Available online at www.jpsscientificpublications.com
Volume – 2; Issue - 5; Year – 2016; Page: 737 – 742
DOI: 10.21276.iajmr.2016.2.5.2

Indo – Asian Journal of Multidisciplinary Research (IAJMR)
ISSN: 2454-1370

ANTIHYPERGLYCEMIC EFFECT OF GERANIOL, A MONOTERPENE ON HFD AND LOW DOSE OF STZ- INDUCED TYPE 2 DIABETIC RATS WITH SPECIAL REFERENCE TO GLYCOPROTEIN COMPONENTS

Asaithambi Karthikeyan and Leelavinothan Pari*,
Department of Biochemistry and Biotechnology, Faculty of Science, Annamalai University, Annamalainagar – 608 002, Tamilnadu, India.

Abstract

Objective: To evaluate the antidiabetic effect of geraniol, a monoterpene, on the levels of glycoprotein components in plasma and liver of high fat diet (HFD) and low dose of streptozotocin (STZ) induced type 2 diabetic rats. **Methods:** Diabetes was induced in male albino Wistar rats by HFD in combination with a low dose of STZ (25 mg/kg body weight). Geraniol was administered orally at a dose of (100 mg/kg body weight (b.w)) for 8 weeks. The effect of geraniol was studied on plasma and liver of glycoproteins. **Results:** Oral administration of geraniol (100 mg/kg b.w) for 8 weeks controlled the altered levels of plasma and liver glycoprotein components to near normal. No significant changes were observed in normal rats treated with geraniol. **Conclusion:** The present findings suggest that geraniol possesses a protective effect on altered glycoprotein metabolism in addition to its antihyperglycemic activity.

Key words: Diabetes, High fat diet, Streptozotocin, Glycoprotein and Geraniol.

1. Introduction

Diabetes mellitus is a heterogeneous disorder characterized by a progressive decline in insulin action (insulin resistance), followed by the inability of beta cells to compensate for insulin resistance (pancreatic beta cell dysfunction). It is characterized by hyperglycemia arising as a consequence of a relative or absolute deficiency of insulin secretion, resistance to insulin action or both (ADA, 2010). The development of diabetic complications such as retinopathy, neuropathy, nephropathy, and ketoacidosis are the chief causes of morbidity and mortality in diabetic populations (Ferris *et al*., 1999). The International Diabetes Federation has expected a global increase from 8.3 % to 9.9 % by the year 2030, with China and India projected to comprise the largest number of diabetic population (IDF, 2012).

Among these avowed possibilities, glycosylation of proteins has been the key subject of much interest Glycoproteins are the carbohydrate linked protein macromolecules serves as the major constituent of animal cells. They function as hormones, enzymes, blood group antigens, and extracellular membranes constituents (Zimmet *et al*., 2001). It has become widely accepted that the carbohydrate moieties of glycoproteins such as hexose, hexosamine, fucose and sialic acid have a vital role in protein stability, function and turnover. Nearly, 70 % of total sialic acid is found on the surface of cell, where it plays an essential role in cell-to-cell and cell to matrix interactions (Yarema, 2006). L-fucose, a deoxyhexose is a component of many N- and O-linked glycoproteins and participates in the events

*Corresponding author: **Leelavinothan Pari**
E.mail: jayampari@gmail.com
Received: 26.06.2016; *Revised*: 19.07.2016;
Accepted: 14.08.2016.

of biological recognition (Orczyk-Pawilowicz, 2007). Impaired metabolism of glycoprotein leads to the pathogenesis of diabetes mellitus (Michael and Fowler, 2008). The contribution of glycoproteins in diabetic complications has been confirmed by many other studies (Pari and Rajarajeswari, 2010). The enhanced biosynthesis or a decline in the glycoproteins metabolism cause these materials deposit in basal membrane of pancreatic β-cells (Ramkumar *et al*., 2007). Many classes of antidiabetic drugs are in use for long-term therapy are associated with undesirable side effects owing to which the developmental process in antidiabetic drug discovery has shifted its focus on plant-derived phytochemicals having minimal side effects (Unnikrishnan, 2010).

