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Research Article

Antihyperglycemic effect of *trans*-anethole in streptozotocin induced diabetic rats with special reference to glycoprotein components

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Abstract

Objective: To evaluate the antidiabetic effect of *trans*-anethole, a natural terpenoid on the levels of glycoprotein components in plasma and tissues of streptozotocin induced diabetic rats. **Methods:** Diabetes was induced in male albino Wistar rats by a single intraperitoneal injection of streptozotocin (40 mg/kg b.w). *Trans*-anethole was administered orally at a dose of (80 mg/kg b.w) for 45 days. The effect of *trans*-anethole was studied on plasma glucose, insulin, plasma and tissue glycoproteins. **Results:** Oral administration of *trans*-anethole (80 mg/kg b.w) for 45 days modulated the altered levels of plasma and tissue glycoprotein components to near normal. No significant changes were observed in normal rats treated with *trans*-anethole. **Conclusion:** The present findings suggest that *trans*-anethole possesses a protective effect on altered glycoprotein metabolism in addition to its antihyperglycemic activity.

Keywords: Diabetes, Streptozotocin, Glycoprotein, *Trans*-anehole, Hexose, Hexosamine, Fucose, Sialic acid.

1.Introduction

Diabetes mellitus (DM) is one of the most widespread metabolic disorders leading to the risk of long-term complications in multiple organs and systems (Perkins and Bril). 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 predicted 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). Hyperglycemia-induced cell damage is caused by alterations in glucose metabolism via four key metabolic pathways, viz., increased polyol pathway flux, increased glycation of proteins (enzymatic or non enzymatic), increased hexosamine pathway flux and activation of protein kinase C (PKC) isoforms (Rolo and Palmeira, 2006). 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. Nearly70% 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 of biological recognition (Orczyk-Pawiłowicz, 2007)[8]. 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 and 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).

Dietary phytochemicals present in medicinal plants especially *Foeniculum vulgare* have received a remarkable attention because of its many recognized health benefits. TA is a principle constituent of many essential oils from aromatic plants (e.g. *Foeniculum vulgare*) and is used in a wide range of food products. *Foeniculum vulgare* is a well-known medicinal plant traditionally used for its antioxidant, anti-inflammatory, anti-genotoxic, antipyretic, and anti-carcinogenic properties and TA appears to be responsible for all above properties (Kang et al.,2013; Oktay et al., 2003; Abraham, 2001; Chen and Linda, 2012). Prolonged treatment with the petroleum ether fraction of the *F.vulgare* extract and confirmed the improvement in blood glucose, lipid profile, HbA1c and other parameters in streptozotocin-induced diabetic rats (Dongare et al., 2012). Recently, we have reported the antihyperglycaemic activity of TA in STZ induced diabetic rats (Sheikh et al., 2015). To our knowledge, no such information exists to explain the effect of TA 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 streptozotocin induced diabetic rats.

2.Materials and Methods

2.1. *Chemicals*

Streptozotocin and *trans*-anethole 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 Himedia (Mumbai, India) and S.D-Fine Chemicals (Mumbai, India).

2.2. *Experimental Animals*

Adult Male albino Wistar rats weighing about 180–200 g were obtained from Central Animal House, Department of Experimental Medicine, Rajah Muthiah Medical College and Hospital, Annamalai University. Rats were housed in clean, sterile and polypropylene cages under standard vivarium conditions 12 h light/12 h dark cycle constant temperature (25± 1 ◦C) with free access to standard chow (Pranav Agro Industries Ltd., Pune, Maharashtra, India) and water. The experimental protocol was approved by the Animal Ethics Committee of Rajah Muthiah Medical College and Hospital (Reg No. 160/1999/CPCSEA, Proposal No.1090), Annamalai University, Annamalainagar.

2.3. *Induction of experimental diabetes*

Experimental diabetes was induced in overnight fasted experimental rats by single interaperitoneal injection of STZ (40 mg/kg BW) dissolved in freshly prepared cold citrate buffer (0.1 M, pH 4.5). STZ-injected animals were allowed to drink 20% glucose solution for 24 h to prevent initial drug-induced hypoglycemic mortality. STZ-injected animals exhibited hyperglycemia within a few days. Diabetic rats were confirmed by measuring the elevated plasma glucose (by glucose oxidase method) 72 h after injection with STZ. The animals with glucose above 250 mg/dL were selected for the experiment.

