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Attenuation of Renoinflammatory Cascade in Experimental Model of Diabetic Nephropathy by Sesamol

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Diabetes has become the most common single cause of end-stage renal disease (ESRD) in the United States and Europe. Approximately 30−40% of patients with type I and 15% with type II diabetes mellitus develop end ESRD. The study was designed to evaluate the impact of sesamol on renal function and renoinflammatory cascade in streptozotocin (STZ)-induced diabetes. STZ-induced diabetic rats were treated with sesamol (2, 4, and 8 mg/kg/day; po) or with vehicle from the fifth to eighth weeks. After 8 weeks, urine albumin excretion, urine output, serum creatinine, blood urea nitrogen, creatinine, and urea clearance were measured. Cytoplasmic and nuclear fractions of kidney were prepared for the quantification of oxidative-nitrosative stress (lipid peroxidation, superoxide dismutase, catalase, nonprotein thiols, total nitric oxide), tumor necrosis factor-alpha (TNF-α), tissue growth factor-1 beta (TGF-β1), p65 subunit of NFκβ, and caspase-3. After 8 weeks of STZ injection, the rats produced significant alteration in renal function, increased oxidative−nitrosative stress, TNF-α, TGF-β1, caspase-3 activity in cytoplasmic lysate, and active p65 subunit of NFκβ in nuclear lysate of kidney of diabetic rats. Interestingly, co-administration of sesamol significantly and dose-dependently prevented biochemical and molecular changes associated with diabetes. Moreover, diabetic rats treated with insulin−sesamol combination produced more pronounced effect on molecular parameters as compared to their respective groups. The data reveal that sesamol modulates the release of profibrotic cytokines, oxidative stress, ongoing chronic inflammation, and apoptosis and thus exerts a marked renoprotective effect.

KEYWORDS: Apoptosis; caspase-3; diabetic nephropathy; sesamol; NFκβ; oxidative−nitrosative stress; TGF-β1; TNF-α

INTRODUCTION

The diabetic pandemic is a major cause of morbidity and mortality worldwide, with diabetic complications being a very important public health issue. Diabetic nephropathy is the largest single cause of end-stage renal disease and a medical catastrophe of worldwide dimensions (*1*). In recent years, our knowledge of the pathophysiological processes that lead to diabetic nephropathy has notably improved on genetic and molecular levels. Thus, the classic view of metabolic and hemodynamic alterations as the main causes of renal injury in diabetes has been transformed significantly, with clear evidence indicating that these traditional factors are only a partial aspect of a much more complex picture (*1*). One of the most important changes is related to the participation of immune-mediated inflammatory processes in the pathophysiology of diabetes mellitus and its complications (*2*, *3*). Although diabetic nephropathy is traditionally considered to be a nonimmune disease, accumulating evidence now indicates that immunologic and inflammatory mechanisms play a significant role in its development and progression (*4*). Therefore, diverse cells, including leukocytes, monocytes, and macrophages as well as other molecules, such as chemokines (monocyte chemoattractant protein-1), adhesion molecules (intercellular adhesion molecule-1, enzymes such as cyclooxygenase-2 and nitric oxide synthase) (*5*, *6*), growth factors (vascular endothelial growth factor, growth hormone, IGF, TGF-β1) (*7*), and nuclear factors (NFκB) (*8*, *9*) are implicated in processes related to diabetic nephropathy.

Despite the benefits derived from the current therapeutics for diabetic nephropathy, mainly strict control of glucose blood pressure and blockade of the renin−angiotensin system, these strategies still provide imperfect protection against renal progression. This imperfection points to the need for newer therapeutic agents that have potential to affect primary mechanisms contributing to the pathogenesis of diabetic nephropathy (*1*). Growing evidence highlights the importance of the inflammatory cascade in the development and progression of diabetic nephropathy. Therefore, investigations into anti-inflammatory strategies may offer new approaches of further effect.

