A single dose of sesame oil alleviates liver injury and steatosis in steatohepatitis via modulation of oxidative stress, cytokines and PPAR-α

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ABSTRACT

Sesame oil is a component of traditional health food; it is a natural product with anti-inflammatory property. Nonalcoholic steatohepatitis (NASH) is a condition categorized by concurrent fat accumulation and inflammation in liver. NASH is associated with increased liver-related mortality. Disorders in hepatic lipid homeostasis elicit oxidative stress and inflammation, leading to the progression of NASH. A possible hepatoprotective effect of sesame oil on methionine-choline deficient (MCD) diet-induced NASH in C57BL/6 J mice has never been studied. Mice were fed with MCD diet for 28 days to induce NASH. Single doses of sesame oil (1, 2, and 4 mL/kg) were given on 27th day. Aspartate transaminase, alanine transaminase, steatosis, triglycerides, peroxisome proliferator-activated receptor-α, nitric oxide, malondialdehyde, tumor necrosis factor-α, and interleukin-6 were assessed after 28 days. All tested parameters were higher in MCD-treated mice than in normal control mice. In MCD plus sesame oil-treated mice, all tested parameters were significantly attenuated compared with MCD-treated mice. We conclude that a single dose of sesame oil protects against steatohepatitic injury by decreasing oxidative stress and inflammatory cytokines, but increasing peroxisome proliferator-activated receptor-α expression.

KEYWORDS: Sesame oil, Non-alcoholic steatohepatitis, Hepatic fibrosis, Inflammatory cytokines, Oxidative stress, Peroxisome proliferator-activated receptor-α
INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is a common chronic liver condition in the general public, probably leading to hepatic complications and cardiovascular disease (Lazo et al., 2010). The rapid rise in the prevalence of obesity and diabetes in the general population has contributed to a parallel increase in NAFLD in many parts of the world. Up to 46% of the adult U.S. population may have hepatosteatosis (Williams et al., 2011). Steatosis results from an imbalance between hepatic lipid mainly triglycerides (TG) input and output (Koteish and Diehl, 2001). The earliest stage of NAFLD is simple steatosis, a benign condition associated with the accumulation of TG, which is attributed to excess caloric intake, insulin resistance, and dysregulation of lipid metabolism. Further, steatosis can progress to more serious diseases such as non-alcoholic steatohepatitis (NASH), cirrhosis, or hepatocellular carcinoma (Cohen et al., 2011). NASH is characterized by liver injury with increased levels of aspartate transaminase (AST), alanine transaminase (ALT), steatosis, TG, oxidative stress, and inflammatory cytokines (Povero et al., 2010; Donnelly et al., 2005; George et al., 2003; Sanyal et al., 2001), but decreased PPAR-α (Pachikian et al., 2013).

Sesame oil, from the seeds of Sesamum indicum L., is a nutrient-rich antioxidant popular in alternative medicine. It protects against hepatic injury after cecal ligation and puncture (Hsu et al., 2004), septic hepatic injury (Hsu et al., 2008), and monocrotaline-induced sinusoidal obstruction syndrome (Periasamy et al., 2013). Sesame oil as a dietary supplement and nutraceutical alleviates various diseases in rodent models (Gauthaman and Saleem, 2009; Namiki, 1995; Fukuda et al., 1994). Sesame oil contains sesamin, sesamol, and sesamolin, all of which contribute to its antioxidant property (White, 1992). Sesame oil offers better protection than other dietary oils, such as peanut oil, against hypertension, hyperlipidemia, and lipid peroxidation by modulating in vivo antioxidants (Sankar et al., 2005). Therefore, we hypothesized that a single dose of sesame oil would attenuate liver injury and reduce inflammation in methionine-choline deficient (MCD)-induced NASH in mice.