2.4. *Experimental design*

A total of 42 rats (30 diabetic rats and 12 normal rats) were used and experimental animals were divided into seven groups, each group consists of a minimum of six rats (n=6) detailed as given below. *Trans*-anethole was dissolved in corn oil and administered orally at different doses using an intragastric tube for a period of 45 days. Glibenclamide was dissolved in distilled water used as standard drug.

Group I: Normal control rats
Group II: Normal + TA (80 mg/kg BW)
Group III: Diabetic control rats
Group IV: Diabetic + TA (80 mg/kg BW)
Group V: Diabetic + glibenclamide (600 µg/kg BW)

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, liver, and kidney 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.

2.5 *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 0.1N H2SO4 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 tissues 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 TA resulted in a significant ($p \leq 0.05$) reduction of glycoprotein components in plasma and tissues of diabetic rats when compared with untreated diabetic control rats. No significant changes were observed in TA alone treated rats.

Table 2 shows the levels of protein-bound fucose, and sialic acid in plasma, liver and kidney tissues 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 TA to diabetic rats significantly ($p \leq 0.05$) reversed these changes in plasma and tissues to near normal.

4.Discussion

STZ has been widely used to induce diabetes mellitus in experimental rat models. The intraperitoneal administration of single dose of STZ (40 mg/kg) selectively destroy some population of insulin secreting β-cells of pancreas resulting in insulin deficiency and causing type 2 diabetes (Chandramohan et al., 2015; Balamurugan et al., 2011). *F.vulgare* is repoted to be rich in TA which is responsible for its antihyperglycemic effect (Politeo et al., 2006). In the present study, we demonstrated TA attenuated the elevation of glycoprotein components in normal and diabetic rats.

The structural and functional alterations of both circulating and membrane bound proteins is the result of prolonged elevation of blood glucose and insulin deficiency in diabetes and also the deficiency of insulin results in the thickening of basal membrane of pancreatic beta cells (Ciftci and Yarim, 2011). The alterations in the composition of carbohydrate components of glycoproteins of serum and basement membrane have been reported in the condition like diabetes (Buse, 2006). The increased 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 salic acid in the plasma of STZ induced diabetic rats. Administration of TA ameliorates the levels of plasma glycoproteins near normal. Our results are in agreement with Muthukumaran et al., who reported that Syringic acid improved glycoprotein levels in diabetic rats (Muthukumaran et al., 2013).

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). 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 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

Table 1Effect of TA on hexoses and hexosamines in the plasma, liver and kidney of normal and STZ-diabetic rats (n = 6).

Groups	Hexoses			Hexoseamines		
	Plasma (mg/dL)	**Liver (mg/100 g tissue)**	**Kidney (mg/100 g tissue)**	**Plasma (mg/dL)**	**Liver (mg/100 g tissue)**	**Kidney (mg/100 g tissue)**
Normal Control	89.09 ± 6.78[a]	25.32 ± 1.93[a]	22.23 ± 1.69[a]	67.07 ± 5.11[a]	16.98 ± 1.29[a]	8.78 ± 0.67[a]
Normal + TA (80 mg/kg b.w.)	90.16 ± 6.90[a]	24.78 ± 1.90[a]	21.58 ± 1.65[a]	67.94 ± 5.20[a]	16.55 ± 1.27[a]	7.98 ± 0.61[a]
Diabetic Control	135.04 ± 10.28[b]	45.45 ± 3.46[b]	36.25 ± 2.76[b]	96.14 ± 7.32[b]	26.78 ± 2.04[b]	23.70 ± 1.80[b]
Diabetic +TA (80 mg/kg b.w.)	100.03 ± 7.66[c]	31.00 ± 2.37[c]	27.12 ± 2.08[c]	78.27 ± 5.99[c]	21.46 ± 1.64[c]	12.58 ± 0.96[c]
Diabetic + Glibenclamide (600µg/kg b.w)	105.92 ± 8.11[c]	28.24 ± 2.16[c]	25.22 ± 1.93[c]	74.24 ± 5.68[a,c]	19.80 ± 1.52[c]	11.44 ± 0.88[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 TA on the Fucose and Salic acid in the plasma, liver and kidney of normal and STZ-diabetic rats (n = 6).

