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Antihyperglycemic effect of carvone: Effect on the levels of glycoprotein components in streptozotocin-induced diabetic rats

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ABSTRACT

Objective: To investigate the effect of carvone on dearrangement in glycoprotein levels in the streptozotocin(STZ)-induced diabetic model. **Methods:** Diabetes was induced in male Wistar rats by a single intraperitoneal injection of STZ (40 mg/kg b.w). The levels of glycoproteins were altered in experimental diabetes mellitus. Carvone were administered to diabetic rats intragastrically at 25, 50, 100 mg/kg bw for 30 d. The effects of carvone on plasma glucose, insulin, plasma and tissue glycoproteins were studied. **Results:** Oral administration of carvone (50 mg/kg b.w) for 30 d, dose dependently improved the glycemic status in STZ-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 present findings suggest that carvone can potentially ameliorate glycoprotein components abnormalities in addition to its antihyperglycemic effect in experimental diabetes. In light of these advantageous results, it is advisable to broaden the scale of use of carvone in a trial to alleviate the adverse effects of diabetes.

1. Introduction

Diabetes mellitus poses a major health problem on both clinical and social plan, not only for the high number of patients, but also for the onset of serious invaliding complications that frequently appear[1]. It is a prototypical, growing, costly chronic non-communicable disease causing increasing morbidity and mortality worldwide, often disproportionately hurting the poor and young sub-populations in developing countries[2]. More than 220 million people worldwide are affected by Diabetes and its incidence is expected to increase to 400 million by 2030[3]. About 95% of diabetic patients suffer from type2 diabetes. Diabetes is chronic, multifaceted, dynamic expression of pathological disequilibria, resulting in various micro and macro vascular complications. The pathophysiology of diabetes involves a very complex cascade of several interrelated mechanism. The chronic hyperglycemia of diabetes is associated with long term damage, dysfunction and failure of various organs, especially the eyes, kidneys, nerves, heart and blood vessels[4].

Glycoproteins can be simply defined as proteins that have carbohydrate moiety covalently attached to their peptide portion. They have multiple and complex function and are found as enzymes, hormones, blood group substances and as constituents of extracellular membranes[5]. The commonest glycoproteins are those in which the carbohydrate is linked to the protein by glycosyl linkages, usually hexose, hexosamine, fucose and sialic acid, joined together covalently linked to polypeptide chain. Hyperglycemia in experimental diabetic rats leads to a decreased utilization of glucose by insulin dependent pathways, thereby enhancing the formation of glycoproteins[6]. At the cell surface or inside the cells, the glycoprotein components such as fucose and sialic acid form specific structures, called glycanic chains covalently linked to lipids or proteins. An increase in the biosynthesis

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or a decrease in the metabolism of glycoproteins could be related to the deposition of these materials in the basal membrane of pancreatic cells. Alterations in glycoproteins level leads to the pathogenesis of diabetes mellitus. Many studies confirm the involvement of glycoproteins in diabetic complications[7].

Experimental induction of diabetes mellitus in animal models is essential for understanding various aspects of its pathogenesis and ultimately finding new therapies and cure. Several methods have been used to induce diabetes mellitus in laboratory animals with variable success. Streptozotocin (STZ), an antibiotic produced by *Streptomyces achromogenes*, has been widely used for inducing diabetes in the experimental animals through its toxic effects on pancreatic β-cells[8]. In recent years, there has been a growing interest in anti-diabetic agents from natural products. They represent an alternative mode for diabetes therapy because most of the anti-diabetic drugs have some side effects and fail to significantly alter the course of the disease. Terpenes are the largest group of natural substances, biosynthetically derived from isoprene units[9]. They are abundantly found in fruits, vegetables and aromatic and medicinal plants. Moreover, they are endowed with many beneficial health effects and can be used to treat different health disorders including diabetes[10]. Monoterpenes are naturally occurring hydrocarbons composed of the condensation of two isoprenes. They are widely distributed in the plant kingdom and are best known in plant essential oils. Carvone (5-isopropenyl-2-methyl-2-cyclohexenone), a monocyclic monoterpene ketone, is produced by over 70 different plants. It is the main component of caraway oil. This monoterpene exhibits some interesting biological activities. It shows, for instance, antimicrobial[11], nematicidal[12] and antitumor properties[13].