Phenolic compounds are widely present in plants, and they have recently received considerable attention due to their antioxidant property. Sesamol (5-hydroxy-1,3-benzodioxole or 3,4-methylenedioxyphenol) is the major constituent of sesame seed oil, which makes it more resistant to oxidative deterioration than

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other vegetable oils (*10*). Sesamol is a powerful antioxidant and inhibits UV- and Fe^{3+}/ascorbate-induced lipid peroxidation in rat brain (*11*, *12*). Sesamol reduces ferric ions, and its unique solubility in both aqueous and oily phases increases its local concentration in cell membranes and makes it a chain-breaking antioxidant (*12*). Sesamol scavenges hydroxyl and lipid peroxyl radicals and reduces radiation-induced deoxyribose degradation (*13*). It also inhibits the formation of single-strand DNA breaks by γ-radiation (*14*). It has been shown that sesamol inhibits several steps in the generation of neoplasia and mutagenesis (*15*). Recently, sesamol has been shown to possess neuroprotective (*16*), hepatoprotective (*17*), anti-inflammatory (*16*), chemopreventive, and antiaging properties (*18*). In the present study, we focused on the effect of sesamol on the resultant oxidative−nitrosative stress, inflammatory cascade, and apoptotic pathways in the kidneys of streptozotocin-induced diabetic rats.

MATERIALS AND METHODS

Animals. Male Wistar rats (250−280 g), bred in the Central Animal House Facility of Panjab University, Chandigarh, India, were used. The animals were housed under standard laboratory conditions, maintained on a 12 h light/dark cycle, and had free access to food (Hindustan Lever Products, Kolkata, India) and water. The experimental protocols were approved by the Institutional Animal Ethics Committee of Panjab University, Chandigarh, and conducted according to the Indian National Science Academy Guidelines for the use and care of experimental animals.

Drugs. Streptozotocin and sesamol was purchased from Sigma (St. Louis, MO). A glucose oxidase peroxidase diagnostic enzyme kit was purchased from Span Diagnostic Chemicals, India. TNF-α, TGF-β1, and caspase-3 ELISA kits (R&D Systems, Minneapolis, MN), NF-κB/p65 Active ELISA kit (Imgenex Systems), and insulin ELISA kit (DRG, Germany) were purchased. All other chemicals used for biochemical estimations were of analytical grade.

Induction and Assessment of Diabetes. A single dose of 55 mg/kg streptozotocin prepared in citrate buffer (pH 4.4, 0.1 M) was injected intraperitoneally to induce diabetes. The age-matched control rats received an equal volume of citrate buffer and were used along with diabetic animals. Diabetes was confirmed after 48 h of streptozotocin injection; blood samples were collected through the tail vein, and plasma glucose levels were estimated by enzymatic GOD-PAP (glucose oxidase peroxidase) diagnostic kit method. The rats having plasma glucose levels of >250 mg/dL (*19*−*21*) were selected and used for the present study. Streptozotocin (STZ), a β-cytotoxin, increases pancreatic islet O-linked protein glycosylation in a dose-dependent, irreversible fashion and also inhibits GlcNAcase, the enzyme that removes O-GlcNAc from protein. This is the mechanism that accounts for its diabetogenic toxicity (*22*). Body weight, plasma glucose, and insulin levels were measured before and at the end of the experiment to see the effect of sesamol on these parameters.