MATERIALS AND METHODS

Animals

Male C57BL/6 J mice 7-8 weeks old and weighing 25-30 g was purchased from our institution’s Laboratory Animal Center. They were given pellet feed MCD diet and water ad libitum. They had a 12-h light/dark cycle and central air conditioning (25°C, 70% humidity) throughout the experiment. The animal care and experimental protocols were in accordance with nationally approved guidelines (No. 99054).

Diet and Chemicals

MCD diet and methionine and choline sufficient (MCS) diet were purchased from Test Diet (A Purina Mills, LLC/PMI Nutrition International Company, TX, USA). All the chemicals used in this study were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Model

MCD-treated mice were used as an experimental model of NASH (Inoue et al., 2011).

Experimental protocol

The mice were divided into 5 groups (N = 6-8). Group I, mice were fed with MCS diet for 28 days. Groups II, III, IV, and V, mice were fed MCD diet to induce fibrosing steatohepatitis. A single dose of mineral oil (4 mL/kg/day) was given orally on 27th day to
Group I and II. Single doses of sesame oil (1, 2, and 4 mL/kg/day) were given orally on 27th day to Group III, IV, and V, respectively. The body weights were measured once every three days. The mice were killed on 28th day. Serum was collected for ALT and AST. Pieces of liver tissue were harvested for hematoxylin and eosin (H&E) stain, TG, PPAR-α, malondialdehyde (MDA), nitric oxide (NO), tumor necrosis factor-α (TNF-α), and interleukin-6 (IL-6).

**Blood collection**

Blood samples were collected from the interior vena cava under light ethyl ether anesthesia. Blood was drawn by venipuncture into serum separation tube, allowed to clot for 20-30 min at room temperature, and then centrifuged at 15000 rpm at 4°C for 15 min.

**Assessing hepatic injury and serum TG**

Hepatic injury and serum TG were assessed by measuring the levels of serum AST, ALT, and TG using a biochemistry analyzer (Fujifilm Dri-Chem 3500s; Fujifilm, Kanagawa, Japan).

**Steatosis assessment**

Pieces of liver tissue from mice were cut and placed in 10% formalin. The tissues were dehydrated using a graded percentage of ethanol and then fixed in paraffin wax for 1 h to form blocks. The blocks were trimmed and cut into 4 µm thick section, stained with H&E, and then mounted sections using Depex-Polystyrene dissolved in xylene mountant. The sections of liver tissue were examined under microscope (magnification, 100X) to assess hepatic steatosis. To quantify the degree of steatosis, we used the modified scoring system described by Silva et al. (2011). The intensity of total steatosis was classified from (0) to (4), as follows: (0) < 5% of affected tissue; (1) 5% to 25% of affected tissue; (2) 25 to 50% of affected tissue; (3) 50 to 75% of tissue affected; and (4) more than 75% of affected tissue.

**PPAR-α assessment**

Approximately 100 mg of liver tissue were washed in PBS to remove blood components. Liver tissue nuclear extract was extracted by using Nuclear and Cytoplasmic Extraction Reagents Kit (Thermo Fisher Scientific, MA, USA). The content of nuclear PPAR-α was measured by using PPAR-α transcription factor assay kit (Cayman Chemical, MI, USA).

**Measuring nitrite content**

Briefly, the amount of nitrite in liver tissue was measured after the Griess reaction. Liver tissue was homogenized in deionized water (1:10, wt/vol). Tissue homogenate (500 µL) was centrifuged at 2500 g for 10 min at 4°C. Supernatant (100 µL) was incubated with 100 µL of Griess reagent at room temperature for 20 min. The absorbance was measured at 550 nm using the spectrophotometer. Nitrite concentration was calculated by comparing it with a standard solution of known sodium nitrite concentration (Hsu et al., 2013).

**Measuring lipid peroxidation levels**

Liver tissue was homogenized in Tris HCl (20 mmol/L; pH 7.4). Tissue homogenate (500 µL) was centrifuged at 2500 g for 10 min at 4°C, and the supernatant (200 µL) was measured for lipid peroxidation (Lipid Peroxidase Assay Kit; Calbiochem-Novabiochem, Darmstadt, Germany) using the spectrophotometer read at 586 nm.