Geraniol is a monoterpenes and it's found in essential oils of fruits and herbs as like *Curcuma longa* (turmeric), *Pelargonium graveolens, Sphaeranthus indicus, Cymbopogon martini*. It have been suggested to represent a new class of agents for biological activities such as anti-cancer, anti-microbial, anti-oxidant and anti-inflammatory, hypotension, hypocholesterolemic activity. *Rose-scented geranium* is an important aromatic plant that grows in temperate areas of the world including those in Tunisia. Geranium oil has a fine rosy odor with pronounced fruity minty undertone and a rich long lasting sweet-rosy dry out. As the cost of its highly valued essential oil, which is used in soaps, perfumery, and cosmetic industries, is increasing in international market, attempts were made to promote its cultivation by employing various agro technologies. (Madankumar *et al*., 2013). To our knowledge, no such information exists to explain the effect of geraniol on the glycoprotein components in diabetic rats. Therefore, the primary objectives of this study were to assess its effect on disarrangement in glycoprotein levels in the HFD and low dose STZ induced diabetic rats.

2. Materials and Methods

Chemicals

Streptozotocin and geraniol were purchased from Sigma–Aldrich (St. Louis, MO, USA). All other chemicals used in this study were of analytical grade and purchased from E. Merck and Hi-media (Mumbai, India) and S.D-Fine Chemicals (Mumbai, India).

Experimental animals

Male albino Wistar rats, weighting about 180 - 220 g were procured from central animal house, Department of Experimental Medicine, Rajah Muthiah Medical College and Hospital, Annamalai University. They were housed in clean, sterile, polypropylene cages under standard vivarium conditions (12 hrs light/dark cycles) with fixed amount of feed (Hindustan Lever Ltd., Bangalore, India) and water. The experimental protocol was approved by the Institutional Animal Ethical Committee, Annamalai University (Reg No. 1119, 2015).

Induction of Experimental DM

The experimental groups were received beef tallow based high fat diet mixed with the commercial diet to diabetic complications (25 % fat, 15 % protein, 51 % starch and 5 % fiber) (Jian *et al*., 1998). After 1 month on their respective diets, experimental rats were injected intraperitoneally with streptozotocin (25 mg /kg body weight) dissolved in freshly prepared citrate buffer (0.1 M, pH 4.5) (Sakr, 2010). STZ Injected rats were allowed to drink 20 % glucose solution overnight to overcome the initial drug - induced hypoglycemic mortality. The induction of DM in rats was confirmed by estimating the elevated plasma glucose levels, 72 hrs after STZ Injection. Rats with fasting plasma glucose levels more than 250 mg/dl were considered diabetic and chosen for the study.

Experimental Design

A total of 36 adult male albino Wistar rats were used and they were divided into six groups of 6 rats in each group as below. Geraniol was dissolved in corn oil and administered orally at different doses using an intragastric tube for a period of 8 weeks.

Group I : Normal control

Group II : Normal rats were administered geraniol (100 mg/kg b.w/day/p.o) dissolved in corn oil

Group III : Diabetic control rats

Group IV : Diabetic rats were administered geraniol (100 mg/kg b.w/day/p.o) dissolved in corn oil

At the end of the experimental period, animals were fasted overnight, anesthetized using ketamine (24 mg/ kg BW, intramuscular injection), and sacrificed by cervical decapitation. All biochemical studies are carried out on plasma and tissues of control and experimental rats. Plasma proteins are precipitated by adding 95 % ethanol and the precipitate was used for the estimation of protein-bound hexose and hexosamine.

