Available online at www.sciencedirect.com

SciVerse ScienceDirect

Journal of Nutritional Biochemistry 23 (2012) 1482–1489

Journal of Nutritional Biochemistry

Sesamol alleviates diet-induced cardiometabolic syndrome in rats via up-regulating PPARγ, PPARα and e-NOS☆

Ashok Kumar Sharma[a,1], Saurabh Bharti[a,1], Jagriti Bhatia[a], Saroj Nepal[a], Salma Malik[a], Ruma Ray[b], Santosh Kumari[c], Dharamvir Singh Arya[a,*]

[a]Department of Pharmacology, Cardiovascular Research Laboratory, All India Institute of Medical Sciences, New Delhi-110029, India
[b]Department of Pathology, All India Institute of Medical Sciences, New Delhi-110029, India
[c]Department of Plant Physiology, Indian Agricultural Research Institute, Pusa, New Delhi-110012, India

Received 1 March 2011; received in revised form 21 July 2011; accepted 27 September 2011

Abstract

Increased oxidative stress and inflammation in obesity are the central and causal components in the pathogenesis and progression of cardiometabolic syndrome (CMetS). The aim of the study was to determine the potential role of sesamol (a natural powerful antioxidant and anti-inflammatory phenol derivative of sesame oil) in chronic high-cholesterol/high-fat diet (HFD)–induced CMetS in rats and to explore the molecular mechanism driving this activity. Rats were fed with HFD (55% calorie from fat and 2% cholesterol) for 60 days to induce obesity, dyslipidemia, insulin resistance (IR), hepatic steatosis and hypertension. On the 30th day, rats with total cholesterol >150 mg/dl were considered hypercholesterolemic and administered sesamol 2, 4 and 8 mg/kg per day for the next 30 days. Sesamol treatment decreased IR, hyperinsulinemia, hyperglycemia, dyslipidemia, TNF-α, IL-6, leptin, resistin, highly sensitive C-reactive protein (hs-CRP), hepatic transaminases and alkaline phosphatase, along with normalization of adiponectin, nitric oxide and arterial pressures in a dose-dependent fashion. Increased TBARS, nitrotyrosine and decreased antioxidant enzyme activities were also amended in HFD rats. Similarly, sesamol normalized hepatic steatosis and ultrastructural pathological alteration in hepatocytes, although the effect was more pronounced at 8 mg/kg. Furthermore, hepatic PPARγ, PPARα and e-NOS protein expressions were increased, whereas LXRα, SERBP-1c, P-JNK and NF-κB expression were decreased by sesamol treatment. These results suggest that sesamol attenuates oxidative stress, inflammation, IR, hepatic steatosis and hypertension in HFD-fed rats via modulating PPARγ, NF-κB, P-JNK, PPARα, LXRα, SREBP-1c and e-NOS protein expressions, thereby preventing CMetS. Thus, the present study demonstrates the therapeutic potential of sesamol in alleviating CMetS.

Keywords: Sesamol; Cardiometabolic syndrome; PPARγ; Insulin resistance; SERBP-1c; e-NOS

1. Introduction

The rising frequency of cardiometabolic syndrome (CMetS) is in direct proportion to the increasing prevalence of obesity and demands the development of multifactorial strategies directed at its underlying etiology. Increased oxidative stress and inflammation in obesity are key factors leading to sequential development of insulin resistance (IR), type 2 diabetes, dyslipidemia, hepatic steatosis, hypertension and, finally, to increased frequency of CMetS, which in turn contribute to an increased morbidity, mortality and escalation in health care costs [1-7]. Dyslipidemia is a continuum of CMetS and is characterized by raised plasma triglycerides (TG), small dense low-density lipoprotein cholesterol (LDL-C) levels, free fatty acids (FFAs) and decreased high-density lipoprotein cholesterol (HDL-C). Previously, fatty liver was considered to be benign, but now it is thought to be a precursor of more advanced liver disease, such as nonalcoholic steatohepatitis (NASH), a condition that may lead to cirrhosis and hepatocellular carcinoma [7,8]. NASH is also associated with persistently elevated liver enzymes such as aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) [8,9].

Several experimental and clinical studies have suggested a strong linkage between the metabolic syndrome and chronic inflammation. The latter is characterized by increased production of various inflammatory mediators such as TNFα, IL-6, leptin, highly sensitive C-reactive protein (hs-CRP) and decreased adiponectin resulting in activation of a network of inflammatory signaling pathways and increased IR [1,6,10]. Furthermore, amplified inflammation and oxidative stress in CMetS also predispose to hypertension by causing NO inactivation, endothelial dysfunction and vasoconstriction [4,5,10,11].

Sesamol (3,4-methylenedioxyphenol), a potent antioxidant and anti-inflammatory phenolic component of lignans and a major constituent of sesame seed oil, has been shown to possess hepatoprotective, cardioprotective, antiatherogenic, chemopreventive and anti-aging properties [12-14]. It has also been demonstrated that sesamol reversed the diabetic state and associated neuropathic

☆ Conflict of interest: None.

* Corresponding author. Tel.: +91 11 26594266; fax: +91 11 26584121.
E-mail address: dsarya16@hotmail.com (D.S. Arya).

[1] These authors contributed equally to this work.

0955-2863/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
doi:10.1016/j.jnutbio.2011.09.011

pain in rats by attenuating oxidative–nitrosative stress and inflammation [12]. Of note, some recent studies reported that sesamol inhibited nuclear factor-κB (NF- κB) and IκB kinase (IKK) activation and up-regulated phosphatidylinositol 3-kinase/Akt/endothelial nitric oxide synthase (e-NOS) pathways [13,14].

In view of the above facts, we undertook the present study to investigate whether sesamol improves diet-induced CMetS in rats, if any, in relation to its effect on oxidative stress, dysregulated adipocytokines production, IR, hepatic steatosis and hypertension, and then explore the complex molecular mechanism responsible for this activity. Overall, our results indicated that sesamol prevents CMetS in high-fat diet (HFD)–fed rats through increasing PPARγ, e-NOS and PPARα, and decreasing phosphorylated c-Jun N-terminal kinases (P-JNK), IKK, NF-κB, liver X receptor α (LXRα), sterol regulatory element binding protein 1c (SREBP-1c) and fatty acid synthase (FAS) protein expressions. Although our results need to be tested further in clinical studies, our findings nevertheless suggest a role for sesamol in the management of CMetS in addition to the conventional medications.

2. Materials and methods

2.1. Animals and diets

Male Wistar albino rats weighing between 140 and 170 g (6–8 weeks old) were obtained from the Central Animal House Facility of All India Institute of Medical Sciences, New Delhi, India, and fed either regular rats chow or a high-fat diet for 60 days. The high-fat diet contained 25% coconut oil, 2% cholesterol and 73% normal chow (w/w), which provided 55% of the animals' energy as fat [10]. The rats had free access to food and water and were kept on a 12-h light/12-h dark cycle. The experimental protocol was approved by the Institutional Animal Ethics Committee and conformed to the Indian National Science Academy (INSA) Guidelines for the use and care of experimental animals in research.