Treatment Schedule. Rats were randomly selected and divided in eight groups of 8−10 animals each. The first group consisted of nondiabetic control animals; the second group was the diabetic control; the third group comprised diabetic animals treated with insulin (10 IU/kg/day, sc); the fourth, fifth, and sixth groups consisted of diabetic animals treated with sesamol (2, 4, and 8 mg/kg/day; oral gavage, respectively) (*33*); the seventh group consisted of diabetic animals treated with insulin (10 IU/kg/day, sc) and sesamol (8 mg/kg, po); and group eight consisted of diabetic animals treated with vitamin E (α-tocopherol) (100 mg/kg/day, po). Starting from the fifth week after STZ injection until the end of eighth week, the control and diabetic control groups received vehicle of sesamol. Sesamol was dissolved in double-distilled water before administration in a constant volume of 5 mL/kg of body weight. The animals were sacrificed under deep anesthesia, blood was collected by carotid bleeding, and serum was separated. Kidneys were rapidly removed and weighed. Kidneys were incubated with 5 mL of ice-cold 1× hypotonic buffer supplemented with 1 mM DTT and 1% detergent solution for 30 min on ice. After incubation, the samples were centrifuged for 10 min at 10000 rpm at 4 °C. The supernatant (cytoplasmic fraction) was transferred into a 15 mL tube and stored at 4 °C. The nuclear pellet was resuspended in 500 μL of nuclear lysis buffer by pipetting up and down. The samples were vortexed vigorously, and the suspension was incubated at 4 °C for 30 min on a rocking platform and again vortexed for 30 s. The suspension was centrifuged at 14000 rpm for 10 min at 4 °C in a microcentrifuge. The supernatant was transferred (nuclear fraction) into a prechilled microcentrifuge tube. The nuclear fraction was stored at −80 °C until further use. Cytoplasmic and nuclear fractions were used for the quantification of caspase-3 colorimeteric detection, total nitric oxide assay, TNF-α ELISA, TGF-β1 ELISA, and NF$\kappa\beta$ p65 active ELISA. The samples were stored at −80 °C until processed for biochemical estimations.

Biochemical Analysis. *Renal Functions*. At the end of the eighth week, rats were kept individually in metabolic cages for 24 h to collect urine for the measurement of urine output and renal function. Renal function was assessed by measuring plasma and urine levels of creatinine, urea, and urine albumin excretion using semiautoanalyzer (Erba Chem-5 plus; Transasia, Mumbai, India). Creatinine and urea clearance were measured as an index of glomerular filtration rate (GFR). Plasma glucose and insulin levels were also measured at 4 weeks and at the end of the experiment to investigate the effect of sesamol on glucose levels.

Assessment of Oxidative Stress. The malondialdehyde content, a measure of lipid peroxidation, was assayed in the form of thiobarbituric acid-reactive substances (TBARS) according to the method of Okhawa et al. (*23*). TBARS were quantified using an extinction coefficient of 1.56×10^5 M^{-1} cm^{-1} and expressed as nanomoles of malondialdehyde per milligram of protein. Tissue protein was estimated using the biuret method and renal malondialdehyde content expressed as nanomoles of malondialdehyde per milligram of protein. Nonprotein thiols were assayed according to the method of Jollow et al. (*24*). The result was expressed as nanomoles of NPSH per milligram of protein. Cytosolic superoxide dismutase activity was assayed according to the method of Kono et al. (*25*). Catalase activity was assayed according to the method of Claiborne et al.(*26*).

Estimation of Nitrosative Stress: Total Nitric Oxide. The quantification of total nitric oxide was done with the help and instructions provided by the R&D Systems Total Nitric Oxide Assay Kit, which involves the conversion of nitrate to nitrite by the enzyme nitrate reductase. The detection of total nitrite is then determined as a colored azo dye product of the Griess reaction.

Rat Insulin ELISA. Plasma insulin levels were measured by using a Rat Insulin ELISA kit (DRG, Germany). The analysis was done according to the manufacturer's instructions. The results were expressed as picomoles per liter.

Rat TNF-α and TGF-β1 ELISA. The quantification of TNF-α and TGF-β1 in the cytoplasmic fraction was done according to the manufacturer's instructions provided with the Quantikine Rat TNF-α and TGF-β1 immunoassay kits (R&D Systems). The results were expressed as picograms per milligram of protein.

Quantification of NFκB p65 Unit. The nuclear levels of p65 may correlate positively with the activation of the NF-κB pathway. The NF-κB/p65 ActivELISA (Imgenex, San Diego, CA) kit was used to measure NF-κB free p65 in the nuclear fraction. The analysis was done according to the manufacturer's instructions. The results were expressed as nanograms per milligram of protein.

