K.S. AL-NUMAIR,
C. VEERAMANI, M.A. ALSAIF,
G. CHANDRAMOHAN

Influence of kaempferol on lipid metabolic changes in streptozotocin-induced diabetic rats

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TITOLO
Influenza del kaempferolo sui cambiamenti metabolici lipidici in ratti affetti da diabete indotto da streptozotocina

KEY WORDS
Diabetes, STZ, dyslipidemia, triglyceride, free fatty acid, phospholipids, kaepmferol

PAROLE CHIAVE
Diabete, STZ, dyslipidemia, triglyceride, acidi grassi liberi, fosfolipidi, kaempferolo

Department of Community Health Sciences, College of Applied Medical Sciences,
King Saud University, P.O. Box 10219, Riyadh 11433, Saudi Arabia.

Address for correspondence:
Dr. G. Chandramohan, Ph.D
Assistant Professor
Department of Community Health Sciences
College of Applied Medical Sciences
King Saud University
P.O. Box 10219, Riyadh 11433
Saudi Arabia
Fax: +96614697502 - Off: +96614697434
Mobile: +966508393530
E-mail: gcmohanphd@yahoo.com

Summary
Diabetes mellitus is associated with dyslipidemia, which is a significant risk factor for cardiovascular complications. This study was designed to investigate the effect of kaempferol on plasma and tissues lipid profiles in streptozotocin-induced diabetic rats. Diabetes was induced in adult male albino rats of the Wistar strain, weighing 180–200 g, by administration of streptozotocin (STZ) (40 mg/kg of body weight) intraperitoneally. The increased levels of plasma glucose and decreased levels of insulin were observed in diabetic rats and treatment with kaempferol significantly decreased the plasma glucose and increased the insulin levels towards normalcy. The levels of total cholesterol, triglycerides, free fatty acids and phospholipids were assayed in the plasma and tissues (liver, kidney and heart) besides lipoprotein-cholesterol (high density lipoprotein-cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C) and very low density lipoprotein-cholesterol (VLDL-C)) were assayed in plasma. Total cholesterol, triglyceride, free fatty acid and phospholipid (LDL-C and VLDL-C in plasma only) levels significantly were increased in plasma and tissues, while plasma HDL-cholesterol significantly decreased in diabetic rats. Treatment with kaempferol prevented the above changes and improved towards normalcy. These results indicate that kaempferol can potentially ameliorate lipid abnormalities related to the risk of diabetes mellitus.

Riassunto
Il diabete mellito è correlato con la dislipidemia, un significativo fattore di rischio per le complicanze cardiovascolari. Questo studio è stato disegnato per investigare l'effetto del kaempferolo sui profili lipidici e plasmatici tissutali in ratti affetti da diabete indotto da streptozotocina. Il diabete è stato indotto in ratti albini maschi adulti del ceppo Wistar, del peso di 180-200 g, mediante somministrazione di streptozotocina (STZ) (40 mg/kg di peso corporeo) per via intraperitoneale. Nei ratti diabetici sono stati osservati un aumento dei livelli di glucosio nel plasma e una

diminuzione dei livelli di insulina e il trattamento con kaempferolo ha diminuito significativamente la glicemia e ha aumentato i livelli di insulina verso la normalità. Sono stati analizzati nel plasma e nei tessuti (fegato, rene e cuore) i livelli di colesterolo totale, trigliceridi, acidi grassi liberi e fosfolipidi oltre ai livelli di colesterolo HDL (HDL-C), colesterolo LDL (LDL-C) e colesterolo VLDL (VLDL-C) che sono stati analizzati nel plasma. I livelli di colesterolo totale, trigliceridi, acidi grassi liberi e fosfolipidi (LDL-C e VLDL-C solo nel plasma) sono aumentati in modo significativo nel plasma e nei tessuti, mentre il HDL-C plasmatico è diminuito significativamente nei ratti diabetici. Il trattamento con kaempferolo ha impedito i cambiamenti sopraccitati e ha migliorato verso la normalità. Questi risultati indicano che kaempferolo può potenzialmente migliorare le dislipidemie correlate al rischio di diabete mellito.

Introduction

The worldwide epidemic of type 2 diabetes (NIDDM) has been stimulating the search for new concepts and targets for the treatment of this incurable disease. Globally diabetes has shadowed the spread of modern lifestyle and it can be linked to an increase overweight and sedentary population (1). Hyperglycemia and hyperlipidemia are two important characters of diabetes mellitus, an endocrine based disease. Diabetic patients experience various vascular complications, such as atherosclerosis, diabetic nephropathy and neuropathy (2). It is now well established that the hyperlipidemia represents a major risk factor for the premature development of atherosclerosis and its cardiovascular complications (3, 4).