2.2. Drugs and chemicals

Sesamol was purchased from Sigma (St. Louis, MO, USA). Rat TNF-α (Diaclone Tepnel Company, UK), IL-6, hs-CRP (Bender Med Systems, A-1030 Vienna, Austria), adiponectin, leptin, resistin (Linco Research, St. Charles, MO, USA) and insulin (Macrodia, Sweden) ELISA Kit were also purchased. The kits for blood glucose, lipid profile, AST, ALT and ALP were purchased from Logotech (New Delhi, India). Pioglitazone was obtained as a gift sample from Zydus Cadila, Ahmedabad, India. All primary and secondary antibodies were procured from Santa Cruz Biotechnology (USA). All other chemicals used in the study were purchased from Sigma.

2.3. Experimental design

After 30 days of HFD feeding, 2 ml of blood was withdrawn from the tail vein of overnight (12 h) fasted rats for confirming hypercholesterolemia [10]. The animals with serum cholesterol level >150 mg/dl were considered hypercholesterolemic and included in the study. The animals were randomly divided into six groups; each group consisted of 12 animals and treated with different doses of sesamol and pioglitazone by oral gavage for the next 30 days while being maintained on HFD.

Group I: (Normal control) Rats given normal diet, neither HFD diet nor any drug.
Group II: (HFD control) Rats given HFD only.
Groups III–V: HFD rats given different doses of sesamol: 2, 4 or 8 mg/kg per day po, respectively.
Group VI: HFD rats given pioglitazone 10 mg/kg per day po.

On the last day of the experiment, 2 ml of blood sample was withdrawn from the tail veins of overnight fasted rats [10]. Serum was separated by centrifugation (Heraeus, Biofuge, Germany) at 2000×g for 5 min and analyzed for glucose, total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), insulin, leptin, adiponectin, resistin, IL-6, hs-CRP and TNF-α.

2.4. Evaluation of hemodynamic parameters

After 1 h of blood withdrawal, the animals were anesthetized with pentobarbitone sodium (60 mg/kg ip), and atropine (0.6 mg/kg ip) was injected to maintain heart rate and reduce tracheobronchial secretions during the surgical procedure. Tracheostomy was performed and ventilated with room air from a positive pressure ventilator (Inco, India) at a rate of 90 strokes/min and a tidal volume of 10 ml/kg. The right carotid artery was cannulated and connected via a pressure transducer (CARDIOSYSCO-101, Experirnentria, Hungary) for recording systolic arterial pressures (SAP), diastolic arterial pressures (DAP), mean arterial blood pressures (MAP) and heart rate. The animals were then sacrificed with an overdose of anesthesia, and the liver was excised and processed for biochemical and microscopic analysis [10].

2.5. Estimation of serum glucose, lipid profile, AST, ALT and ALP

Serum glucose, lipid profiles (TC, TG, LDL-C and HDL-C), AST, ALT and ALP were estimated spectrophotometrically using commercial kits.

2.6. Estimation of serum insulin, TNF-α, IL-6, hs-CRP, adiponectin, resistin and leptin

Serum insulin, TNF-α, IL-6, hs-CRP, adiponectin, resistin and leptin levels were measured by ELISA kits following the manufacturer's instructions.

2.7. Estimation of thiobarbituric acid-reactive substances content, superoxide dismutase and glutathione peroxidase activity in serum and liver

For biochemical estimations, the left lobe of the liver excised from a rat was perfused with chilled phosphate buffer (pH 7.4), minced and a 10% homogenate was prepared in ice-chilled 0.1 M phosphate buffer (pH 7.4) using a Teflon/glass tissue grinder (Bharat Electricals, India) and was used to measure thiobarbituric acid-reactive substances (TBARS) [15]. To the tissue homogenate, 8.1% sodium dodecyl sulfate (0.2 ml), 20% acetic acid (1.5 ml) and 0.8% thiobarbituric acid (1.5 ml) were added, mixed thoroughly and heated for 60 min in a boiling water bath. After incubation for 15 min at room temperature, the pink-colored supernatant was separated by adding butanol/pyridine (5 ml, 15:1). Finally, absorbance of organic layer was observed at 532 nm (Specord 200, Germany) and plotted against standard graph and expressed as millimoles per gram of tissue and the supernatant was used to assess superoxide dismutase (SOD) [16], glutathione peroxidase (GSH-Px) [17] and total protein [18]. Similarly, following the above methods, we measured TBARS, SOD and GSH-Px and total protein in serum.

2.8. Evaluation of IR

IR was calculated by the homeostasis model assessment method (HOMA) [19]. We used the following equations:

$$\text{IR (HOMA}-\text{IR)} = [\text{Fasting glucose (mmol/L)} \times \text{fasting insulin } (\mu\text{IU/ml})] / 22.5$$

2.9. Western blot analysis

Protein samples (15 μg) were separated by 12% SDS–PAGE; transferred to a nitrocellulose membrane (MDI, Ambala, India), which was blocked for 1 h with 5% (w/v) dry milk in Tris-buffered saline; and incubated overnight at 4°C with the primary antibodies. The primary antibodies used were as follows: insulin receptor substrate 1 (IRS1), phosphorylated tyrosine 612 IRS1 [P-IRS1 (Tyr162)], PPARγ, PPARα, LXRα, SREBP-1c, FAS, c-Jun N-terminal kinases (JNK), P-JNK, IKK, NF-κB, e-NOS, nitrotyrosine (NT) and β-actin. The primary antibody was detected with HRP-conjugated secondary antibody and Bio-Rad Protein Assay Reagent and visualized by Bio-Rad Quantity One 4.4.0 software (Bio-Rad, Hercules, CA, USA).

2.10. Microscopic examination

Histology and ultrastructural studies of the liver were performed and graded as described in our previous study [10].

2.11. Statistical analysis

All results were expressed as mean±S.D. (n=12/group). Statistical analysis was performed using the SPSS software package version 11.5. The values were analyzed by one-way analysis of variance (ANOVA) followed by Bonferroni post hoc test. P<.05 was considered significant.