Caspase-3 Colorimetric Assay. The caspase-3 enzymatic activity in the nuclear fraction was carried out by using a caspase-3 colorimetric kit (R&D Systems). The analysis was done according to the manufacturer's instructions. The results were expressed as nanograms per milligram of protein.

Statistical Analysis. Results were expressed as mean ± SEM. The intergroup variation was measured by one-way analysis of variance (ANOVA) followed by Tukey's test. Statistical significance was considered at $P < 0.05$. The statistical analysis was done using SPSS Statistical Software version 14.

RESULTS

Effect of Sesamol on Blood Glucose, Body Weight, Water Intake, Food Intake, Blood Pressure, Insulin Level, and Renal Function of Diabetic Rats. Four weeks after STZ injection,

Table 1. Effect of Sesamol on Plasma Glucose and Plasma Insulin Levels and Renal Functions in Diabetic Rats (Mean ± SEM)[a]

group	control	STZ	STZ + insulin (10)	STZ + sesamol (2)	STZ + sesamol (4)	STZ + sesamol (8)	STZ + insulin (10) + sesamol (8)	STZ + vitamin E (100)
plasma glucose (mg/dL)	111 ± 8	402 ± 11*	199 ± 10**	345 ± 4**	294 ± 6**	222 ± 4**	157 ± 5**	289 ± 8**
plasma insulin (pmol/L)	102 ± 6	25 ± 18*	65 ± 13**	55 ± 25	52 ± 19	43 ± 21	33 ± 12**	29 ± 25
body wt (g)	232 ± 2	171 ± 1*	215 ± 4**	161 ± 10	191 ± 7**	215 ± 3**	234 ± 6**	176 ± 4**
food intake (g)	28 ± 2	58 ± 3*	33 ± 1**	51 ± 2**	42 ± 4**	37 ± 2**	29 ± 2**	44 ± 1**
water intake (mL)	35 ± 1.23	108 ± 1.65*	44 ± 3.02**	93 ± 4.28**	76 ± 2.49**	54 ± 3.54**	42 ± 4.35**	72 ± 3**
urine output (mL)	9 ± 1	43 ± 1*	24 ± 1**	37 ± 1**	28 ± 2**	21 ± 3**	16 ± 3**	28 ± 2**
urine albumin (mg/dL)	3.9 ± 0.5	11.4 ± 0.5*	8.2 ± 0.6**	9.1 ± 0.3**	8.0 ± 0.1**	6.5 ± 0.4**	4.5 ± 0.2**	9.6 ± 0.7**
serum creatinine (mg/dL)	0.31 ± 0.02	1.42 ± 0.11*	0.73 ± 0.03**	1.34 ± 0.13**	0.99 ± 0.05**	0.71 ± 0.06**	0.50 ± 0.06**	0.92 ± 0.12**
blood urea N (mg/dL)	24 ± 1	58 ± 2*	45 ± 1**	53 ± 2**	48 ± 2**	39 ± 2**	28 ± 2**	48 ± 3**
creatinine clearance (mL/min)	0.71 ± 0.01	0.25 ± 0.02*	0.50 ± 0.01**	0.29 ± 0.02**	0.42 ± 0.04**	0.57 ± 0.05**	0.70 ± 0.01**	0.43 ± 0.10**
urea clearance (mL/24 h)	0.8 ± 0.06	0.31 ± 0.06*	0.57 ± 0.05**	0.35 ± 0.06**	0.51 ± 0.03**	0.65 ± 0.04**	0.74 ± 0.05**	0.48 ± 0.07**
blood pressure (mmHg)	112	205*	132**	185	155**	137**	121**	151**

[a] Sesamol (2) = sesamol 2 mg/kg; sesamol (4) = sesamol 4 mg/kg; sesamol (8) = sesamol 8 mg/kg; vitamin E (100) = α-tocopherol 100 mg/kg. *, $P < 0.05$ as compared to control group; **, $P < 0.05$ as compared to streptozotocin (STZ)-treated group.

