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Original article

Antidiabetic effect of kaempferol a flavonoid compound, on streptozotocin-induced diabetic rats with special reference to glycoprotein components

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Summary. Flavonoids have anti-inflammatory and antioxidative effects and thus may protect against diabetic complications. In our early study, kaempferol at an optimum dose of 100 mg was decreased plasma glucose level and increased the insulin levels and also kaempferol exhibited hypolipidemic action in streptozotocin-diabetic rats after 45 days of treatment. The aim of this study was planned to investigate the effect of kaempferol a flavonoid compound on plasma and tissues glycoprotein components in streptozotocin (STZ)-induced diabetic rats. Diabetes was induced into adult male albino rats of the Wistar strain, weighing 180–200 g, by intraperitoneal administration of streptozotocin (40 mg/kg of body weight (BW)). Kaempferol at a dose of 100 mg/kg BW was administered once daily for 45 days to normal and STZ-induced diabetic rats. The levels of plasma and tissues (liver, kidney and heart) glycoproteins containing hexoses, hexosamines and fucose were increased significantly in diabetic rats. In addition, the level of sialic acid significantly increased in plasma, liver and heart while decreased in kidney of STZ-induced diabetic rats. Treatment of kaempferol to diabetic rats, the above metabolic alteration of glycoprotein reverted towards normal level. These results indicate that kaempferol can potentially ameliorate glycoprotein abnormalities related to the risk of diabetes mellitus.

Key words: Plasma glucose, diabetes, glycoproteins, kaempferol, streptozotocin

«Effetto antidiabetico di kaempferolo, un composto flavonoide, in ratti con diabete da streptozotocina con particolare riferimento ai componenti glicoproteici»

Riassunto. I flavonoidi hanno effetti anti-infiammatori e antiossidanti e possono pertanto avere un ruolo protettivo nelle complicanze del diabete. Nel nostro studio iniziale, il kaempferolo, ad una dose ottimale di 100 mg, ha diminuito il livello di glucosio nel sangue, ha aumentato i livelli di insulina e ha mostrato un'azione ipolipemizzante in ratti con diabete streptozotocina-indotto dopo 45 giorni di trattamento. Lo scopo di questo studio è quello di studiare l'effetto del kaempferolo, un composto flavonoide, sui componenti glicoproteici plasmatici e tissutali in ratti con diabete streptozotocina (STZ)-indotto. Il diabete è stato indotto in ratti adulti albini maschi del ceppo Wistar, del peso di 180-200 g, mediante somministrazione intraperitoneale di streptozotocina [40 mg/kg di peso corporeo (BW)]. Il kaempferolo alla dose di 100 mg/kg BW è stato somministrato 1 volta al giorno per 45 giorni a ratti normali e diabetici STZ-indotti. I livelli di glicoproteine plasmatiche e tissutali (fegato, rene e cuore) contenenti esosi, esosamine e fucosio sono aumentate in modo significativo nei ratti diabetici. Inoltre, il livello di acido sialico è significativamente aumentato nel plasma, fegato e cuore, mentre è diminuito nei reni dei ratti diabetici STZ-indotti.

Il trattamento dei ratti diabetici con kaempferolo riporta l'alterazione metabolica delle glicoproteine, vista qui sopra, a livelli normali. Questi risultati indicano che kaempferolo potenzialmente può migliorare le alterazioni glicoproteiche correlate al rischio di diabete mellito.

Parole chiave: Glicemia, diabete, glicoproteine, kaempferolo, streptozotocina

Introduction

The elevated level of blood glucose in diabetes is the major causal factor for the development of diabetic complications (1, 2). Diabetes mellitus is possibly the world's largest growing metabolic disorder, and as the knowledge on the heterogeneity of this disorder is advanced, the need for more appropriate therapy increases (3). In spite of the availability of various antihyperglycemic agents, diabetes and its secondary complications continue to be a major problem in the world population. Glycoproteins are carbohydrate linked protein macromolecules found in the cell surface, which is the principal component of animal cells. Hexoses, hexosamines, fucose and sialic acid are the basic components of the glycoproteins. In these glycoproteins 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 (4). Its also play a major role in the pathogenesis of diabetes mellitus due to impaired metabolism (5). Previous study has reported that alterations occur in the concentrations of various glycoproteins in human diabetes (6). Recent studies have suggested that elevated levels of glycoproteins in diabetic condition could be a consequence of impaired carbohydrate metabolism (7). Insulin deficiency and high levels of plasma glucose in the diabetic condition may result in an increased synthesis of glycoproteins (8). Thus, increase glycoproteins synthesis has been associated with the severity and duration of diabetes.