**Measuring TNF-α and IL-6 levels in liver tissue**

Liver tissue was homogenized in deionized water (1:10; wt/vol) and centrifuged at 12500 g for 10 min at 4°C. TNF-α and IL-6 levels in the tissue supernatant were determined using an enzyme-linked immunosorbent assay (R&D Systems, Inc, Minneap-
Cytokines were assessed by measuring absorbance at 450 nm and extrapolating from a standard curve with a sensitivity limit of 32.5 pg/mL. Protein concentration (pg/mg) in liver tissue was determined using protein assay dye (Bio-Rad Laboratories, Hercules, Calif, USA).

Statistical analysis

All statistical analyses were done using SPSS 11.0.1 (SPSS Inc., Chicago, IL, USA). Data are means±SD. Differences in the measured variables between each group were assessed using Fisher’s Least Significant Difference test. Significance was set at $P<0.05$.

RESULTS

Sesame oil attenuated hepatic injury

Serum ALT and AST were significantly higher ($P<0.05$) in Group II than Group I. A single dose of sesame oil significantly attenuated the levels of serum AST (Fig. 1a) and ALT (Fig. 1b) in Group III compared with Group II. Group IV and V showed no significant change compared with Group II.

Sesame oil attenuated steatosis

Liver histopathology from Group II-V showed hepatic steatosis with varying degrees of steatosis. In contrast, liver sections from single doses of sesame oil administered in Group IV and V showed significant ($P<0.05$) decrease in the degree of steatosis compared with Group II (Fig. 2 and 3a).

Sesame oil decreased TG level

TG level was significantly higher ($P<0.05$) in Group II than Group I. Single doses of sesame oil significantly attenuated the level of TG in Group IV and V compared with Group II (Fig. 3b).

Sesame oil increased the PPAR-α expression

We quantified PPAR-α to evaluate the effect of single doses of sesame oil in MCD-induced NASH mice. PPAR-α expression in Group II-V significantly decreased compared with Group I. Group III and IV significantly increased the expression of PPAR-α compared with Group II (Fig. 4).

Figure 1: Effect of single doses of sesame oil on liver injury in MCD-fed NASH mice. Group I mice ($n=6$) were fed with MCS diet and gavaged with mineral oil on 27th day; Group II mice ($n=6$) were fed with MCD diet and gavaged with mineral oil on 27th day; Group III-V mice ($n=6$ each) were fed with MCD diet and gavaged with oral sesame oil (1, 2, and 4 mL/kg, respectively) on 27th day. (a) ALT; (b) AST. Data are means±SD. $a,b,c$ The differences between treatments with different letters are significant ($P<0.05$).
Sesame oil decreased NO and MDA

To substantiate the anti-oxidative effect of sesame oil on NASH, NO and MDA were assessed. NO and MDA were significantly ($P<0.05$) higher in Group II compared with Group I. Group III significantly ($P<0.05$) decreased the level of NO (Fig. 5a) and MDA (Fig. 5b) compared with Group II.

Figure 2: Effect of single doses of sesame oil on liver histopathology in MCD-fed NASH mice. (See groups and treatment details in Fig. 1 legend). Photomicrographs of liver histology at $[10\times] \times [10\times]$.

DISCUSSION

A single dose of sesame oil reduced MCD-induced steatohepatic liver injury in mice. Sesame oil (1 mL/kg) significantly decreased hepatic injury, NO, MDA, TNF-α, and IL-6; and 2 and 4 mL/kg reduced steatosis and TG, but increased the expression of PPAR-α. However, sesame oil (4 mL/kg) may contribute to increase steatohepatitis (Periasamy et al., 2013). Sesame oil is harmless when taken in recommended doses, but in a large dose does not have a cumulative antioxidative effect; instead, its antioxidative effect is significantly decreased (Hsu et al., 2008). Dietary fat with polyunsaturated fatty acid contributes to steatohepatitis (Lee et al., 2007). Therefore, a high dose of sesame oil may increase acute liver injury, but substantially reduce steatosis.