Table 2. Effect of Sesamol on Lipid Peroxide, Nonprotein Thiols, Superoxide Dismutase, Catalase, and Nitric Oxide Levels (Mean ± SEM)[a]

treatment	LPO (nmol/mg of protein)	nonprotein thiols (mol)	SOD (units/mg of protein)	catalase (k/min)	total NO (μmol/L)
control	43.78 ± 3.42	56.61 ± 4.37	22.34 ± 1.56	8.41 ± 0.06	99 ± 6
STZ	175.45 ± 4.25^{a}	16.34 ± 1.89^{a}	3.98 ± 2.01^{a}	0.87 ± 0.10^{a}	897 ± 24^{a}
STZ + insulin (10)	131.78 ± 3.89^{b}	20.34 ± 2.31^{b}	7.01 ± 1.10^{b}	1.89 ± 0.11^{b}	648 ± 10^{b}
STZ + sesamol (2)	125.34 ± 2.21^{bc}	22.46 ± 1.63^{bc}	7.89 ± 1.03^{bc}	2.27 ± 0.08^{bc}	598 ± 16^{bc}
STZ + sesamol (4)	98.72 ± 3.28^{bc}	32.92 ± 2.12^{bc}	11.32 ± 1.11^{bc}	3.46 ± 0.13^{bc}	443 ± 13^{bc}
STZ + sesamol (8)	67.34 ± 1.78^{bc}	44.79 ± 3.46^{bc}	16.84 ± 1.03^{bc}	5.97 ± 0.09^{bc}	154 ± 17^{bc}
STZ + insulin (10) + sesamol (8)	51.21 ± 2.14^{bd}	52.38 ± 2.98^{bd}	20.11 ± 1.31^{bd}	8.02 ± 0.12^{bd}	109 ± 12^{bd}
STZ + vitamin E (100)	77.62 ± 2.84^{bc}	27.67 ± 2.54^{bc}	10.53 ± 1.10^{bc}	3.82 ± 0.10^{bc}	212 ± 18^{bc}

[a] Sesamol (2) = sesamol 2 mg/kg; sesamol (4) = sesamol 4 mg/kg; sesamol (8) = sesamol 8 mg/kg; vitamin E (100) = α-tocopherol 100 mg/kg. Letters following entries indicate the following: a, different from control; b, different from diabetic; c, different from one another; d, different from their per se group ($P < 0.05$).

diabetic animals exhibited increased blood glucose levels (111 ± 7.94 and 402 ± 10.52 mg/dL; for control and diabetic rats, respectively; $P < 0.01$) and decreased body weight (232 ± 2.15 and 171 ± 1.32 g for control and diabetic rats, respectively; $P < 0.05$) compared with control rats. Chronic treatment of sesamol in diabetic rats from the fifth to the eighth weeks ameliorated plasma glucose levels as well as body weight as compared with vehicle-treated diabetic rats. Treatment with higher doses of sesamol attenuated the decrease in body weight in diabetic rats (**Table 1**). Furthermore, significant increases in food intake, water intake, and systolic blood pressure in STZ-injected rats were attenuated with sesamol treatment.

Diabetic rats exhibited marked polyuria, increased urinary albumin excretion, and high serum creatinine as well as blood urea nitrogen. Chronic treatment with sesamol (2, 4, and 8 mg/kg/day) significantly and dose-dependently reduced diabetic proteinuria and polyuria and increased serum creatinine and blood urea nitrogen. Creatinine and urea clearance were also significantly improved following the administration of sesamol (2, 4, and 8 mg/kg/day) to diabetic rats compared with untreated diabetic rats. (**Table 1**). However, insulin levels were not significantly altered in sesamol-treated diabetic rats. Moreover, diabetic rats treated with an insulin−sesamol combination significantly prevented this alteration in renal function as compared to their respective groups (diabetic rats treated with insulin and sesamol alone).