3. Results

3.1. Effect on body weight

Table 1 shows the effect of sesamol on body weight changes in different experimental groups. At the end of the experiment, HFD control rats showed a significant increase in body weight as compared to the normal control group (P<.001), which can be solely attributed to the presence of high fat in the diet. Rats treated with sesamol (4 and 8 mg/kg per day) for 30 days showed significantly decreased body weight gain in HFD-fed rats. This reduction can be

Table 1
Biochemical findings and heart rate in different experimental groups at the end of study

Parameters	Normal control	HFD Control	HFD+Sesamol			HFD+Pioglitazone, 10 mg/kg
			2 mg/kg	4 mg/kg	8 mg/kg	
Body weight (g)						
0 day	150.7±13.9	145.3±15.2	153.8±11.4	149.8±12.8	146.5±9.4	154.8±13.4
30th day	193.5±11.4	230.0±14.2	225.3±10.6	220.3±9.5	223.0±10.8	218.6±14.7
60th day	229.4±15.0	270.3±19.1***	261.0±12.5	246.7±18.0##	237.6±11.8###	244.5±20.0##
Glucose (mg/dl)	94.6±10.7	173.4±14.1***	154.9±11.3	139.6±10.9##	113.0±10.5###	105.9±13.4###
Insulin (±U/mL)	12.3±2.5	44.7±5.2***	38.4±2.7	26.1±3.5###	17.3±1.9###	14.5±4.2###
HOMA-IR	2.8±1.4	14.9±2.5***	11.6±1.5#	7.3±1.3###	4.5±1.7###	3.7±2.5###
HR (beats min)	350±20	359±18	348±24	363±15	353±22	368±28

HOMA-IR: Homeostasis model assessment of insulin resistance; HR: heart rate. All values are expressed as mean±S.D. (n=12/group). ***P<.001 vs. normal control; #P<.05, ##P<.01, ###P<.001 vs. HFD control.

credited to sesamol as both HFD and sesamol groups were subjected to the same diet except for the presence of sesamol in the sesamol group.

3.2. Effect on hyperglycemia, hyperinsulinemia and IR

Serum glucose, insulin and IR (HOMA-IR) were significantly elevated by 1.8-, 2.9- and 5.3-fold, respectively, in HFD control rats compared to normal control (Table 1). Sesamol dose dependently attenuated hyperglycemia, hyperinsulinemia and IR in such a way that the sesamol 8 mg/kg group was not different from the standard pioglitazone drug (Table 1).

3.3. Effect on dyslipidemia

Table 2 depicts the effect of sesamol on serum lipid profile in different experimental groups. A significant increase in TC (2.7-fold), TG (4.1-fold), LDL-C (3.5-fold) and FFAs (3.8-fold) with concomitant decrease in HDL-C was seen in HFD control animals as compared to the normal control group (P<.001). Administration of sesamol (2–8 mg/kg per day) dose dependently decreased TC, TG and LDL-C with concomitant increase in HDL-C in HFD-fed rats. However, the effect was most prominent at 8 mg/kg per day doses and it was higher than that of pioglitazone (P<.01).

3.4. Effect on TBARS level, GSH-Px and SOD activity

HFD control rats showed a significant reduction in SOD and GSH-Px enzyme activities and an increase in TBARS level in liver and serum (Table 3). Sesamol treatment significantly decreased TBARS and increased SOD and GSH-Px activity as compared with HFD. The effect of sesamol 8 mg/kg per day induced increase in enzymatic antioxidant activity and decrease in TBARS level was comparable to that of pioglitazone.

3.5. Effects on hemodynamic parameters and serum NO levels

The HFD group exhibited a significantly (P<.001) increased systolic, diastolic and mean arterial blood pressure as compared to the normal group (Fig. 1A). In contrast, administration of sesamol (2–8 mg/kg per day) for 30 days decreased SAP, DAP and MAP. This effect was significant (P<.01) at 4 and 8 mg/kg per day doses of sesamol. Moreover, no significant change in heart rate was observed in all the experimental groups (Table 1). Furthermore, the low serum NO level in HFD control rats was significantly increased by sesamol treatment and sesamol 8 mg/kg brought back the serum NO level to almost near normal (Fig. 1B).

3.6. Effect on serum AST, ALT and ALP

A significant (P<.001) increase in serum levels of ALT (4-fold), AST (2.7-fold) and ALP (3.5-fold) was observed in the HFD control rats as compared to the normal controls. On the other hand, sesamol 4 and 8 mg/kg per day significantly (P<.01) decreased the serum levels of these hepatic enzymes to near normal levels (Fig. 2A).

3.7. Effect on serum TNF-α, IL-6, leptin, resistin, hs-CRP and adiponectin

HFD resulted in a significant increase in serum TNF-α (3.7-fold), IL-6 (1.8), leptin (3.6-fold), resistin (2-fold), hs-CRP (3.9-fold) and in a decrease in adiponectin level as compared to the normal control group (Fig. 3B–D). Administration of sesamol (4 and 8 mg/kg per day) for 30 days resulted in a significant (P<.01) reduction in serum TNF-α, IL-6, leptin, resistin and hs-CRP levels and an increase in adiponectin level as compared with the HFD control group (Fig. 3B–D).

Table 2
Lipid profile in different experimental groups

Groups	TC (mg/dl)		TG (mg/dl), 60th day	LDL-C (mg/dl), 60th day	HDL-C (mg/dl), 60th day	FFAs (mmol/L), 60th day
	30th day	60th day				
Normal control	93.2±18.9	98.5±10.7	44.8±4.2	38.2±6.1	55.3±6.5	1.2±0.3
HFD Control	210.1±20.3	266.4±30.8***	153.7±10.3***	182.0±16.3***	36.5±4.9***	4.5±0.6***
HFD+Sesamol						
2 mg/kg	225.7±13.3	239.5±15.0	140.3±11.7	165.7±12.5	38.5±4.2	3.7±0.8
4 mg/kg	213.0±19.7	202.8±13.4##	133.6±14.7#	148.1±12.7##	42.3±7.5	2.6±0.4##
8 mg/kg	216.5±16.6	130.3±14.0###	81.4±15.6###	72.3±13.0###	47.8±6.2##	1.5±0.6###
HFD+Pioglitazone 10 mg/kg	221.5±14.2	143.6±12.7###	92.4±14.5###	89.2±17.4###	45.7±4.6##	1.9±0.5###

TC: Total cholesterol; TG: triglyceride; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol. All values are expressed as mean±S.D. (n=12/group). ***P<.001 vs. normal control; #P<.05, ##P<.01, ###P<.001 vs. HFD control.

Table 3
Antioxidant status and TBARS level in different experimental groups at the end of study

Parameters	Normal control	HFD Control	HFD+Sesamol			HFD+Pioglitazone, 10 mg/kg
			2 mg/kg	4 mg/kg	8 mg/kg	
TBARS						
Serum (nmol/L)	2.8±0.6	4.3±0.8***	3.7±1.2	3.2±0.6#	2.5±1.0##	2.9±0.7##
Liver (nmol/g)	52.7±8.4	102.6±15.6***	97.2±13.1	72.5±9.3##	50.6±14.6##	69.3±13.8###
SOD						
Serum (U/mg protein)	137.4±15.6	109.6±14.5***	112.3±11.8	131.4±17.5#	138.6±10.5###	132.8±12.6##
Liver (U/mg protein)	164.6±20.8	115.7±13.5***	126.4±16.3	138.9±20.0##	167.4±15.2###	144.5±17.3###
GSH-Px						
Serum (U/mg protein)	17.3±1.4	10.5±1.8***	12.1±1.3	15.6±3.2##	16.8±1.7##	15.1±2.8##
Liver (U/mg protein)	24.9±2.3	15.7±2.4***	18.2±1.6	20.9±1.5##	26.3±2.1###	22.8±2.6###

TBARS: Thiobarbituric acid reactive substances; SOD: superoxide dismutase; GSH-Px: glutathione peroxidase. All values are expressed as mean±S.D. (n=12/group). ***P<.001 vs. normal control; #P<.05, ##P<.01, ###P<.001 vs. HFD control.