Determination of glycoprotein levels in plasma and tissues

For the estimation of glycoproteins, the tissues were defatted by the method of Folch *et al*. (1957) and hydrolyzed with 0.1 N H_2SO_4 at 80 °C for 1 hour, and aliquots were used for sialic acid estimation by the method of Warren (1959). To the remaining solution, 0.1 N NaOH was added and aliquots were used for the estimation of hexose and hexosamine and fucose by the methods of Niebes (1972), Wagner (1979) and Dische and Shettles (1948) respectively.

3. Results

Table - 1 shows the changes in the levels of protein bound hexose, hexosamine in plasma and liver of control and experimental rats. The levels of glycoprotein components in plasma were significantly ($p \leq 0.05$) increased in diabetic rats when compared with normal control rats. Administration of Geraniol resulted in a significant ($p \leq 0.05$) reduction of glycoprotein components in plasma and liver of diabetic rats when compared with untreated diabetic control rats. No significant changes were observed in geraniol alone treated rats.

Table - 2 shows the levels of protein-bound fucose, and sialic acid in plasma and liver of control and experimental rats. The levels of sialic acid were significantly ($p \leq 0.05$) increased in plasma and decreased in the tissues of diabetic rats and also increase in protein-bound fucose was observed. Oral administration of geraniol to diabetic rats significantly ($p \leq 0.05$) reversed these changes in plasma and liver to near normal.

4. Discussion

In our study plasma glycoprotein components have been associated with the severity of diabetes. The elevation in the levels of plasma glycoprotein components might be due to the secretion from cell membrane glycoconjugates in the circulation (Pari and Srinivasan, 2010). In the present study, we observed the increased levels of hexose, hexoseamine, fucose and sialic acid in the plasma of HFD and low dose STZ induced diabetic rats. Liver plays pivotal role in producing a large amount of glycoproteins present in blood. The elevated levels of plasma glycoproteins in diabetic condition could be a consequence of abnormal carbohydrate metabolism (Saravanan *et al*., 2010). Prolonged hyperglycemia due to insulin deficiency associated with oxidative stress increases the expression of GFAT (Glutamine: Fructose 6- phosphate amino transferase), the rate-limiting enzyme of this pathway leading to an increase in the levels of hexosamine (Brownlee, 2005). Hexosamines function as physiologic glucose sensors that serve as an adaptor in redirecting excess calories just before storage as fat (Wellen *et al*., 2010). Our study indicates that the elevated levels of hexosamine were observed in plasma and tissues of diabetic rats when compared with normal control rats. Diabetic rats

Table – 1: Effect of geraniol on hexoses and hexoseamines in the plasma and liver of normal and diabetic rats (n = 6)

Groups	Hexoses		Hexoseamines	
	Plasma (mg/dL)	Liver (mg/100 g tissue)	Plasma (mg/dL)	Liver (mg/100 g tissue)
Normal Control	88.08±7.28[a]	24.16±1.91[a]	66.95±5.55[a]	16.78±1.40[a]
Normal + Geraniol (100 mg/kg b.w.)	89.26±6.97[a]	25.02±2.02[a]	67.02±5.65[a]	16.99±1.43[a]
Diabetic Control	133.02±11.26[b]	44.41±3.78[b]	95.11±7.94[b]	25.78±2.36[b]
Diabetic + Geraniol (100 mg/kg b.w.)	101.06±8.36[c]	30.06±2.53[c]	77.21±6.45[c]	20.98±1.67[c]

Values in each group are represented as means ± S.D. for 6 rats in each group.
Values not sharing a common superscript letter (a-c) differ significantly at $p < 0.05$ (DMRT).