Effect of Sesamol on Diabetes-Induced Changes in Lipid Peroxidation. TBARS levels were increased significantly in the kidneys of diabetic rats as compared to control group (**Table 2**). Chronic treatment with sesamol produced a significant and dose-dependent reduction in TBARS levels in STZ-treated rats. Sesamol was more potent in inhibiting TBARS than α-tocopherol. However, diabetic rats treated with the insulin−sesamol combination significantly prevented this rise in lipid peroxidation as compared to their respective groups (diabetic rats treated with insulin and sesamol alone) [$F(8,76) = 44.58$ ($P < 0.05$)].

Effect of Sesamol on Diabetes-Induced Changes in the Antioxidant Profile. The nonprotein thiols [$F(8,76) = 35.49$ ($P < 0.05$)] and enzyme activities of superoxide dismutase [$F(8,76) = 21.76$ ($P < 0.05$)] and catalase [$F(8,76) = 38.52$ ($P < 0.05$)] significantly decreased in the kidneys of diabetic rats as compared to the control group (**Table 2**). This reduction was significantly and dose-dependently improved by treatment with sesamol in the kidneys of STZ-treated rats. Sesamol produced more pronounced effects in comparison to α-tocopherol. However, diabetic rats treated with the insulin−sesamol combination significantly restored the endogenous antioxidant profile as compared to their respective groups (diabetic rats treated with insulin and sesamol alone).

Effect of Sesamol on Diabetes-Induced Nitrosative Stress. Total nitric oxide levels were significantly elevated in the diabetic rat kidney (**Table 2**). Sesamol treatment significantly and dose-dependently inhibited this increase in nitric oxide levels, which was further prevented by insulin−sesamol combination in the STZ-treated rats [$F(8,76) = 86.47$ ($P < 0.01$)]. However, sesamol produced more pronounced effects in comparison to α-tocopherol.

Effect of Sesamol on Tumor Necrosis Factor-α (TNF-α) and Tissue Growth Factor-1β (TGF-β1). TNF-α and TGF-β1 (**Figure 1**) levels were significantly elevated in the kidneys of diabetic animals. Sesamol treatment significantly and dose-dependently inhibited TNF-α and TGF-β1 levels in the STZ-treated rats. Sesamol produced more pronounced effects in comparison to α-tocopherol. Moreover, diabetic rats treated with the insulin−sesamol combination more significantly inhibited TNF-α [$F(8,76) = 58.69$ ($P < 0.01$)] and TGF-β1 [$F(8,76) = 135.68$

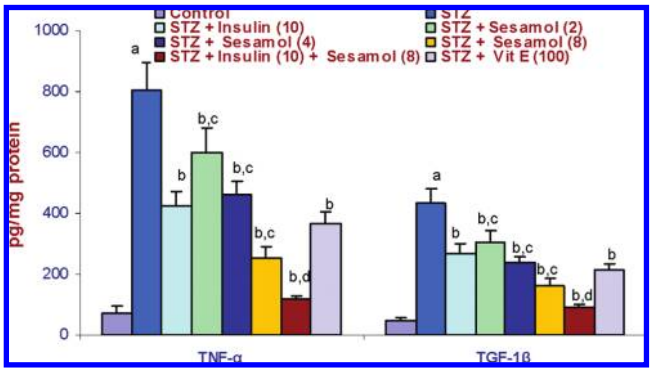

Figure 1. Effect of sesamol and its combination with insulin on TNF-α and TGF-β1 levels in the kidneys of diabetic rats. Data are expressed as mean ± SEM. a, different from control; b, different from diabetic group; c, different from one another; d, different from sesamol and insulin per se groups. Sesamol (2) = sesamol 2 mg/kg; sesamol (4) = sesamol 4 mg/kg; sesamol (8) = sesamol 8 mg/kg; Vit E (100) = α-tocopherol 100 mg/kg.