3.8. Effect on PPARγ, PPARα, LXRα, SERBP-1c, FAS, e-NOS P-JNK, IKK and NF-κB protein expression and NT accumulation in liver tissue

Fig. 3 shows the effects of sesamol treatment on the protein expression of liver PPARγ, PPARα, P-JNK, NF-κB, LXRα, SERBP-1c, FAS and e-NOS. The HFD control group showed reduced PPARγ expression. However, it was not significantly different as compared to the normal control. Furthermore, sesamol 4 and 8 mg/kg and pioglitazone significantly (P<.001) increased PPARγ expression (Fig. 3A). The sesamol-mediated inhibition of oxidative stress and dysregulated adipocytokines production was associated with significantly decreased IKK, NF-κB and P-JNK (Fig. 3B and C) translation activity which further confirms its anti-inflammatory and antioxidant activity. To investigate whether decreased dyslipidemia and hepatic steatosis in sesamol-treated rats were associated with modulation of PPARα, LXRα, SERBP-1c and FAS protein expression which are involved in lipid metabolism and lipogenesis, we examined their protein expressions in liver tissue. Interestingly, sesamol treatment significantly increased PPARα expression (Fig. 3A) and decreased LXRα, SERBP-1c and FAS expression (Fig. 3D), and the effect of sesamol 8 mg/kg was more pronounced than that of pioglitazone. Furthermore, the decreased IR with sesamol 4 and 8 mg/kg treatment was also associated with significantly increased P-IRS1 (Tyr162) expression without any change in total IRS1 expression in liver (Fig. 3E). We also found significantly decreased expression of e-NOS as well as increased accumulation of NT, an inactivation product of NO in HFD rats (Fig. 3F). Interestingly, sesamol dose dependently increased e-NOS expression and decreased NT, although the effect was significant at 4 and 8 mg/kg.

3.9. Histopathological and ultrastructural studies

To evaluate the effect of sesamol on diet-induced hepatic steatosis, we performed a histopathological and an ultrastructural analysis of liver tissue (Fig. 4A–L and Table 4). On histopathological examination, the livers of HFD control rats showed severe swelling of hepatocytes, fat accumulation (macrovesicular and microvesicular) and loss of nucleus and inflammatory cells, indicating hepatic steatosis (Fig. 4B), whereas the electron microscopic analysis of hepatocytes revealed

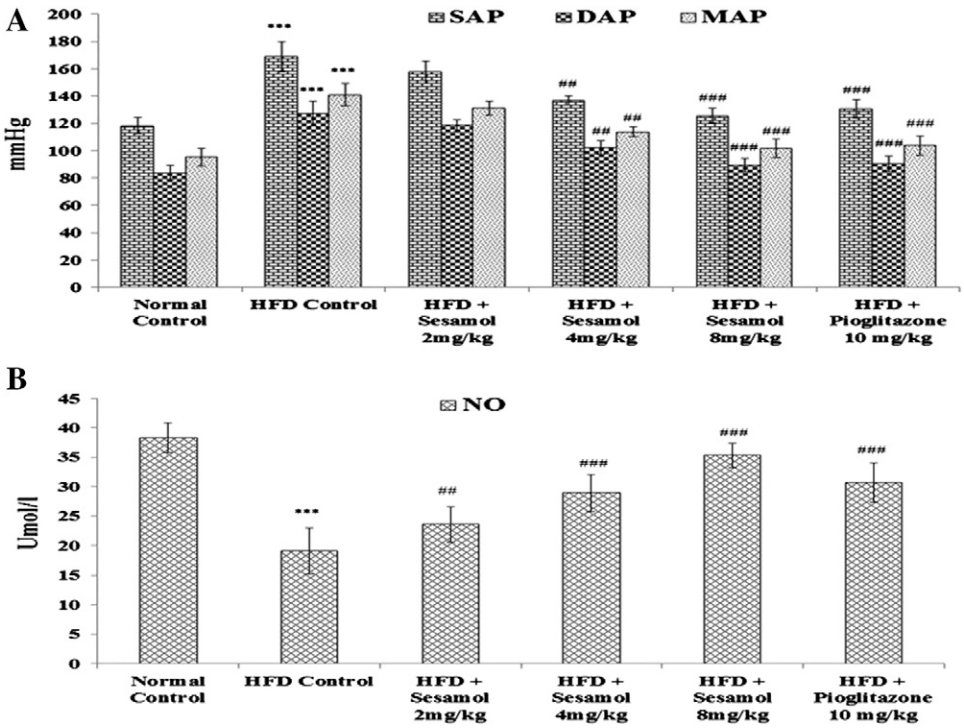

Fig. 1. (A) Hemodynamic parameters. (B) Serum NO levels in different experimental groups at the end of study. SAP: Systolic arterial pressure; DAP: diastolic arterial pressure; MAP: mean arterial pressure. All values are expressed as mean±S.D. (n=12/group). ***P<.001 vs. normal control; #P<.05, ##P<.01, ###P<.001 vs. HFD control.