Medicinal plants and their bioactive constituents are used for the treatment of diabetes through out the world and popularized as neutraceutical. Many indigenous medicinal plants have been used to successfully manage diabetes (9, 10). In addition, many of the currently available drugs have been derived directly or indirectly from plant source. Even the discovery of the widely used hypoglycemic drug metformin came from the traditional approach of using Galegaofficinalis (11). Flavonoids a group of natural substances with variable phenolic structures, which is found in fruits, vegetables, grains, bark, roots, stems, flowers, tea, and wine. These natural products were known for their beneficial effects on health long before flavonoids were isolated as the effective compounds. More than 4000 varieties of flavonoids have been identified (12). Kaempferol a (Fig. 1) flavonoid and its naturally occurs in a variety of fruits, vegetables, wine and tea. It can be isolated from tea, broccoli, witch-hazel, propslis, grapefruit, and other plant source (13). The medicinal properties of kaempferol contain antioxidant (14), anti-inflammatory (15) and anticancer activity (16). Several studies have indicated that intake of kaempferol containing foods is associated with reductions in mortality, the incidence of myocardial infarction (17) and the incidence of cerebrovascular disease (18), as well as a slightly reduced risk of

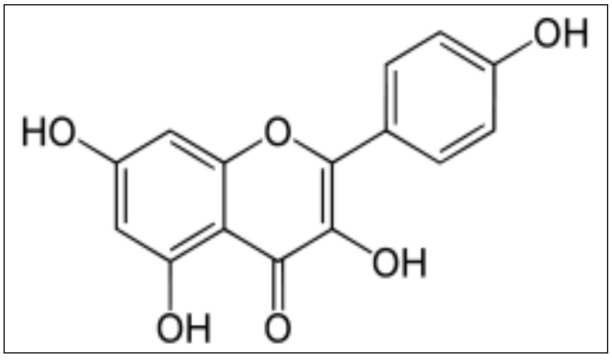

Figure 1. Chemical structure of kaempferol

coronary heart disease (19). Previously *in vitro* study was shown that kaempferol to ameliorate hyperglycemia by improved insulin stimulated glucose uptake in adipocytes (20). Kaempferol also beneficial role on diabetes by preventing oxidative damage in pancreatic β cells (21).

In our early study, kaempferol at an optimum dose of 100 mg was decreased plasma glucose level and increased the insulin levels and also kaempferol exhibited hypolipidemic action in streptozotocin-diabetic rats after 45 days of treatment (22). No study has been conducted on the effect of kaempferol on glycoprotein components in diabetic rats. Hence, the present study was designed to investigate the effect of kaempferol on plasma and tissues glycoprotein components in streptozotocin-induced diabetic rats.

Materials and methods

Drugs and Chemicals

STZ and kaempferol were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals were of analytical grade.

Experimental animals

Male albino rats of Wistar strain of body weight (BW) ranging from 180 to 200 g were procured from Central Animal House, King Saud University, and they were maintained in an air-conditioned room (25 ± 1°C) with a 12-hour light/12-hour dark cycle. The animals were fed *ad libitum* with normal laboratory pellet diet used in the study and procedures involving animals and their care were accordance with the Policy of Research Centre, King Saud University.

Experimental induction of diabetes

Adult (180-200 g) male Wistar albino rats were rendered diabetic via an intraperitoneal injection of STZ, (40 mg/kg BW). Diabetes was confirmed in STZ-induced rats by assess the fasting plasma glucose concentrations at 96 h post-injection. Albino rats with plasma glucose levels above 220 mg/dL were considered diabetic and were used in the experiments.

Experimental Design

The animals were randomly divided into five groups consisting of six animals each. Kaempferol at a dose of 100 mg/ Kg BW or glibenclamide was dissolved in 5% DMSO and administered by intubation (p.o.) once a day, between 9 a.m. and 10 a.m., for 45 days.

Group I: Normal rats (5% DMSO alone)

Group II: Normal rats + kaempferol (100 mg/kg BW)

Group III: Diabetic control

Group IV: Diabetic rats + kaempferol (100 mg/kg BW)

Group V: Diabetic rats + glibenclamide (600 μg/kg BW)

After 45 days administration of kaempferol and glibenclamide, the rats were fasted for 12 h, anesthetized by ketamine (24 mg/kg BW via intramuscular injection) and sacrificed by decapitation. Blood sample was collected in tubes containing a mixture of potassium oxalate and sodium fluoride (1:3) for the estimation of glycoprotein components. Tissue was sliced into pieces and homogenised in appropriate buffer in cold condition (pH 7.0) to give 20% homogenate. The homogenate were centrifuged at 1000 rpm for 10 min at 0°C in cold centrifuge. The supernatant was separated and used for various biochemical estimations.