Table – 2: Effect of geraniol on fucose andsialic acid in the plasma and liver of normal and diabetic rats (n = 6)

Groups	Fucose		Sialic acid	
	Plasma (mg/dL)	Liver (mg/100 g tissue)	Plasma (mg/dL)	Liver (mg/100g tissue)
Normal Control	27.02±2.30[a]	12.02±0.91[a]	56.98±4.68[a]	10.25±0.87[a]
Normal + Geraniol (100mg/kg b.w.)	27.72±2.32[a]	12.09±0.99[a]	57.01±4.77[a]	10.71±0.88[a]
Diabetic Control	45.32±3.74[b]	25.12±2.09[b]	77.12±6.51[b]	4.92±0.44[b]
Diabetic+Geraniol (100 mg/kg b.w.)	32.58±2.60[c]	15.98±1.53[c]	66.79±5.68[c]	7.02±0.56[c]

Values in each group are represented as means ± S.D. for 6 rats in each group.
Values not sharing a common superscript letter (a-c) differ significantly at $p < 0.05$ (DMRT).

treated with Geraniol showed significantly decreased hexosamines in the plasma and tissues when compared to diabetic rats, which could be due to improved glycemic control. Fucose (6-deoxy-L-galactose) is a distinguishing component of essential sugars that are required for the functioning of cell to cell communication in the body and its metabolism appears to be altered in various disease conditions like diabetes mellitus (Pari and Karthikesan, 2009). The increase in fucose levels might be the result of increased glycosylation in diabetic condition. Complex protein -carbohydrate molecules could contribute to an increase in plasma glycoproteins. The diminished glucose consumption was observed by insulin dependent pathways, thereby enhancing the formation of hexose, hexosamine and fucose for the accumulation of glycoproteins (Spiro and Spiro, 1971). The insulin secretary potential of geraniol has been recently reported (Sheikh *et al*., 2015). The results of the present study are in agreement with Muruganathan *et al.* (2013) who reported that the elevated levels of fucose were lowered by insulin secretion in carvone treated diabetic rats. The hyperglycemia mediated oxidative stress and inflammation may contribute to bring about damages to cellular membranes and increases serum sialic acid levels. The diminished content of sialic acid in the tissues might be due to the utilization for the synthesis of fibronectin,

which have sialic acid residues in the core structure. The administration of Geraniol increased the content of sialic acid in the liver and decreased the levels of sialic acid levels in the plasma. This is attributed to insulinoropic potential of geraniol which restored the altered glycoprotein components in the plasma and liver of diabetic animals to near normal. Our results are in accordance with the previous reports (Saravanan *et al*., 2010).

In conclusion, in our study the intragastric administration of geraniol (100 mg/kg BW) to the diabetic rats resulted in modulation of glycoprotein content in plasma and liver. Supplementation of geraniol was shown to be effective in diabetic complications by the enhancement of insulin action, as evident by the decreased level of plasma glucose in diabetic rats treated with geraniol. In addition, our findings provided an insight that geraniol could be developed as a new drug ingredient for the prevention of diabetes mellitus.