The aim of the present study was experimentally to validate the neutraceutical potential of carvone in the management of diabetes and also mediated through its salubrious effect on plasma and tissue glycoproteins in STZ-induced diabetic rats and the efficacy of carvone was compared with glyclazide, a standard oral antihyperglycemic drug. The outcome would provide important information about its usefulness as a therapeutic agent for the treatment of diabetes and related complications.

2. Materials and methods

2.1. Chemicals

Carvone and STZ were procured from Sigma Chemicals Co., St. Louis, MO, USA, stored at 2-4 °C and protected from sunlight. All other chemicals were of analytical grade and were obtained from standard commercial suppliers.

2.2. Animals

Male Wistar rats weighing approximately 180-200 g were grown in the Central Animal House, Rajah Muthiah Medical College, Annamalai University, India. Before starting the experiments all the animals were acclimatized to the laboratory conditions for 1 week. They were housed at an ambient temperature of (25±2) °C, 12/12 h of light-dark cycle with *ad libitum* food and water. 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 study protocol was approved (Reg No.160/1999/CPCSEA, Vide No. 986/2012) by the Committee for the Purpose of Control and Supervision on Experimental Animals at Annamalai University, Annamalainagar, India.

2.3. Induction of diabetes

Diabetes was induced in overnight fasted experimental rats by a single intraperitoneal injection of STZ (40 mg/kg b.w) dissolved in freshly prepared citrate buffer (0.1 M, pH 4.5). STZ injected animals were allowed to drink 20% glucose solution overnight to overcome the initial drug-induced hypoglycemic mortality. Control rats were injected with same volume of citrate buffer alone. After 96 h, plasma glucose was determined and those rats with fasting blood glucose greater than 250 mg/dL were used in the present study.

2.4. Experimental design

The animals were randomly divided into seven groups of six animals in each group (30 diabetic surviving and 12 normal). Carvone was dissolved in vehicle solution of corn oil and administered to experimental rats.

Group I	Normal control (vehicle treated)
Group II	Normal rats received carvone (100 mg/kg b.w) intra gastrically suspended in 1 mL corn oil for 30 d
Group III	Diabetic control
Group IV	Diabetic rats received carvone (25 mg/kg b.w) intra gastrically suspended in 1 mL corn oil for 30 d
Group V	Diabetic rats received carvone (50 mg/kg b.w) intra gastrically suspended in 1 mL corn oil for 30 d
Group VI	Diabetic rats received carvone (100 mg/kg b.w) intra gastrically suspended in 1 mL corn oil for 30 d
Group VII	Diabetic rats received glyclazide (5 mg/kg b.w) intra gastrically in aqueous solution for 30 d

During the experimental period, body weight, blood glucose, food and water consumption and physical examinations were determined at regular intervals. The dosage was adjusted every week according to any change in body weight to maintain similar dose per kg body weight

of rat over the entire period of study for each group. At the end of the treatment period, the rats were fasted overnight, anaesthetized with ketamine (24 mg/kg body weight; i.p.) and killed by cervical decapitation. Blood sample was collected in a tube containing potassium oxalate and sodium fluoride (3:1) for the estimation of plasma glucose, insulin and glycoproteins. Liver and kidney were dissected out, washed in ice-cold saline, patted dry and weighed.

2.5. Extraction of glycoproteins

To 0.1 mL of plasma, 5.0 mL of methanol was added, mixed well and centrifuged for 10 min at 3 000×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 glycoproteins. This was used for the estimation of hexose and hexosamine. For extraction of glycoproteins from the tissues, a known weight of the tissue was homogenized in 7.0 mL of methanol. The contents were filtered and homogenized with 14.0 mL of chloroform. This was filtered and the residue was successively homogenized in chloroform-methanol (2:1 v/v) and each time the extract was filtered. The residue (defatted tissues) was obtained and the filtrate decanted. A weighed amount of defatted tissue was suspended in 3.0 mL of 2 N HCl and heated at 90 °C for 4 h. The sample was cooled and neutralized with 3.0 mL of 2 N NaOH. Aliquots from this were used for estimation of fucose, hexose, hexosamine and sialic acid.

