic cats and dogs". J Vet Sci., vol. 12(4): pp. 325-331.

[23] Buse M.G. (2006). "Hexosamines, insulin resistance, and the complications of diabetes: current status". Am. J. Physiol Endocrinol Metab., vol. 290: E1-E8.

[24] Saravanan G, Ponmurugan G. P., Senthil Kumar, Rajrajan T. (2010). "Antidiabetic effect of S-allylcysteine: Effect on plasma and tissue glycoproteins in experimental diabetes". Phytomedicine, vol.17: pp. 1086-1089.

[25] Obici J, Wang R, Chowdury Z, Feng U, Siddhanta K, Morgan. (2002). "Identification of a biochemical link between energy intake and energy expenditure". J Clin Invest., vol.109: pp.1599-605.

[26] Brownlee M. (2005). "The pathobiology of diabetic complications: A unifying mechanism". Diabetes, vol. 54: pp. 1615-25.

[27] Wellen K. E., Lu C, Mancuso A, Lemons J.M.,Ryczko M, Dennis J.W.,et al. (2010). "The hexosamine biosynthetic pathway couples growth factor-induced glutamine uptake to glucose metabolism". Genes Dev., vol. 24: pp. 2784-2799.

[28] NarendraSilawat,VipinBihari Gupta. (2013). "Antidiabetic effect of chebulic acid in streptozotocin induced diabetic rats". Asian J Complem Alternat Med., 01 (01): 16-23.

[29] Pari L, Karthikesan K. (2009). "Protective role of tetrahydrocurcumin and chlorogenic acid on glycoprotein changes in streptozotocin nicotinamide- induced diabetic rats". J Pharm Sci Res., vol. 1(4): pp.173-180.

[30] Muruganathan U, Srinivasan S, Indumathi D. (2013). "Antihyperglycemic effect of carvone: Effect on the levels of glycoprotein components in streptozotocin-induced diabetic rats". J Acu Dis., pp.310-315.

[31] Jayachandran M, Srinivasan S et al., (2013). "Syringic acid, a novel natural phenolic acid, normalizes hyperglycemia with special reference to glycoprotein components in experimental diabetic rats". J Acu Dis, 304-309.

[32] Calles-Escandon J, Cipolla M. (2001). "Diabetes and endothelial dysfunction: A clinical perspective". Endocr Rev., 22: 6-52.

[33] Leelavinothan Pari, Bashir Ahmad Sheikh. (2015). "Antihyperglycemic effect of *trans*-anethole in streptozotocin induced diabetic rats with special reference to glycoprotein components". Int. J. Adv. Res. Biol.Sci, vol. 2(5): pp. 28–34.