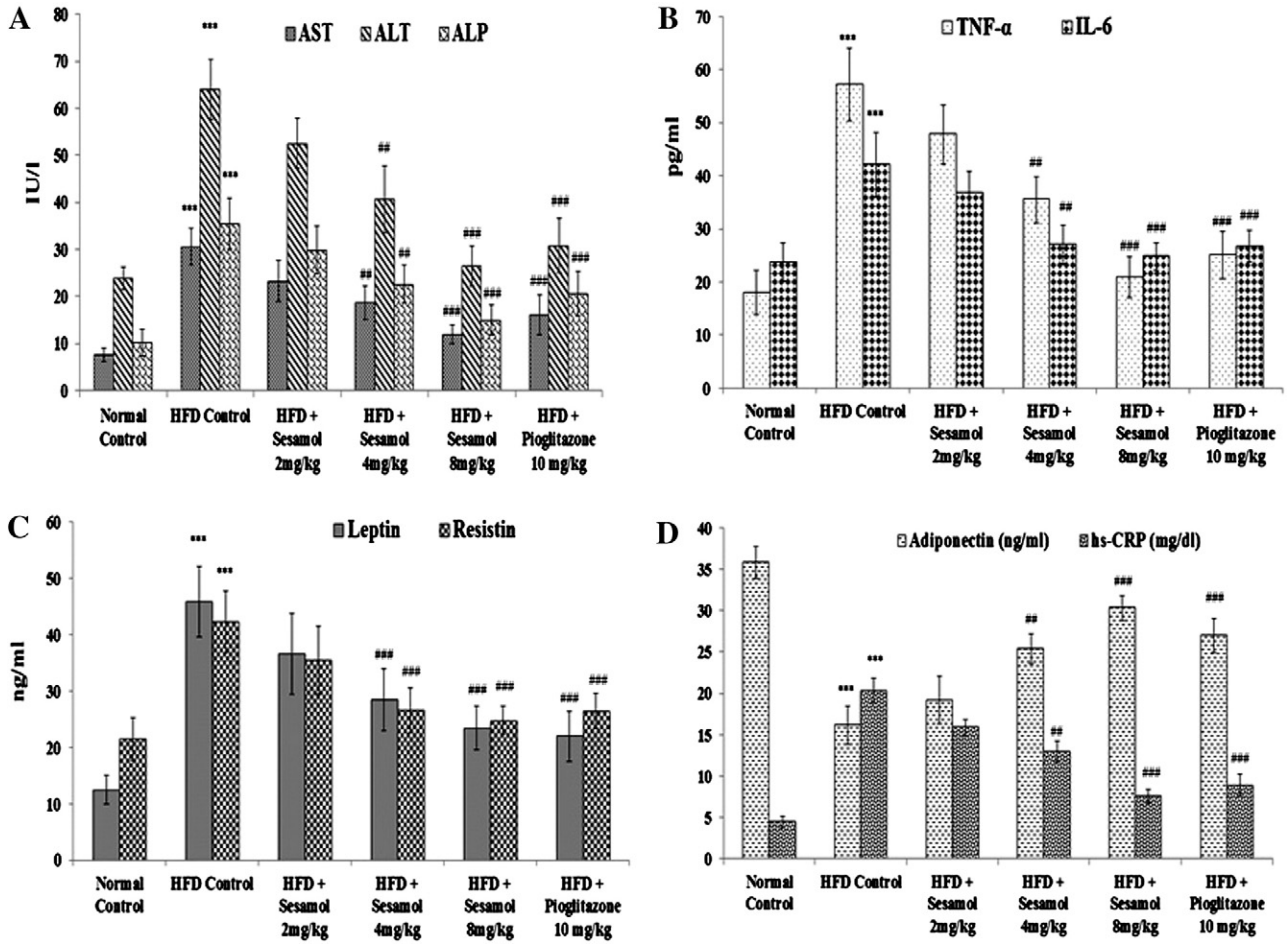

Fig. 2. Biochemical findings in different experimental groups at the end of study. (A) Serum levels of AST, ALT, and ALP; (B) TNFα and IL-6; (C) leptin and resistin; (D) adiponectin and hs-CRP. AST: Aspartate aminotransferase; ALT: alanine aminotransferase; ALP: alkaline phosphate; TNFα: tumor necrosis factor alpha; IL-6: interleukin-6; hs-CRP: highly sensitive C-reactive protein. All values are expressed as mean±S.D. (n=12/group). $^{***}P<.001$ vs. normal control; $^{##}P<.01$, $^{###}P<.001$ vs. HFD control.

rarefied matrix, lipid accumulation, distension of rough endoplasmic reticulum, swelling of mitochondria and loss of cristae (Fig. 4H). Moreover, sesamol 8 mg/kg per day reversed these pathological changes and restored the normal architecture of the liver (Fig. 4E and K). Thus, light microscopic and ultrastructural examinations established that sesamol alleviates the diet-induced hepatic steatosis.

4. Discussion

The results of the present study suggest a potential role for sesamol in the regulation of body weight, oxidative stress, inflammation, IR, hypertension and hepatic steatosis in HFD-induced metabolic syndrome in rats. This beneficial effect of sesamol is attributable to increased antioxidant enzyme activity, decreased hs-CRP and attenuation of dysregulated production of adipocytokines such as TNF-α, IL-6, leptin, resistin and adiponectin. Importantly, the attenuation of IR and inflammation by sesamol are largely on account of the up-regulation of PPARγ. This leads to suppression of pro-inflammatory P-JNK, IKK and NF-κB pathways and increased phosphorylated tyrosine IRS1. Notably, in our study, improvement in hepatic steatosis and dyslipidemia by sesamol treatment is strongly related to modulation of PPARα, LXRα, SREBP-1c and FAS. Furthermore, it was also observed that reversal of HFD-induced hypertension by sesamol is mediated by enhanced expression of e-NOS and normalization of serum NO levels.

Increased incidence of CMetS worldwide is a matter of great concern. The establishment of a suitable target and new therapeutic approaches are the need of the hour to further facilitate its management. Increased visceral obesity augments oxidative stress and inflammation and is believed to be the major culprit in the pathogenesis of this cluster of diseases [1,2]. In the present study, after being fed with HFD diet for 2 months, the rats developed CMetS characterized by increased body weight, oxidative stress and inflammation, along with development of IR, dyslipidemia, hepatic steatosis and hypertension. In parallel to our findings, various important studies established that chronic administration of HFD causes CMetS in rats [4,5,9,10,20].

In our study, HFD rats showed development of IR as evidenced by significantly increased HOMA-IR, hyperinsulinemia, hyperglycemia and decreased tyrosine phosphorylation of IRS1. Furthermore, inflammatory mediators like TNFα, IL-6, hs-CRP and resistin were increased and anti-inflammatory adiponectin was decreased in HFD rats along with overexpression of P-JNK, IKK and NF-κB. Decreased tyrosine phosphorylation of IRS1 seems to be a consequence of activation of JNK and IKK, a phenomenon that is also attributed to increased oxidative stress and inflammation in metabolic syndrome [1,2,20]. Notably, sesamol treatment decreased P-JNK, IKK and NF-κB protein expression as well as inflammatory mediators that promote IR such as TNFα, IL6, resistin and hs-CRP, while increased adiponectin attenuates IR, resulting in increased tyrosine phosphorylation of IRS1 and insulin sensitivity. Furthermore, we observed a significant