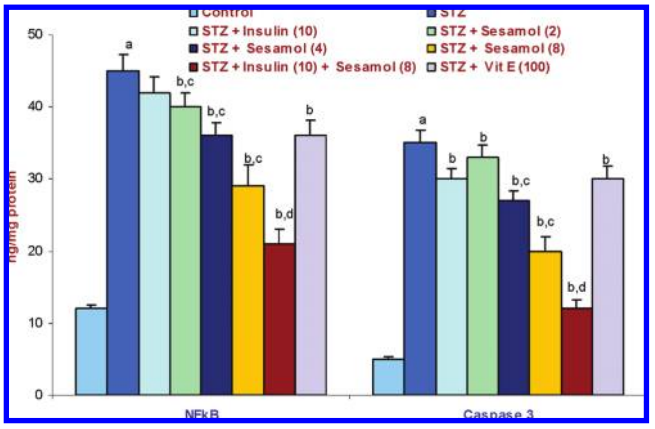

Figure 2. Effect of sesamol and its combination with insulin on p65 subunit of NFκβ and caspase-3 levels in the kidneys of diabetic rats. Data are expressed as mean ± SEM. a, different from control; b, different from diabetic group; c, different from one another; d, different from sesamol and insulin per se groups. Sesamol (2) = sesamol 2 mg/kg; sesamol (4) = sesamol 4 mg/kg; sesamol (8) = sesamol 8 mg/kg; Vit E (100) = α-tocopherol 100 mg/kg.

($P < 0.01$)] levels as compared to their respective groups (diabetic rats treated with insulin and sesamol alone).

Effect of Sesamol on Nuclear Factor κβ (NFκβ). NFκβ p65 subunit was significantly elevated in the kidneys of diabetic animals (**Figure 2**). Sesamol treatment significantly and dose-dependently prevented reactive oxygen species induced NFκβ p65 subunit expression in the nuclear fraction of STZ-treated rats. Sesamol produced more pronounced effects in comparison to α-tocopherol. The levels of NFκβ p65 subunit were further significantly reduced by the insulin−sesamol combination as compared to their respective groups (diabetic rats treated with insulin and sesamol alone) [$F(8,76) = 183.46$ ($P < 0.05$)].

Effect of Sesamol on Caspase-3. Caspase-3 levels were significantly elevated in the diabetic rat kidney (**Figure 2**). Sesamol treatment significantly and dose-dependently inhibited apoptosis in the STZ-injected rats. Sesamol again displayed more marked inhibition of caspase-3 as compared to α-tocopherol. However, diabetic rats treated with the insulin−sesamol combination produced a more pronounced attenuation of caspase-3 expression as compared to their respective groups (diabetic rats treated with insulin and sesamol alone) [$F(8,76) = 87.24$ ($P < 0.05$)].

DISCUSSION

In the present study, STZ-injected rats demonstrated typical characteristics of diabetes mellitus such as hyperglycemia, polyuria, growth retardation, and an increase in urinary albumin excretion. With the onset of diabetes mellitus, there is a subsequent decrease in creatinine and urea clearance. It has also been observed that increased blood urea nitrogen and serum creatinine in diabetic rats indicate progressive renal damage, which is taken as an index of altered glomerular filtration rate (GFR) in diabetic nephropathy. There have been reports that decreased GFR is associated with the formation of reactive oxygen intermediates. Sesamol administration significantly restored renal functions. Our previous studies showed that diabetes induces oxidative−nitrosative stress in rat kidneys (*27*, *28*). In the present study, treatment with sesamol returned the levels of lipid peroxides, nitric oxide, nonprotein thiols, superoxide dismutase, and catalase toward their control values. Sesamol is a powerful antioxidant and inhibits UV- and Fe^{3+}/ascorbate-induced lipid peroxidation in rat brain (*11*, *12*). Sesamol reduces ferric ions, and its unique solubility in both aqueous and oily phases increases its local concentration in cell membranes and makes it a chain-breaking antioxidant (*12*). Sesamol scavenges hydroxyl and lipid peroxyl radicals and reduces radiation-induced deoxyribose degradation (*13*). It also inhibits the formation of single-strand DNA breaks by γ-radiation (*14*). Sesamol attenuated systemic oxidative stress by reducing xanthine oxidase and improving hepatic function in iron-intoxicated mice (*29*).