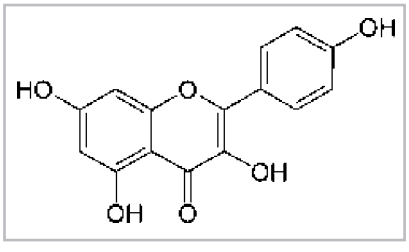
Accumulation of lipids in diabetes is mediated through a variety of derangements in metabolic and regulatory processes, especially insulin deficiency, thereby rendering the diabetic patient more prone to hypercholesterolemia and hypertriglyceridemia (5). One of the major pathogenesis of lipid metabolism disturbances in diabetes is the increased mobilization of fatty acids from adipose tissue and secondary elevation of free fatty acid level in the blood (6). Excessive lipolysis has been found to occur during diabetes. One of the consequences of excessive mobilization of fatty acid is the production of ketone bodies in the liver. The excessive lipolysis in diabetic adipose tissue leads to increase free fatty acids in circulation. They enter the liver and are esterified to form triglycerides (7). Fatty acids are required for both the structure and function of every cell in the body and they form an important component of cell membranes. Several authors have reported that, the fatty acid compositions of various tissues are altered in both experimental and human diabetes (8, 9).

Plant-based pharmaceuticals have been employed in the management of various mankind diseases (10). 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 (11). 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 (12). The medicinal properties of kaempferol contain antioxidant (13, 14, 15), anti-inflammatory (16, 17, 18) and anticancer activity (19, 20). Several studies have indicated that intake of kaempferol containing foods is associated with reductions in mortality, the incidence of myocardial infarction (21) and the incidence of cerebrovascular disease (22), as well as a slightly reduced risk of coronary heart disease (23). Previously *in vitro* study was shown that kaempferol to ameliorate hyperglycemia by improved insulin stimulated glucose uptake in adipocytes (24). Kaempferol also beneficial role on diabetes by preventing oxidative damage in pancreatic cells (25).

No study has been carried out on the effect of kaempferol on lipid profile in diabetic rats. Hence, the present study was designed to investigate the effect of kaempferol on lipid profile in plasma and tissues such as liver, kidney and heart of STZ-diabetic rats, which was compared with glibenclamide as an anti-diabetic drug.

Figura 1 - Shows the chemical structure of kaempferol



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 body weight). 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
Dose determination study

The animals were randomly separated into seven groups consisting of six animals each. Three different doses of kaempferol (50, 100 and 200 mg/kg BW) and glibenclamide dissolved in 5% dimethyl sulfoxide (DMSO) were administered to the diabetic rats for 45 days. The dose of 100 mg/kg BW showed the maximum glucose lowering effect compared to other two doses. So, the 100 mg/kg BW fixed as an optimum dose and used for further study.

Experimental protocol for further study

The animals were randomly divided into five groups consisting of six animals each. Kaempferol 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.

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 plasma glucose and insulin or ethylene diamine tetra acetic acid (EDTA) for the estimation of plasma lipid profiles. Tissues lipids were extracted by the methods of Folch et al. (28) and used for various biochemical estimations.

Biochemical assays

Glucose was estimated by the method of Trinder using reagent Kit (26). Plasma insulin was assayed by the method of Burgi et al. (27). Plasma and tissue lipids were extracted by the methods of Folch et al. (28). Plasma and tissue total cholesterol, triglycerides, free fatty acids, and phospholipids were estimated by the methods of Siedel et al. (29), Foster and Dunn (30), Falholt et al. (31), and Zilversmit and Davis (32), respectively. Plasma high density lipoprotein-C was estimated by the method of Warnick et al. (33). Low density lipoprotein-C and very low density lipoprotein-C were calculated by Friedwald et al. (34) formula.

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.