5. References

1) American Diabetes Association. 2010. Diagnosis and classification of diabetes mellitus. *Diabetes Care*, 34: 62 – 69.
2) Brownlee, M. 2005. The pathobiology of diabetic complications: A unifying mechanism. *Diabetes*, 54: 1615 - 1625.
3) Dische, Z and Shettles, L. B. 1948. A specific color reaction of methyl pentoses and a spectrophotometric micro method for their determination. *Journal of Biological Chemistry*, 175: 596 - 603.
4) Ferris, F. L., Davis, M. D and Aiello, L. M. 1999. Treatment of diabetic retinopathy. *English Journal of Medicine*, 341: 667 - 678.
5) Folch, J., Lees, M and Solane, S. G. 1957. A simple method for isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry*, 26: 497 - 509.
6) IDF. 2012. Diabetes Atlas, 5th edition.
7) Jian, L. Q., Josephine, T., Joyce, K. H., Christina, S and Cynthia, L. 1998. Non-genetic mouse models of non-insulin dependent diabetes mellitus. *Metabolism*, 47: 663 - 668.
8) Madankumar, A., Jayakumar, S., Gokuldhas, K., Rajan, B., Raghunandhakumar, S and Asokkumar Devaki, T. 2013. GOH modulates tongue and hepatic phase I and phase II conjugation activities and may contribute directly to the chemopreventive activity against experimental oral carcinogenesis. *European Journal of Pharmacology*, 705: 148 – 155.
9) Muruganathan, U., Srinivasan, S and Indumathi, D. 2013. Antihyperglycemic effect of carvone: Effect on the levels of glycoprotein components in streptozotocin-induced diabetic rats. *Journal of Disease*, 310 - 315.
10) Niebes, P. 1972. Determination of enzymes and degradation products of glycosamino glycans metabolism in the serum of healthy and varicose subjects. *Clinical Chemistry Acta*, 42: 399 - 408.
11) Orczyk – Pawiłowicz, M. 2007. The role of fucosylation of glycoconjugates in health and disease. *Postepy Hig Med Dosw*, 61: 240 - 252.
12) Pari, L and Karthikesan, K. 2009. Protective role of tetrahydrocurcumin and chlorogenic acid on glycoprotein changes in streptozotocin nicotinamide - induced diabetic rats. *Journal of Pharmaceutical Science and Research*, 1(4): 173 - 180.
13) Pari, L and Rajarajeswari, N. 2010. Protective role of coumarin on plasma and tissue glycoprotein components in Streptozotocin nicotinamide induced hyperglycemic rats. *International Journal of Biological and Medical Research*, 1: 61 - 65.
14) Pari, L and Srinivasan, S. 2010. Preventive effect of diosmin, a bioflavonoid, on glycoprotein changes in Streptozotocin nicotinamide - induced type 2 diabetic rats. *International Journal of Pharmaceutical Science and Research*, 10: 89 - 95.
15) Ramkumar, K. M., Rajaguru, P., Latha M and Ananthan, R. 2007. Ethanol extract of *Gymnema montanum* leaves reduces

glycoprotein components in experimental diabetes. *Nutrition and Research,* 27: 97 - 103.
16) Sakr, H. F. 2010. Endothelial Dysfunction Induced by Type 2 Diabetes Mellitus and Fibrinolytic Activity. *American Journal of Biochemistry and Biotechnology*, 6: 103 -110.
17) Saravanan, G., Ponmurugan, P., Senthil Kumar, G. P and Rajrajan, T. 2010. Antidiabetic effect of S-allylcysteine: Effect on plasma and tissue glycoproteinsins in experimental diabetes. *Phytomedicine,*17: 1086 - 1089.
18) Sheikh, B. A., Pari, L., Rathinam, A and Chandramohan, R. 2015. Trans-anethole, a terpenoid ameliorates hyperglycemia by regulating key enzymes of carbohydrate metabolism in streptozotocin induced diabeticrats. *Biochimie*, 112: 57 - 65.
19) Spiro, R. G and Spiro, M. J. 1971. Effect diabetes on the biosynthesis of the renal glomerular basement membrane. Studies on the glycosyl transferase. *Diabetes,* 20: 641 - 648.
20) Unnikrishnan, M. K. 2010. Antidiabetic effect of *Dodonaea viscosa* L. Lacq. Aerial parts in high fructose-fedinsulin resistant rats: a mechanism based study. *Indian Journal of Experimental Biology*, 48: 800 - 810.
21) Wagner, W. D. 1979. A more sensitive assay discriminating galactosamine and glucosamine in mixture. *Annals of Biochemistry*, 94: 394 - 396.
22) Warren, L. 1959. The thiobarbituric acid assay of sialic acids. *Journal of Biological Chemistry*, 234: 1971 - 1975.
23) Wellen, K. E., Lu, C., Mancuso, A., Lemons, J. M., Ryczko, M and Dennis, J. W. 2010. The hexosamine biosynthetic pathway couples growth factor - induced glutamine uptake to glucose metabolism. *Genes Dev*, 24 (24): 2784 - 2799.
24) Yarema, K. 2006. The sialic acid pathway in human cells. Baltimore: John Hopkins University.
25) Zimmet, P., Alberti, K. G and Show, J. 2001. Global and societal implications of the diabetes epidemic. *Nature*, 414: 782 - 787.