2.6. Estimation of blood glucose and plasma insulin

Blood glucose was determined spectrophotometrically using a commercial diagnostic kit Agappe Diagnostics Pvt Ltd., India) by the method of Trinder[14]. Plasma insulin was assayed by ELISA using a Boehringer-Mannheim kit with an ES300 Boehringer analyzer (Mannheim, Germany). Both the analyses were done according to the manufacturer's instructions.

2.7. Determination of glycoproteins

The plasma and tissue hexose content was estimated by the method of Niebes[15], sialic acid in plasma and tissues were estimated by the method of Warren[16] and hexosamine by the method of Wagner[17]. Fucose was estimated by the method of Dische and Shettles[18]respectively.

2.8. Statistical analysis

The values are expressed as mean values of six rats in each group±standard error mean (S.E.M). Data analysis was done with SPSS 17.0 (SPSS, Cary, NC, USA) student software. Hypothesis testing method included one way analysis of variance (Anova) followed by Duncan's Multiple Range Test (DMRT)[19]. Values are considered statistically significant when $P<0.05$.

3. Results

3.1. Effect of carvone on the levels of plasma glucose and insulin

Table 1 showed the levels of plasma glucose and insulin in normal control and experimental rats. The level of plasma glucose as significantly increased whereas plasma insulin level was significantly decreased in diabetic control rats. Administration of carvone (all doses), as well as glyclazide brings a significant decrease in plasma glucose and increase in insulin level were observed at the end of the experimental period. Carvone at a dose of 50 and 100 mg/kg b.w showed a highly significant effect than other dose (25 mg/kg b.w). Based on these data, the effective dose was fixed as 50 mg/kg b.w. and used for further analysis.

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

Groups	Plasma glucose (mg/dL)	Plasma insulin (μU/mL)
Normal control	78.20±5.95*	16.24±1.24*
Normal + carvone (100 mg/kg)	77.95±5.97*	16.43±1.26*
Diabetic control	295.23±22.48†	6.91±0.53†
Diabetic + carvone (25 mg/kg)	143.78±11.01‡	10.06±0.81‡
Diabetic + carvone (50 mg/kg)	115.33±8.83**	13.63±1.04**
Diabetic + carvone (100 mg/kg)	111.57±8.54**	13.99±1.07**
Diabetic + glyclazide (5 mg/kg)	103.32±7.91***	14.34±1.10***

Values are given as means±S.D. for six rats in each group. Values in a column not sharing a common superscript symbol (* -***) differ significantly at $P<0.05$. Duncan's Multiple Range Test (DMRT).

3.2. Effect of carvone on the levels of plasma glycoproteins

Table 2.
Effect of carvone on plasma glycoprotein levels in normal and experimental rats (mg/dL).

Groups	Hexose	Hexosamine	Fucose	Sialic acid
Normal control	96.20±7.32*	72.20±5.50*	34.73±2.64*	55.85±4.27*
Normal + carvone (50 mg/kg)	93.97±7.19*	70.58±5.40*	32.83±2.51*	54.34±4.14*
Diabetic control	162.36±12.36†	101.29±7.75†	51.28±3.90†	82.94±6.35†
Diabetic + carvone (50 mg/kg)	115.33±8.83‡	88.29±6.76‡	42.93±3.29‡	63.66±4.85‡
Diabetic + glyclazide (5 mg/kg)	107.31±8.21‡	80.33±6.15‡	38.28±2.93‡	60.37±4.62

Values are given as means±S.D. for six rats in each group. Values in a column not sharing a common superscript symbol ($^{*-\ddagger}$) differ significantly at $P<0.05$. Duncan's Multiple Range Test (DMRT).

Table 2 shows the changes in the levels protein bound hexose, hexosamine, fucose and sialic acid in plasma of control and experimental rats. Significantly higher levels of glycoprotein components were observed in the plasma of diabetic rats when compared to normal control rats. Oral

administration of carvone as well as glyclazide to diabetic rats resulted in a significant reduction of protein bound hexose, hexosamine, fucose and sialic acid in plasma when compared to diabetic control rats.

3.3. Effect of carvone on the levels of tissue glycoproteins

The levels of liver and kidney glycoprotein of control and experimental rats were shown in Tables 3 and 4. The level of hexose, hexosamine and fucose were significantly increased whereas the level of sialic acid was significantly decreased and those levels were brought back to near normal by treatment with carvone and glyclazid.

Table 3.
Effect of carvone on liver glycoprotein levels in normal and experimental rats (mg/g defatted tissue).