Tabella A - Dose determination study

Group I:	Normal rats (5% DMSO alone)
Group II:	Normal + kaempferol (200 mg/kg BW)
Group III:	Diabetic control
Group IV:	Diabetic + kaempferol (50 mg/kg BW)
Group V:	Diabetic + kaempferol (100 mg/kg BW)
Group VI:	Diabetic + kaempferol (200 mg/kg BW)
Group VII:	Diabetic + glibenclamide (600 μg/kg BW)

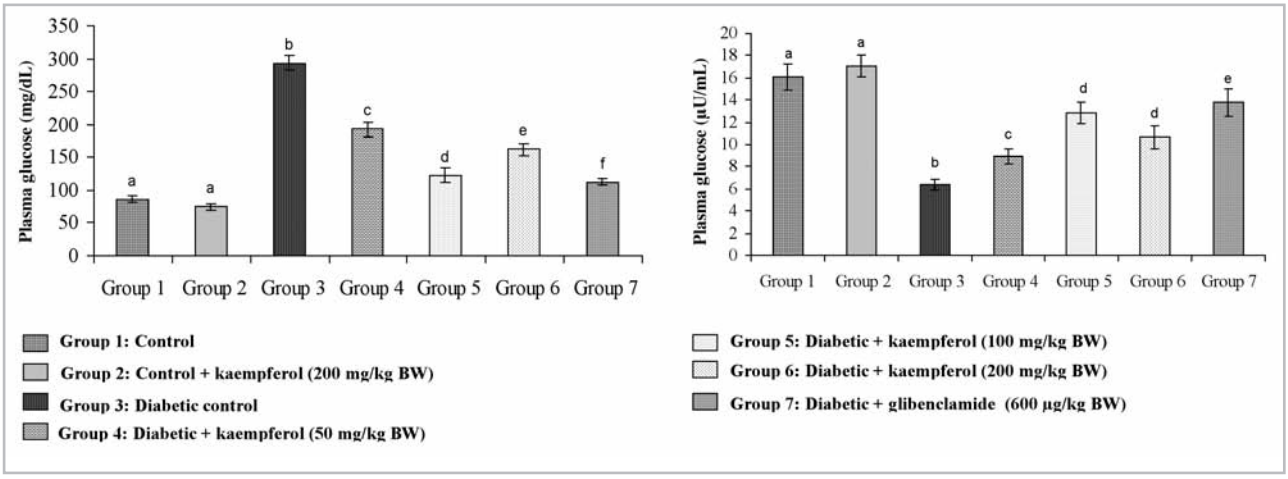
Tabella B - Experimental protocol for further study

Group I:	Normal rats (5% DMSO alone)
Group II:	Normal + kaempferol (100 mg/kg BW)
Group III:	Diabetic control
Group IV:	Diabetic + kaempferol (100 mg/kg BW)
Group V:	Diabetic + glibenclamide (600 μg/kg BW)

Results and discussion

The currently available drug regimens for management of diabetes mellitus have certain drawbacks and therefore there is a need to find safer and more effective antidiabetic drugs (35). The present study was to evaluate the hypoglycemic and hypolipidemic effects of kaempferol in STZ-induced diabetic rats. Figure 2 represents the effect of the 45 days oral administration of kaempferol at three different doses (50,100, 200 mg/kg BW) on plasma glucose and insulin levels in normal and STZ-diabetic rats. Diabetic rats showed an elevated level of plasma glucose and decreased level of insulin when compared to normal rats. Oral administration of kaempferol or glibenclamide in diabetic rats showed decreased plasma glucose and increased insulin levels when compared to the diabetic control rats. The dose of 100 mg/kg BW showed the maximum glucose lowering effect compared to other two doses such 50 and 200 mg/kg BW. So, the 100

Figura 2 - Shows the effect of kaempferol on plasma glucose and insulin in STZ-diabetic rats



mg/kg BW fixed as an optimum dose and used for further study. In kaempferol treated diabetic rats, the significant elevation of plasma insulin may be due to the stimulation of insulin secretion from the existing β-cells of the pancreas. The decrease in blood glucose level of diabetic rats treated with kaempferol might be due to elevated secretion of insulin, which in turn, increases the utilization of glucose by the tissues. Previously *in vitro* study was observed that kaempferol to ameliorate hyperglycemia by improved insulin stimulated glucose uptake in adipocytes (24). Kaempferol also beneficial role on diabetes by preventing oxidative damage in pancreatic cells (25).