Biochemical assays

Total hexoses, hexosamines, fucose and sialic acid were estimated by the methods of Niebes (23), Elson and Morgon (24), Dische and Shettles (25) and Welmer *et al*. (26), respectively.

Statistical analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) using SPSS software package 9.05. Results were expressed as mean ± S.D. from six rats in each group. P values < 0.05 were considered as significant.

Results

Effect of kaempferol on total hexoses and hexosamines

Tables 1 and 2 represent the levels of total hexoses and hexosamines in the plasma and tissues (liver, kidney and heart) of normal and STZ-diabetic rats. The diabetic rats had increased levels of total hexoses and hexosamines in the plasma and tissues while treatment with kaemferol and glibenclamide showed reversal of these parameters towards normal levels.

Effect of kaempferol on fucose and sialic acid

Tables 3 and 4 represent the levels of fucose and sialic acid in the plasma and tissues (liver, kidney and heart) of normal and STZ-diabetic rats. The diabetic rats had increased levels of sialic acid (except kidney) and fucose in the plasma and tissues while treatment with kaemferol and glibenclamide showed reversal of these parameters towards normal levels.

Table 1. Effect of kaempferol on total hexoses in the plasma and tissues of control and STZ-induced diabetic rats.

Name of the group	Plasma (mg/dL)	Liver (mg/100 g)	Kidney (mg/100 g)	Heart (mg/100 g)
Normal control	101.20 ± 8.41^{a,d}	19.52 ± 1.25^{a}	18.45 ± 1.50^{a}	16.20 ± 1.03^{a}
Normal + kaempferol (100 mg/kg/d)	98.35 ± 7.08^{a}	19.02 ± 1.16^{a}	17.81 ± 1.19^{a}	15.91 ± 1.47^{a}
Diabetic control	152.14 ± 12.60^{b}	41.26 ± 3.12^{b}	34.28 ± 3.06^{b}	37.73 ± 2.81^{b}
Diabetic + kaempferol (100 mg/kg/d)	113.51 ± 10.15^{c}	23.84 ± 2.12^{c}	21.95 ± 1.50^{c}	19.57 ± 1.85^{c}
Diabetic + glibenclamide (600 μg/kg/d)	108.13 ± 9.05^{c,d}	21.05 ± 1.85^{d}	20.90 ± 1.62^{c}	17.06 ± 1.30^{a}

Values are given as means ± S.D from six rats in each group. Values not sharing a common superscript vertically differ significantly at p< 0.05 (DMRT).

Table 2. Effect of kaempferol on hexosamines in the plasma and tissues of control and STZ-induced diabetic rats.

Name of the group	Plasma (mg/dL)	Liver (mg/100 g)	Kidney (mg/100 g)	Heart (mg/100 g)
Normal control	56.15 ± 4.02^{a}	7.28 ± 0.50^{a}	7.54 ± 0.58^{a}	11.47 ± 0.97^{a}
Normal + kaempferol (100 mg/kg/d)	54.78 ± 3.54^{a}	7.15 ± 0.35^{a}	7.31 ± 0.45^{a}	11.20 ± 1.01^{a}
Diabetic control	72.36 ± 6.08^{b}	14.85 ± 1.02^{b}	17.09 ± 1.60^{b}	28.82 ± 2.08^{b}
Diabetic + kaempferol (100 mg/kg/d)	59.14 ± 5.10^{a}	8.84 ± 0.75^{c}	9.36 ± 0.85^{c}	9.95 ± 0.85^{c}
Diabetic + glibenclamide (600 μg/kg/d)	57.51± 3.88^{a}	8.59 ± 0.55^{c}	8.55 ± 0.62^{d}	9.30 ± 0.63^{c}

Values are given as means ± S.D from six rats in each group. Values not sharing a common superscript vertically differ significantly at p< 0.05 (DMRT).

Table 3. Effect of kaempferol on sialic acid in the plasma and tissues of control and STZ-induced diabetic rats.