Groups	Hexose	Hexosamine	Fucose	Sialic acid
Normal control	30.53±2.32*	13.25±1.01*	17.38±1.32*	11.02±0.84*
Normal + carvone (50 mg/kg)	28.79±2.20*	12.88±0.99*	16.93±2.61*	11.94±0.91*
Diabetic control	51.28±3.07†	24.73±2.03†	34.27±3.02†	4.27±0.33†
Diabetic + carvone (50 mg/kg)	40.17±2.63‡	19.28±1.48‡	24.74±1.89‡	6.90±0.53‡
Diabetic + glyclazide (5 mg/kg)	36.94±2.83‡	16.83±1.29‡	22.58±1.73‡	8.07±0.62‡

Values are given as means±S.D. for six rats in each group. Values in a column not sharing a common superscript symbol (* -‡) differ significantly at P<0.05. Duncan's Multiple Range Test (DMRT).

Table 4.
Effect of carvone on kidney glycoprotein levels in normal and experimental rats (mg/g defatted tissue).

Groups	Hexose	Hexosamine	Fucose	Sialic acid
Normal control	28.46±2.17*	16.92±1.29*	14.68±1.12*	9.47±0.72*
Normal + carvone (50 mg/kg)	27.62±2.11*	16.03±1.23*	14.06±1.08*	10.33±0.79*
Diabetic control	47.28±3.60†	37.87±2.88†	35.56±2.71†	4.87±0.37†
Diabetic + carvone (50 mg/kg)	35.19±2.69‡	22.75±1.74‡	20.44±1.56‡	6.93±0.53‡
Diabetic + glyclazide (5 mg/kg)	32.93±2.52‡	20.55±1.57‡	19.03±1.46‡	7.02±0.54‡

Values are given as means±S.D. for six rats in each group. Values in a column not sharing a common superscript symbol (* -‡) differ significantly at $P<0.05$. Duncan's Multiple Range Test (DMRT).

4. Discussion

Streptozotocin, a glucopyranose derivative of l-methyl-l nitrosourea, is unique for its diabetogenic potential in experimental animals and the presence of 2-deoxy-D-glucose in its structure facilitates its preferential uptake into the pancreatic β-cells, probably via the low affinity glucose transporter, GLUT2[20]. Insulin is the sole hormone synthesized that lowers blood glucose, thereby playing important role in glucose metabolism[21]. In STZ administered diabetic rats, pancreatic β-cells are directly and specifically destroyed, and the resultant chronic hyperglycemia further damages the peripheral organs through increased oxidative stress and inflammation reaction, an effect called glucose toxicity[22]. These in turn potentiate the gradual loss of insulin sensitivity of peripheral organs during the pathogenesis of diabetes[23], which forms a vicious cycle for the progression and development of diabetes. In the present research, we found that oral administration of carvone for 30 d resulted in a significant reduction in plasma glucose concentrations and an increase in insulin levels in diabetic rats. The antidiabetic effect of carvone may be due to the increased release of insulin from the existing β-cells and/or regenerated β-cells of pancreas, restored insulin sensitivity or inhibition of intestinal absorption of glucose or enhanced the utilization of glucose by peripheral tissues. These results are in agreement with Ramachandran and Saravanan[24] who reported that administration of asiatic acid, a terpene to diabetic rats significantly decreased the glucose level to near normal.

Glycation is a time-dependent, non-enzymatic reaction of sugars with proteins. The first step of glycation involving glucose requires a reversible, non-covalent binding interaction between glucose and select sites on the protein. A labile Schiff base may then form between the electrophilic carbonyl group of glucose and free nucleophilic amino groups on protein, followed by an Amadori rearrangement that yields a stable ketoamine adduct[25]. In uncontrolled or poorly controlled diabetes there is an increased glycosylation of a number of proteins including hemoglobin and crystalline of lens. Raised levels of glycoproteins in diabetics may also be a predictor of angiopathic complications.