Diabetes is associated with profound alterations in the plasma lipid, triglycerides and lipoprotein profile and with an increased risk of coronary heart disease (36, 37). Lowering the plasma lipid levels through dietary or drug therapy appears to be associated with a decrease in the risk of vascular disease (38). Tables 1 and 2 represents the levels of total cholesterol, triglycerides, free fatty acids, phospholipids, HDL-C, VLDL-C and LDL-C in the plasma of diabetic rats. The diabetic rats had elevated levels of plasma total cholesterol, triglycerides, free fatty acids, phospholipids, LDL-C, and VLDL-C and decreased level of HDL-C. Treatment with kaempferol or glibenclamide prevented the above changes in diabetic rats and improved towards normal levels. Normally circulating LDL-C undergoes reuptake in the liver via specific receptors and gets cleared from the circulation (39). This increased LDL concentration in the plasma of diabetic rats might be due to the defect in LDL-C receptor either through failure in its production (or) function. HDL-C is protective by reversing cholesterol transport, inhibiting the oxidation of LDL-C and by neutralizing the atherogenic effects of oxidized LDL-C. A greater increase of LDL-C and VLDL-C may also cause a greater decrease of HDL-C as there is a reciprocal relationship between the concentration of VLDL-C and HDL-C. Decreased HDL-C may also be due to diminished lecithin cholesterol acyl transferase activity. In the present study, the diabetic rats treated with kaempferol showed an elevation in HDL-C and re-

Tabella 1 - Effect of kaempferol on total cholesterol, triglycerides, high density lipoprotein-C, very low density lipoprotein-C and low density lipoprotein-C in the plasma of STZ-diabetic rats

Groups	TC (mg/dL)	TG (mg/dL)	HDL-C (mg/dL)	VLDL-C (mg/dL)	LDL-C (mg/dL)
Control	76.94 ± 4.13a	56.89 ± 4.16a	46.84 ± 2.55a	11.37 ± 0.54a	18.73 ± 1.22a
Control + kaempferol (100 mg/kg BW)	72.02 ± 5.11a	53.54 ± 3.82a	45.12 ± 3.21a	10.70 ± 0.69a	16.20 ± 1.14a
Diabetic control	186.71 ± 8.06b	149.56 ± 8.15b	30.25 ± 2.40b	29.91 ± 2.12b	126.55 ± 8.13b
Diabetic + kaempferol (100 mg/kg BW)	93.56 ± 5.67 c	75.21 ± 5.64c	41.29 ± 2.56c	15.04 ± 1.38c	37.23 ± 2.65c
Diabetic + glibenclamide (600 µg/kg BW)	82.31 ± 4.86 d	72.68 ± 6.51c	43.12 ± 3.15c	14.53 ± 1.85c	24.66 ± 1.75d

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

Tabella 2 - Effect of kaempferol on free fatty acids and phospholipids in the plasma of STZ-diabetic rats

Groups	FFA (mg/dL)	PL (mg/dL)
Control	60.19 ± 4.65a	89.54 ± 6.55a
Control + kaempferol (100 mg/kg BW)	57.86 ± 7.62a	86.37 ± 7.06a
Diabetic control	128.35 ± 9.49b	172.49 ± 11.72b
Diabetic + kaempferol (100 mg/kg BW)	72.14 ± 5.70c	101.86 ± 6.93c
Diabetic + glibenclamide (600 µg/kg BW)	69.10 ± 5.23c	96.15 ± 8.23c

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

duction in LDL-C and VLDL-C. Hence, these observed results evidence for kaempferol could alleviate the risk of cardiovascular diseases.

Tables 3-5 represents the levels of total cholesterol, triglycerides, free fatty acids and phospholipids in the tissues of diabetic rats respectively. The diabetic rats had elevated levels of total cholesterol, triglycerides, free fatty acids and phospholipids in liver, kidney and heart. Treatment with kaempferol or glibenclamide prevented the above changes and improved towards normal levels. It is well known that in uncontrolled type 2 diabetes mellitus, while the levels of total cholesterol increase and contributing to secondary complications (40). High levels of total cholesterol and more importantly LDL-cholesterol in blood and tissues are major coronary risk factors. In our study, we have observed higher levels of total cholesterol in the plasma and tissues of diabetic rats and administration of kaempferol to diabetic rats had

Tabella 3 - Effect of kaempferol on total cholesterol, triglycerides, free fatty acids and phospholipids in the liver of STZ-diabetic rats

Groups	(mg/g wet tissue)			
	TC	TG	FFA	PL
Control	4.24 ± 0.31a	4.18 ± 0.30a	8.31 ± 0.54a	22.14 ± 1.45a
Control + kaempferol (100 mg/kg BW)	4.06 ± 0.19a	3.96 ± 0.29a	8.09 ± 0.35a	19.88 ± 1.75a
Diabetic control	8.14 ± 0.47b	8.13 ± 0.54b	15.26 ± 0.98b	54.29 ± 3.12b
Diabetic + kaempferol (100 mg/kg BW)	5.15 ± 0.36c	5.06 ± 0.36c	9.08 ± 0.78c,d	34.72 ± 2.13c
Diabetic + glibenclamide (600 μg/kg BW)	4.54 ± 0.28a,c	4.43 ± 0.25a,d	8.91 ± 0.56d	25.26 ± 1.57a, d