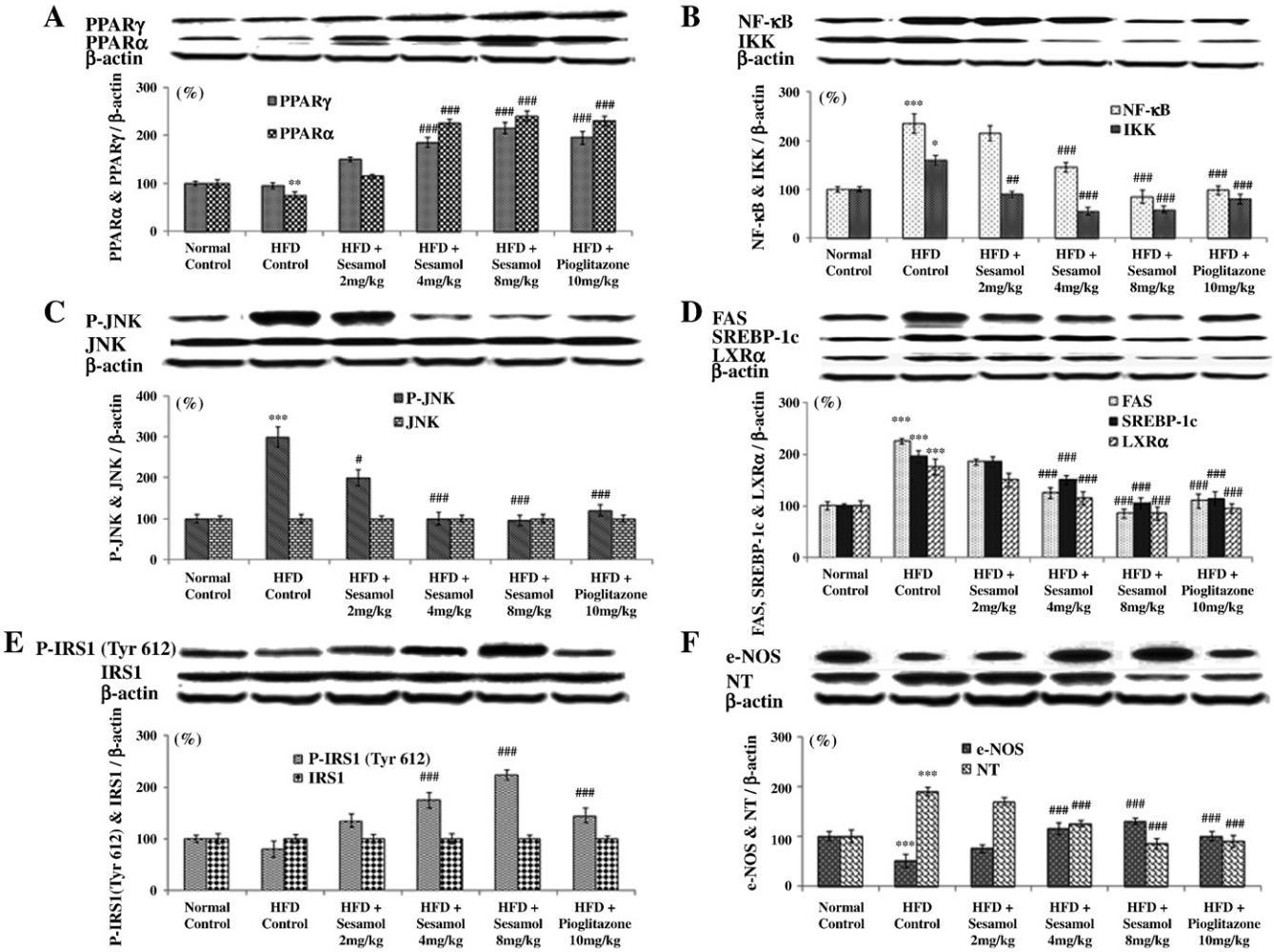

Fig. 3. Protein expressions in liver in different experimental groups. (A) PPARγ and PPARα; (B) NF-κB and IKK; (C) P-JNK and JNK; (D) LXRα, SREBP-1c and FAS; (E) P-IRS1 (Tyr612) and IRS1; (F) e-NOS and NT. Data are expressed as the ratio of normal control value (set to 100%). All values are expressed as mean±S.D. (n=12/group). Representative blots are from the same experiment in panels A and B, in panels C and D, and in panels E and F, respectively. $^{*}P<.05$, $^{**}P<.01$, $^{***}P<.001$ vs. normal control and $^{\#\#}P<.01$, $^{\#\#\#}P<.001$ vs. HFD control.

increase in serum leptin in HFD control rats [9], indicating leptin resistance which was also attenuated by sesamol. Interestingly, in our study, sesamol up-regulated PPARγ expression and it is well known that activation of PPARγ has potential to interfere with transcriptional pathways that are involved in oxidative stress and inflammatory responses [21-24]. We assume that this enhanced expression of PPARγ by sesamol negatively regulates the stimulus-dependent activation of P-JNK, IKK and NF-κB, and dysregulated production of numerous adipocytokines that promote IR [1,2,20,22,25].

In addition, in this experiment, HFD rats showed significantly increased body weight, FFAs, TG, TC and LDL-C with concomitantly decreased HDL-C. HFD-induced dyslipidemia may result from increased absorption of TGs in the form of chylomicrons, elevated endogenous very low density lipoprotein (VLDL) production and decreased TG uptake in peripheral tissues and/or alteration of cholesterol metabolism due to either decreased hepatic LDL-C receptor activity or diminished LDL-C catabolism [2,26,27]. The morphological examination of the liver of rats fed HFD displayed hallmark features of hepatic steatosis such as fat accumulation, microvesicular and macrovesicular hepatic steatosis, inflammatory cells, rarefied matrix, swelling of rough endoplasmic reticulum and mitochondria in hepatocytes. It was also associated with hepatocyte damage as evidenced from increased levels of liver enzymes like AST, ALT and ALP [8,9]. Of note, hepatic steatosis in the metabolic syndrome may be a result of hyperinsulinemia and IR causing increased expression of LXRα and SREBP-1c that leads to decreased FFA oxidation, increased FAS enzyme activity and increased lipogenesis [7,26]. Furthermore, increased oxidative stress, mitochondrial dysfunction and dysregulated adipocytokine production (TNFα, IL-6, leptin, resistin and adiponectin) in obesity are also believed to be the causative factors for NASH [2,8,9,26]. Interestingly, sesamol administration strongly reduced body weight gain, dyslipidemia, hepatic steatosis and hepatic injury marker enzymes in HFD rats. The cellular mechanisms underlying this activity of sesamol are still not fully understood, but some observations, including ours, suggest the role of its potent anti-inflammatory and antioxidant activity which attenuates IR, body weight gain and hepatic steatosis [12-14,28]. Furthermore, we also demonstrated that sesamol amplified hepatic PPARα expression and reduced the activation of LXRα, SREBP-1c and FAS in HFD rats, which in turn markedly attenuated the interrelated steatosis and dyslipidemia through decreased fatty acid synthesis [7,8,11,24,26].