Name of the group	Plasma (mg/dL)	Liver (mg/100 g)	Kidney (mg/100 g)	Heart (mg/100 g)
Normal control	51.13 ± 4.25a	7.80 ± 0.60a	5.88 ± 0.48a	8.10 ± 0.66a
Normal + kaempferol (100 mg/kg/d)	50.02 ± 4.14a	7.35 ± 0.53a	5.76 ± 0.36a	7.94 ± 0.51a
Diabetic control	72.35 ± 6.45b	18.07 ± 1.02b	3.25 ± 0.27b	18.52 ± 1.43b
Diabetic + kaempferol (100 mg/kg/d)	59.10 ± 4.08c	9.25 ± 0.75c	5.20 ± 0.35c	10.35 ± 0.91c
Diabetic + glibenclamide (600 μg/kg/d)	56.38 ± 3.90c	8.44 ± 0.48a,c	5.31 ± 0.40a,c	9.74 ± 0.70 c

Values are given as means ± S.D from six rats in each group. Values not sharing a common superscript vertically differ significantly at p< 0.05 (DMRT).

Table 4. Effect of kaempferol on fucose in the plasma and tissues of control and STZ-induced diabetic rats.

Name of the group	Plasma (mg/dL)	Liver (mg/100 g)	Kidney (mg/100 g)	Heart (mg/100 g)
Normal control	16.25 ± 1.20[a]	12.65 ± 1.09[a]	9.75 ± 0.63[a]	18.90 ± 1.54[a]
Normal + kaempferol (100 mg/kg/d)	15.88 ± 1.31[a]	12.18 ± 0.95[a]	9.51 ± 0.80[a]	18.06 ± 1.10[a]
Diabetic control	26.91 ± 2.10[b]	23.56 ± 2.15[b]	28.20 ± 2.05[b]	32.56 ± 2.85[b]
Diabetic + kaempferol (100 mg/kg/d)	18.54 ± 1.65[c]	14.78 ± 1.30[c]	12.04 ± 0.94[c]	22.12 ± 1.70[c]
Diabetic + glibenclamide (600 µg/kg/d)	17.93 ± 1.45[c]	13.29 ± 1.78[d]	10.65 ± 1.06[d]	20.58 ± 1.52[d]

Values are given as means ± S.D from six rats in each group. Values not sharing a common superscript vertically differ significantly at p< 0.05 (DMRT).

Discussion

Diabetes mellitus is a common endocrine disorder characterized by hyperglycemia, metabolic abnormalities and long-term complications afflicting the eyes, kidneys, nerves, heart and blood vessels (27). Hyperglycaemia in diabetes mellitus involves the overproduction (excessive hepatic glycogenolysis and gluconeogenesis) and decreased utilization of glucose by the tissues (28), and studies have shown that the level of blood glucose was elevated and the level of plasma insulin was decreased in STZ-induced diabetic rats (29, 30). In our early study, kaempferol at an optimum dose of 100 mg was decreased plasma glucose level and increased the insulin levels and also kaempferol exhibited hypolipidemic action in streptozotocin-diabetic rats after 45 days of treatment (22). Studies demonstrated that flavonoid compounds act against diabetes mellitus either through their capacity to avoid glucose absorption or to improve glucose tolerance. *In vitro* studies have shown that a soybean extract containing the isoflavones genistein and daidzein inhibits glucose absorption into the intestinal brush border membrane vesicles of rabbits (31). Naringenin was also found to reduce glucose uptake in the intestinal brush border membrane vesicles of diabetic rats to a level similar to that of normal rats (32). Kaemferol a flavonoid and it may be involved to avoid glucose absorption or to improve glucose tolerance by stimulation of insulin secretion. Previously *in vitro* study was shown that kaempferol to ameliorate hyperglycemia by improved insulin stimulated glucose uptake in adipocytes (20).

Hyperglycemia in experimental diabetic rats leads to a lack of glucose utilization by insulin deficiency or reduced insulin activity, thereby enhancing the glycoproteins formation (33). 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 and or a decrease in the metabolism of glycoproteins could be related to the deposition of these materials in the basal membrane of pancreatic cells (8). Abnormal levels of glycoproteins are important in the pathogenesis of liver, kidney and heart diseases in diabetes. Abnormalities in the metabolism of glycoproteins are observed in both naturally occurring and experimental diabetes (34). In this study, we have observed the increased levels of hexoses, hexosamines, fucose and sialic acid (except kidney) in the plasma and tissues of streptozotocin induced diabetic rats. The raised plasma glycoprotein component has been associated with the severity and duration of diabetes. The secretion or shedding from cell membrane glycol conjugates into the circulation leads to the elevation of plasma glycoprotein components. STZ-induced diabetic rats exhibited a significant modification in the connective tissue macro-molecule (35). This is due to the depressed utilization of glucose by insulin dependent pathways leads to increase the formation of hexoses, hexosamines, sialic acid and fucose for the accumulation of glycoproteins (36). Diabetic rats treated with kaemferol showed significantly decreased levels of plasma glycoprotein components, which could be due to improved glycemic control with increased levels of insulin. Previous study was reported that kaempferol to ameliorate hyperglycemia by improved insulin stimulated glucose uptake in adipocytes (37).