Tubulointerstitial fibrosis is the final manifestation of end-stage renal disease, and renal injury is correlated to the degree of renal interstitial fibrosis. Transforming growth factor-β1 (TGF-β1) is known to be one of the major mediators that lead to fibrosis. GW788388, a new TGF-β type I receptor inhibitor, significantly reduced renal fibrosis and decreased the mRNA levels of key mediators of extracellular matrix deposition in the kidneys of db/db mice (*30*). Furthermore, experimental studies have consistently reported that mRNA encoding TNF-α and protein levels increase in glomerular and proximal tubule cells from diabetic rats (*31*). These investigations demonstrated a significant role of TNF-α in the development of renal hypertrophy and hyperfunction, two main alterations during the initial stage of diabetic nephropathy (*32*). TNF-α has a stimulatory effect on sodium-dependent solute uptake in cultured mouse proximal tubular cells, and in these studies, diabetic rats exhibited enhanced urinary TNF-α excretion, sodium retention, and renal hypertrophy, which were prevented by administration of the anti-TNF-α agent TNFR:Fc, a soluble TNF-α receptor fusion protein (*32*). Uncontrolled diabetes significantly enhanced TNF-α and TFG-β1 levels in diabetic rat kidneys (*27*, *28*). A significant inhibition of TNF-α and TFG-β1 levels by tocotrienol observed in our study is indicative of the fact that sesamol contributes to beneficial effects seen in diabetic nephropathy.

The final common pathway for progressive renal diseases such as diabetic nephropathy is the development of tubular atrophy and chronic interstitial fibrosis, which is generally preceded by or associated with an inflammatory infiltrate. Increased steady-state mRNA levels of inflammatory genes are shown to be associated with interstitial fibrosis and progressive human diabetic nephropathy (*9*). The transcription factor NF-κβ helps to control the expression of numerous genes activated during inflammation. NF-κβ is induced by various cell stress-associated stimuli including growth factors, vasoactive agents, cytokines, and oxidative stress (*33*). NF-κβ in turn controls the regulation of genes encoding proteins involved in immune and inflammatory responses (i.e., cytokines, chemokines, growth factors, immune

receptors, cellular ligands, and adhesion molecules). The activation and nuclear translocation of NF-$\kappa\beta$ in human diabetic nephropathy have been demonstrated in intrinsic cells of the kidney in human diabetic nephropathy (*3*). Further activation of the NF-$\kappa\beta$ signaling pathway leads to renal dysfunction in the diabetic animals, which is positively correlated with increased oxidative−nitrosative stress and inflammation. In the present study, we observed that sesamol significantly and dose-dependently suppressed NF-$\kappa\beta$ signaling via inhibition of oxidative−nitrosative stress and inflammation. Moreover, the combination of sesamol with insulin produced robust suppression of the TNF-mediated NF-$\kappa\beta$ signaling pathway. Very recently, we have reported that sesamol inhibited tumor necrosis factor α in diabetic encephalopathy (*21*).

To better understand the pathway leading to apoptosis in diabetes, we investigated the expression of apoptosis-related proteins such as caspase-3 in of diabetic rat kidney. It has been demonstrated that high glucose causes the generation of peroxynitrite, leading to caspase-mediated apoptosis. Ebselen and a caspase-3 inhibitor provided significant protection against high glucose-mediated apoptosis, implicating peroxynitrite as a proapoptotic reactive oxygen species in early diabetic nephropathy (*34*). In the present study, diabetes produced a marked increase in caspase-3 activity in the kidneys; this effect was reversed by sesamol. In addition, sesamol was found to reverse these biochemical alterations more potently than vitamin E.

The major finding of the study is that insulin alone corrected the hyperglycemia and partially reversed the renal dysfunction in diabetic rats. However, combination with sesamol not only attenuated the diabetic condition but also reversed renal dysfunction through modulation of TNF-α-induced NF-$\kappa\beta$ signaling pathway and caspase-3 in the diabetic rats and thus it may find clinical application to treat renal dysfunction in diabetic patients.

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