In order to investigate the possible role of oxidative stress in HFD-induced CMetS, we measured several oxidant–antioxidant

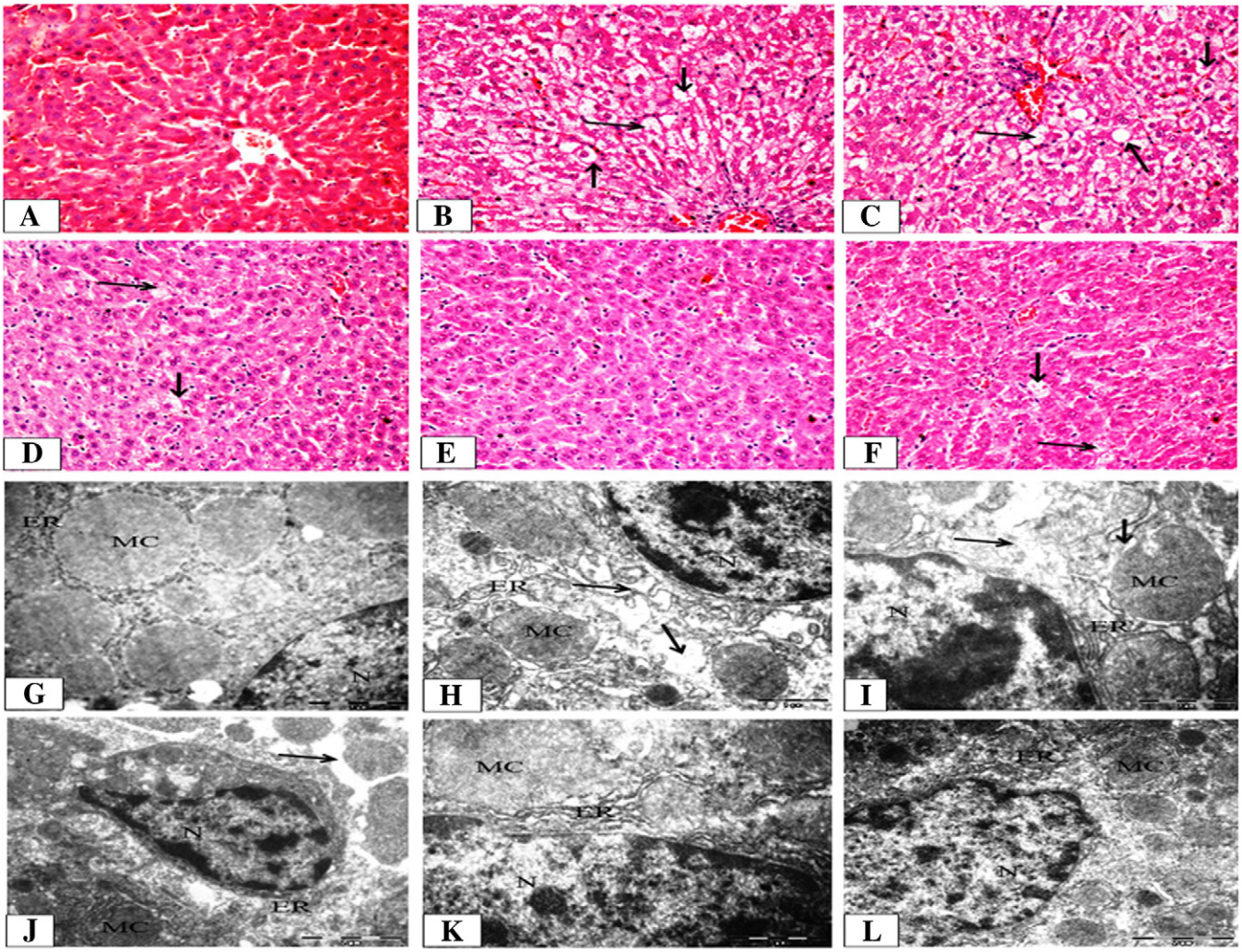

Fig. 4. Light microscopic study of hepatic tissue (A–F, 20×, scale bar 50 μm) and electron microscopic study of hepatocyte (G–L, 4000×, scale bar 1 μm) in different experimental groups. (A and G) Normal control; (B and H) HFD control; (C–E and I–K) sesamol 2, 4 and 8 mg/kg per day treated; (F and L) pioglitazone treated. MC: Mitochondria; ER: endoplasmic reticulum; N: nucleus; →: Fatty changes.

parameters in liver and serum. HFD administration led to marked increase in TBARS, a parameter of oxidant activity, accompanied by decrease in GSH-Px and SOD, parameters of antioxidant activity. These results allowed us to hypothesize that increased systemic oxidative stress in HFD rats might be related to the dysregulated production of adipocytokines, inflammation, IR, hypertension and NASH [1,2,9,10]. These data are in good agreement with recent studies, including ours, suggesting that increased oxidative stress in HFD rats mediates the development of metabolic syndrome [5,9,10,20]. On the other hand, sesamol treatment for 30 days normalized these oxidant–antioxidant parameters. Moreover, the mechanisms that underlie the sesamol-induced decline of oxidative stress and TBARS could be due to direct antioxidant activity of sesamol or indirectly due to augmentation of SOD and GSH-Px activity in liver and serum, which scavenges hydroxyl and lipid peroxyl radicals and normalized the lipid peroxide levels. This is in line with various other findings that sesamol is a potent antioxidant and its solubility in both aqueous and oily phases makes it an inimitable chain-breaking antioxidant by increasing its concentration in cell membranes [12-14]. However, this observed antioxidant activity of sesamol, at least in part, is an indirect consequence of the activation of PPARγ or the suppression of P-JNK, IKK and NF-κB, which further suggests an important mechanistic insight into its antioxidant activity [2,12-14,22]. Thus, our findings established that sesamol may have utility in treating CMetS, now known to involve activation of oxidative stress, as an important contributor in its etiology and progression.

Furthermore, this increased oxidative stress in HFD rats also underlies the pathophysiology of hypertension, because increased reactive oxygen species (ROS), mainly superoxide anions, are potent inactivators of NO and mediators of vascular injury [2,3,25]. Previous studies have reported that various mechanisms

Table 4
Histological observations of hepatic tissue in experimental groups

Groups	Swelling of hepatic cells	Fat accumulation	Displacement of nucleus	Loss of nucleus
Normal control	−	−	−	−
HFD Control	+++++	+++++	+++++	++++
HFD+Sesamol				
2 mg/kg	+++++	+++++	++++	+++
4 mg/kg	++	++	++	+
8 mg/kg	+	+	+	+
HFD+Pioglitazone, 10 mg/kg	++	++	+	+

++++++, High degree; +++++, severe; ++++, moderate; +++, fair; ++, mild; +, very mild; −, no changes.

involved in hypertension, viz., increased oxidative degradation of NO, defective L-arginine availability, decreased expression or activity of the e-NOS enzymes, result in impaired biological activity of the NO [3-5,25]. Furthermore, superoxide combines with NO to produce peroxynitrite that subsequently reacts with proteins, lipids and DNA to induce tissue damage and also reacts with tyrosine residues to produce nitrotyrosine (NT). Increased level of NT in tissues, an established imprint of NO inactivation by ROS, can be used as a surrogate measure for ROS-mediated NO oxidation [4,5]. Moreover, increased hyperlipidemia and inflammatory mediators in CMetS have suppressive action on e-NOS leading to deceased NO synthesis [2-5]. Accordingly, in our study, HFD rats showed significantly increased SAP, DAP and MAP along with accumulation of NT in liver and decreased e-NOS and serum NO levels, key evidences of NO inactivation and decreased NO availability [4,5,10]. Conversely, sesamol treatment abrogated hypertension and normalized serum NO levels in HFD rats. The precise mechanism by which sesamol treatment attenuated hypertension, NT accumulation and improved e-NOS expression and serum NO levels in HFD rats is not fully clear, but the results of this study, as well as those of others, point to the partial role of increased antioxidant enzyme activities, up-regulation of PPARγ and inactivation of P-JNK, IKK and NF-κB inflammatory pathways [10,12-14,25,28].