Groups	Fucose			Salic acid		
	Plasma (mg/dL)	**Liver (mg/100 g tissue)**	**Kidney (mg/100 g tissue)**	**Plasma (mg/dL)**	**Liver (mg/100 g tissue)**	**Kidney (mg/100 g tissue)**
Normal Control	27.87 ± 2.12[a]	12.17 ± 0.93[a]	10.32 ± 0.79[a]	57.38 ± 4.37[a]	10.75 ± 0.82[a]	10.08 ± 0.77[a]
Normal + TA (80 mg/kg b.w.)	28.13 ± 2.15[a]	12.37 ± 0.95[a]	10.21 ± 0.78[a]	57.04 ± 4.37[a]	10.99 ± 0.84[a]	9.98 ± 0.76[a]
Diabetic Control	46.35 ± 3.53[b]	26.14 ± 1.99[b]	23.04 ± 1.75[b]	78.11 ± 5.95[b]	4.50 ± 0.34[b]	4.16 ± 0.32[b]
Diabetic +TA (80 mg/kg b.w.)	34.58 ± 2.65[c]	16.68 ± 1.28[c]	14.35 ± 1.10[c]	65.79 ± 5.04[c]	7.43 ± 0.57[c]	6.94 ± 0.53[c]
Diabetic + Glibenclamide (600µg/kg b.w)	31.79 ± 2.43[c]	15.09 ± 1.15[d]	12.12 ± 0.93[d]	62.14 ± 4.76[a,c]	8.03 ± 0.61[c]	7.60 ± 0.58[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-d]) differ significantly at $p < 0.05$ (DMRT).

metabolic complications of diabetes (Obici et al., 2002). 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). The results of the present study are in harmony with previous studies that diabetic rats showed elevated level of hexosamines, which could be due to, increased expression of GFA and increased plasma glucose. 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 treated with TA and glibenclamide 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. Therefore, vascular complications that involve complex protein-carbohydrate molecules could contribute to an increase in plasma glycoproteins. The serum proteins haptoglobin, α-1 acid glycoprotein and α-1-antitrypsin are synthesized in liver, in the conditions like diabetes metabolism and synthesis of these proteins may be altered leading to changes in serum in the hyperglycemic state accelerates the synthesis of glycoproteins (Radhakrishnamoorthy and Berenson, 1973). 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). Upon the administration of TA the fucose levels were diminished, which might be due to increased secreation of insulin. The insulin secretary potential of TA has been recently reported (Sheikh et al., 2015). The results of the present study are in agreement with Udaiyar Muruganathan (Muruganathan et al., 2013) who reported that the elevated levels of fucose were lowered by insulin secretion in carvone treated diabetic rats.

Renal disease is one of the most common characteristic and severe complication in diabetes (Shanmugasundram et al., 1990). Diabetes leads to the progression of microvascular pathology in renal glomerulus and the end-stage of renal disease is the consequence of its microvascular pathology (Brownlee, 2005). The hyperglycemia mediated oxidative stress and inflammation may contribute to bring about damages to cellular membranes and increases serum sialic acid levels. In addition to this, vascular endothelium is rich in sialic acid moieties where it regulates permeability. Impaired function of insulin and the resulting hyperglycemia are associated with impairment in endothelium, leading to the release of sialic acid into circulation (Calles-Escandon and Cipolla, 2001). The diminished content of salic acid in the tissues might be due to the utilization for the synthesis of fibronectin, which have salic acid residues in the core structure. The epithelial cells of luminal surface in kidney tubules are also lined with a thick carbohydrate rich glycoprotein layer (Mittal et al., 1996). The administration of TA increased the content of salic acid in the tissues and decreased the levels of salic acid levels in the plasma. This decrease may also be related to increased synthesis of fibronectin which contains sialic acid in its core structure. This is attributed to insulinoropic potential of TA which restored the altered glycoprotein components in the plasma and tissues of diabetic animals to near normal. Our results are in accordance with the previous reports (Saravanan et al., 2010).

In conclusion, we put forward that the intragastric administration of TA (80 mg/kg BW) to the diabetic rats resulted in modulation of glycoprotein content in plasma, liver and kidney. Supplementation of TA 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 TA. In addition, our findings provided an insight that TA could be developed as a new food additive or drug ingredient for the prevention of diabetes mellitus.

Conflict of interest

The authors declare that they have no conflicts of interest concerning this article.

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