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

Tabella 4 - Effect of kaempferol on total cholesterol, triglycerides, free fatty acids and phospholipids in the kidney of STZ-diabetic rats

Groups	(mg/g wet tissue)			
	TC	TG	FFA	PL
Control	4.78 ± 0.25a	4.21± 0.29a	4.70 ± 0.32a	14.88 ± 1.34a
Control + kaempferol (100 mg/kg BW)	4.64 ± 0.30a	4.13 ± 0.22a	4.52 ± 0.36a	14.21 ± 1.02a
Diabetic control	8.16 ± 0.46b	7.68 ± 0.55b	11.02 ± 0.65b	29.51 ± 2.11b
Diabetic + kaempferol (100 mg/kg BW)	5.49 ± 0.38c	5.12 ± 0.30c	5.95 ± 0.41c	20.06 ± 1.27c
Diabetic + glibenclamide (600 μg/kg BW)	4.35 ± 0.34d	4.55 ± 0.26d	5.62 ± 0.39c,d	17.69± 1.45d

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

Tabella 5 - Effect of kaempferol on total cholesterol, triglycerides, free fatty acids and phospholipids in the heart of STZ-diabetic rats

Groups	(mg/g wet tissue)			
	TC	TG	FFA	PL
Control	2.52 ± 0.19a	4.46 ± 0.30a	6.64 ± 0.51a	9.86 ± 0.78a
Control + kaempferol (100 mg/kg BW)	2.32 ± 0.10a	4.16 ± 0.39a	6.48 ± 0.40a	9.41 ± 0.81a
Diabetic control	4.56 ± 0.31b	7.02 ± 0.58b	13.24 ± 1.12b	20.56 ± 1.64b
Diabetic + kaempferol (100 mg/kg BW)	3.48 ± 0.22c	3.85 ± 0.28c	7.91± 0.65c	15.64 ± 1.12c
Diabetic + glibenclamide (600 μg/kg BW)	2.89 ± 0.25a	4.82 ± 0.35a,d	7.02 ± 0.53a,d	12.16 ± 1.09d

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

decreased the levels of cholesterol. Hypertriglyceridemia is a common finding in patients with diabetes mellitus and is responsible for vascular complications (41). Bruan and Severson (42) have reported at deficiency of lipoprotein lipase (LPL) activity may contribute significantly to the elevation of triglycerides in diabetes. Lopes-Virella et al. (43) reported that treatment of diabetes with insulin served to lower triglyceride levels by returning lipoprotein lipase levels to normal. Thus decreased triglyceride level by kaempferol treatment might be due to the increased insulin secretion, which in turn increase lipoprotein lipase activity. Previously in vitro study was observed that kaempferol to improved insulin stimulated glucose uptake in adipocytes (24).

Fatty acids undergo changes during the process of injury, repair and cell growth (44). The abnormal high concentration of serum lipids in diabetic subjects is mainly due to the increase in the mobilization of free fatty acids from fat depots (45) since insulin is required for the inhibition of hormone-sensitive lipase. On the other hand, glucagon and other hormones enhance lipolysis. The marked hyperlipidemia that characterizes the diabetic state may, therefore, be regarded as a consequence of uninhibited actions of lipolytic hormones on the fat depots (46). Seigneur et al. (47) have reported that there is a significant alteration in the fatty acid composition of serum and variety of tissues in experimental diabetes. Diabetic rats treated with kaempferol had decreased free fatty acid, which might be due to the increased insulin secretion which, in turn, inhibits hormone-sensitive lipase.

The elevated serum phospholipid levels are a consequence of elevated lipoproteins. Jain et al. (48) suggested that the levels of glycemic control and elevated levels of HDL cholesterol and decreased levels of triglycerides in the blood are significantly correlated with the phospholipid levels. The serum cholesterol/phospholipids ratio is one of the important markers of development of atherosclerosis. Thus decreased phospholipids level by kaempferol treatment may be controlled mobilization of serum triglycerides; controlling the tissue metabolism and improving the level of insulin secretion and action presumably mediate cholesterol and phospholipids.

In conclusion, our findings demonstrate that kaempferol having hypolipidemic effect, which is evidenced by the decreased levels of total cholesterol, triglycerides, free fatty acids, phospholipids, LDL-C, VLDL-C and elevated levels of HDL-C in the plasma and tissues of diabetic rats.

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