In summary, sesamol abolishes several detrimental effects of HFD-induced CMetS in rats: it attenuates inflammatory response, oxidative stress and IR; improves dyslipidemia and hepatic steatosis; and offers protection from development of hypertension. We believe that this is the first study to examine the molecular mechanism underlying this activity of sesamol. We demonstrated that prevention of CMetS by sesamol is associated with up-regulation of PPARγ, PPARα and e-NOS, and suppression of P-JNK, NF-κB, LXRα, SREBP-1c and FAS protein expression. Thus, our results indicate that sesamol may be an effective therapeutic strategy for the treatment of CMetS, although further experimental and clinical studies are required to explore the additional mechanisms and establish its clinical utility.

References

[1] Hotamisligil GS. Inflammation and metabolic disorders. Nature 2006;444:860–7.
[2] Shoelson SE, Herrero L, Naaz A. Obesity, inflammation, and insulin resistance. Gastroenterology 2007;132:2169–80.
[3] Hopps E, Noto D, Caimi G, Averna MR. A novel component of the metabolic syndrome: the oxidative stress. Nutr Metab Cardiovasc Dis 2010;20:72–7.
[4] Roberts CK, Vaziri ND, Barnard RJ. Effect of diet and exercise intervention on blood pressure, insulin, oxidative stress, and nitric oxide availability. Circulation 2002;106:2530–2.
[5] Roberts CK, Vaziri ND, Wang XQ, Barnard RJ. Enhanced NO inactivation and hypertension induced by a high-fat, refined-carbohydrate diet. Hypertension 2000;36:423–9.
[6] Otani H. Oxidative stress as pathogenesis of cardiovascular risk associated with metabolic syndrome. Antioxid Redox Signal 2010 [Epub ahead of print].
[7] Ferré P, Foufelle F. Hepatic steatosis: a role for de novo lipogenesis and the transcription factor SREBP-1c. Diabetes Obes Metab 2010;12:83–92.
[8] Fan JG, Qiao L. Commonly used animal models of non-alcoholic steatohepatitis. Hepatobiliary Pancreat Dis Int 2009;8:233–40.
[9] Sundaresan S, Vijayagopal P, Mills N, Imrhan V, Prasad C. A mouse model for nonalcoholic steatohepatitis. J Nutr Biochem 2010 [Epub ahead of print].
[10] Nepal S, Malik S, Sharma AK, Bharti S, Kumar N, Siddiqui KM, et al. Abresham ameliorates dyslipidemia, hepatic steatosis and hypertension in high-fat diet fed rats by repressing oxidative stress, TNF-α and normalizing NO production. 2011 [Epub ahead of print]
[11] Shin SJ, Lim JH, Chung S, Youn DY, Chung HW, Kim HW, et al. Peroxisome proliferator-activated receptor-alpha activator fenofibrate prevents high-fat diet-induced renal lipotoxicity in spontaneously hypertensive rats. Hypertens Res 2009;32:835–45.
[12] Chopra K, Tiwari V, Arora V, Kuhad A. Sesamol suppresses neuro-inflammatory cascade in experimental model of diabetic neuropathy. J Pain 2010;11:950–7.
[13] Chu PY, Chien SP, Hsu DZ, Liu MY. Protective effect of sesamol on the pulmonary inflammatory response and lung injury in endotoxemic rats. Food Chem Toxicol 2010;48:1821–6.
[14] Ying Z, Kherada N, Kampfrath T, Mihai G, Simonetti O, Desikan R, et al. A modified sesamol derivative inhibits progression of atherosclerosis. Arterioscler Thromb Vasc Biol 2010 [Epub ahead of print].
[15] Okhawa H, Oohishi N, Yagi N. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979;95:351–8.
[16] Marklund S, Marklund G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem 1974;47:469–74.
[17] Lawrence RA, Burk RF. Glutathione peroxidase activity in selenium-deficient rat liver. Biochem Biophys Res Commun 1976;71:952–8.
[18] Bradford MM. A rapid and sensitive method for quantization of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976;72:248–54.
[19] Matthews DR, Hosker JP, Rudenski AS, et al. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28:412–9.
[20] Sabio G, Davis RJ. cJun NH2-terminal kinase 1 (JNK1): roles in metabolic regulation of insulin resistance. Trends Biochem Sci 2010;35:490–6.
[21] Yki-Jarvinen H. Thiazolidinediones. Drug Ther 2004;351:1106–18.
[22] Ding L, Jin D, Chen X. Luteolin enhances insulin sensitivity via activation of PPARγ transcriptional activity in adipocytes. J Nutr Biochem 2010;21:941–7.
[23] Michalik L, Wahli W. Involvement of PPAR nuclear receptors in tissue injury and wound repair. J Clin Invest 2006;116:598–606.
[24] Cornick CL, Strongitharm BH, Sassano G, Rawlins C, Mayes AE, Joseph AN, et al. Identification of a novel agonist of peroxisome proliferator-activated receptors alpha and gamma that may contribute to the anti-diabetic activity of guggulipid in Lep(ob)/Lep(ob) mice. J Nutr Biochem 2009;20:806–15.
[25] Yang J, Park Y, Zhang H, Xu X, Laine GA, Dellsperger KC, et al. Feed-forward signaling of TNF-alpha and NF-kappaB via IKK-beta pathway contributes to insulin resistance and coronary arteriolar dysfunction in type 2 diabetic mice. Am J Physiol Heart Circ Physiol 2009;296:H1850–8.
[26] Kammoun HL, Chabanon H, Hainault I, Luquet S, Magnan C, Koike T, et al. GRP78 expression inhibits insulin and ER stress-induced SREBP-1c activation and reduces hepatic steatosis in mice. J Clin Invest 2009;119:1201–15.
[27] Taskinen MR. Diabetic dyslipidemia: from basic research to clinical practice. Diabetologia 2003;46:733–49.
[28] Chandrasekaran VR, Chien SP, Hsu DZ, Liu MY. Anti-hepatotoxic effects of 3,4-methylenedioxyphenol and *N*-acetylcysteine in acutely acetaminophen-overdosed mice. Hum Exp Toxicol 2011 [Epub ahead of print].