The liver is a major site of plasma glycoprotein biosynthesis which producing a large amount of gly-

coproteins in blood. In diabetic condition the levels of plasma glycoproteins have been elevated which could be a consequence of abnormal carbohydrate metabolism (4). Glucosamine synthesized from glucose is an insulin-dependent pathway through the presence of glucose-6-phosphatase enzyme. It is therefore conceivable that, in insulin deficiency as in diabetes mellitus glucose is redirected to an insulin-dependent pathway. This could lead to the accumulation of high levels of glucose in the blood, which may result in an increased synthesis of glycoproteins (37). Hexoses, hexosamines, and sialic acid are the basic components of the glycosaminoglycans and glycoproteins. They play an important role in membrane transport, cell differentiation and recognition, the adhesion of macromolecules to cell surface and the secretion and absorption of macromolecules. Previously have been reported the increased depositions of these components in the liver of STZ-induced diabetic rats (4). Administration of kaemferol to diabetic rats showed significantly decreased the levels of glycoprotein components in liver. The decreased levels of glycoprotein components in liver this may be kaemferol to increase the insulin level and enhance glucose utilization. In this context, previous reports have shown that a decrease in hyperglycaemia could lead to a decrease in glycoprotein levels (38).

Renal disease is one of the most common and severe complications in diabetes (30). Diabetes mellitus affects the kidney by the factors such as oxidative stress, abnormal lipid metabolism and renal accumulation of lipids and others, abnormal glycoprotein metabolism which results in the pathogenesis of diabetic nephropathy (39). The excess availability of glucose in the diabetic state accelerates the synthesis of basement membrane components, i.e., glycoproteins which leads to the thickening of capillary basement membrane of pancreatic beta cells (40). The kidney luminal surface of epithelial cells also lined with a thick carbohydrate rich glycoprotein layer (36). Stimulation of kidney protein synthesis may contribute to explain the increase in the synthesis of glycoproteins in basement membrane as well as the renal hypertrophy that occurs early in diabetes (41). In this study diabetic rats showed increased level of hexoses, hexosamines and fucose while decreased level of sialic acid in kidney. The elevated level of hexoses, hexosamines and fucose in kidney might be due to insulin deficiency and high levels of plasma glucose. Sialic acid contributes to the negative charges on this membrane, thus possibly playing a role in the selective glomerular permeability to negative charged proteins (42). It has been postulated that an increased activity of sialidase, an enzyme which catalyses the removal of sialic acid residues from sialoconjugates which might be responsible for the depletion of glomerular sialic acid (43). Administration of kaemferol to diabetic rats significantly decreased the levels of hexoses, hexosamines and fucose while significantly decreased the level of sialic acid in kidney. This may be due to the improved glycemic control and increased insulin level.

Diabetes is associated with increased atherosclerosis and other causes of myocardial dysfunction. The pathogenesis of cardiovascular disease (CVD) in diabetes is multifactorial and can be affected by increased oxidative stress, abnormal lipid metabolism and abnormal glycoprotein metabolism and other factors. Studies have shown that glycoproteins are involved in the myocardial necrosis and repair (44). In extracellular matrix the glycation produces changes in macromolecular structure affecting matrix-matrix and matrix cell interactions associated with decreased elasticity and increased fluid filtration across the arterial wall and endothelial cell adhesion (45). When the concentration of advanced glycation end-product (AGEs) increased above a critical level, cell surface AGE receptors become activated. Prolonged elevation of blood glucose in diabetes may result in structural and functional alterations of membrane bound proteins (40). In this study we have observed increased level of hexoses, hexosamines, fucose and sialic acid in the heart of diabetic rats. These increased levels of glycoproteins due to the insulin deficiency and high levels of plasma glucose in diabetic condition (8). In this study diabetic rats showed increased level of hexoses, hexosamines, fucose and sialic acid in heart which could be due to insulin deficiency and high levels of plasma glucose. Treatment with kaemferol to diabetic rats observed significantly decreased the levels of glycoprotein components. This may be due to the activation of glucose transport mechanism and also alters insulin binding receptor specificity (46).

The conclusion of this study oral administration of kaemferol significantly lowered the glycoprotein

components (except sialic acid in kidney) levels in circulation and tissues. This indicates its potent role in carbohydrates and glycoproteins metabolism. The observed effect of kaemferol on reversing the adverse effects of hyperglycemia provides an insight into the pathogenesis of diabetic complications, and may be used to advantage in therapeutic approaches.

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