Generalized abnormalities in glycoprotein metabolism are observed in both naturally occurring and experimental diabetes[26]. Berenson *et al*[27] reported that STZ-induced diabetic rats exhibited a significant modification in the connective tissue macromolecule. Insulin has been shown to increase the incorporation of glucose in the rat submaxillary gland. The requirement of insulin for the biosynthesis of the carbohydrate moiety of mucoproteins from glucose is thus evident. But the deficiency of insulin during diabetes produces derangement of glycoprotein metabolism, resulting in the thickening of the basal membrane of pancreatic β-cells. The increased availability of glucose in the hyperglycemic state accelerates the synthesis of basement-membrane components, that is, glycoproteins[28]. Administration of carvone and glyclazid to diabetic rats significantly reversed these changes to near normal levels. The antihyperglycemic action of carvone, which is mediated via an enhancement of insulin action, as evidenced by the increased level of insulin in carvone treated diabetic rats, may be responsible for the reversal of plasma glycoprotein changes associated with diabetes.

Glucose is the most important component for most living organisms. Hexoses of which glucose is one example evolved as energy sources critical for life. Protein bound hexoses in the cell membrane provides hydrophobic areas, whereas protein bound hexosamine provides cationic charges on

the cell membrane surface and make the membrane more polar. The elevated level of hexoses in diabetic rats may be associated with disturbances with carbohydrate metabolism. Treatment with carvone and glyclazid in diabetic rats showed significantly decreased hexoses due to improved glycemic control.

Hexosamine a nitrogenous sugar in which an amino group replaces a hydroxyl group. It is an excellent precursor for use in the investigation of synthesis of plasma and tissue glycoproteins. The level of hexosamine, increased significantly in the plasma and tissues of diabetic rats which may be due to insulin deficiency, this leads to depressed utilization of glucose by insulin-dependent pathway, thereby enhancing the formation of hexose and hexosamine[29]. In diabetic rats treated with carvone and glyclazid showed significantly decreased hexosamines in the plasma and tissues when compared to diabetic rats, which could be due to improved glycemic control. Our results are find in line with the study of reduced hexosamine by improved glycemic control in S allylcysteine treated diabetic rats[30].

Fucose is a member of the group of eight essential sugars the body requires for optimal function of cell-to-cell communication and its metabolism appears to be altered in various diseases such as diabetes mellitus[31]. A raise in fucose levels could be due to increased glycosylation in the diabetic state. Elevated levels of fucose in experimental diabetes were reported by other researchers[32]. In diabetic rats treated with carvone and glyclazid significantly lowered fucose levels, which might be due to increased secretion of insulin. Our results are finding in line with the study of reduced fucose by improved secretion of insulin in coumarin treated diabetic rats[33].

Sialic acid is the terminal residue of the oligosaccharide side chain of glycoproteins and widely occurs in the exposed positions of molecules like hormones, enzymes and also on tissues[34]. Sialic acids play a role in cell-cell recognition, protein targeting, protease resistance, conformational stabilization, adhesion and intracellular signaling events in biological systems. In diabetes mellitus, the tissue concentration of sialic acid was found to decrease significantly. The decrease in the content of sialic acid in tissues may be due to the utilization for the synthesis of fibronectin, which contains sialic acid residues in the core structure. The synthesis of fibronectin was also reported to increase significantly in various tissues of diabetic patients and rats. In our studies, a significant increase in total sialic acid levels in the plasma was observed when compared with the control group. Various factors might cause an elevation in the concentration of plasma sialic acid. Among these factors, the first is an increase in the synthesis of sialic acid in insulin-independent tissues, such as the liver and the brain, and the second is an increase in the activity of sialytransferase, which transfers the sialic acid residues to the glycolipids and glycoproteins[35]. In our study, administration of carvone and glyclazid increased the content of sialic acid in the tissues and decreased the sialic acid level in the plasma by enhancing the secretion insulin level. Insulin has been found to inhibit cytokine-induced acute phase protein synthesis in rat hepatic cell. This may, consequently, account for the decreased plasma sialic acid levels in the STZ-induced diabetic rats. Previous studies show that diosmin, produce the same effect in experimantal diabetic rats[36].

From the above findings, we conclude that oral administration of carvone exhibits great potential as an antidiabetic agent by improving hyperglycemia in STZ-induced diabetic rats. It also improved plasma insulin levels and decreased glycoprotein components in plasma, liver and kidney. This can be used as an effective indicator to show the beneficial effects of carvone in controlling the progression and complications of diabetes.

Conflict of interest statement

The authors of this article do not have any conflict of interest to disclose. No part of the manuscript has been submitted or is under consideration in any other publication.

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