A single dose of sesame oil attenuated MCD-induced NASH by decreasing liver injury and oxidative stress. MCD-induced NASH involves sequence of pathogenic events such as early steatosis and oxidative stress that lead to hepatocellular injury and inflammation. Oxidative stress and the subsequent cytokine production are two interrelated events that promote NASH to fibrogenesis and cirrhosis (Povero et al., 2010). A single dose of sesame oil decreases oxidative stress by decreasing NO and MDA levels (Periasamy et al., 2010; Hsu et al., 2006; 2005). Sesame oil might intervene in the pathogenic sequence of steatosis, oxidative stress, and inflammation, thereby attenuate MCD-induced NASH.

Sesame oil decreased TNF-α and IL-6 levels

We measured the effect of single doses of sesame oil on TNF-α and IL-6 expression in NASH mice. TNF-α (Fig. 6a) and IL-6 (Fig. 6b) expression in Group II significantly ($P<0.05$) increased compared with Group I. Group III significantly ($P<0.05$) decreased compared with Group II.
Figure 3: Effect of single doses of sesame oil on steatosis and TG in MCD-fed NASH mice. (See groups and treatment details in Fig. 1 legend). (a) Steatosis scoring; (b) TG. Data are means±SD. a,b,c The differences between treatments with different letters are significant ($P<0.05$).

A single dose of sesame oil activated PPAR-α thereby attenuated MCD-induced NASH. PPAR-α is a ligand-activated transcription factor that belongs to the steroid hormone receptor superfamily (Yoon, 2009). PPAR-α plays a pivotal role in ameliorating dyslipidemia by regulating lipid and lipoprotein metabolism (Barter and Rye, 2008; Jay and Ren, 2007; Staels, 2007; Berger et al., 2005). PPAR-α, predominantly expressed in the liver, is a key factor tightly associated with inflammation and insulin resistance (Ahmed and Byrne, 2007; Zambon et al., 2006). Activation of PPAR-α with agonists such as fenofibrate and gemfibrozil reduces high circulating TG levels (Yoon, 2009). Down-regulation and deficient expression of PPAR-α is associated with NAFLD and treatment with an agonist for PPAR-α improves hepatic steatosis (Seo et al., 2008; Haran et al., 2006). Sesame oil activated PPAR-α which might regulate lipid and lipoprotein metabolism that reduced TG level ultimately steatosis; and activated PPAR-α regulated inflammation.

Figure 4: Effect of single doses of sesame oil on PPAR-α level in MCD-fed NASH mice. (See groups and treatment details in Fig. 1 legend). Data are means±SD. a,b,c The differences between treatments with different letters are significant ($P<0.05$).

A single dose of sesame oil attenuated NASH by decreasing TNF-α and IL-6. Oxidative stress and inflammatory cytokines produce liver insults of NASH by inducing inflammation and fibrosis finally lead to end-
stage liver disease (Lee et al., 2007). Inflammation is a key variable in the progression of hepatic steatosis (Povero et al., 2010). TNF-α and IL-6 overproduction is associated with obesity and hepatic inflammation (Shoelson et al., 2007; Bastard et al., 2006; Hu et al., 2004). TNF-α provokes liver inflammation with hepatocyte injury in the steatotic liver (Chitturi and Farrell, 2001). Sesame oil decreased TNF-α and IL-6 levels in MCD-fed mice. Sesame oil decreased proinflammatory cytokines that might attenuate the hepatic inflammation and steatosis.

**Figure 5**: Effect of single doses of sesame oil on oxidative stress in MCD-fed NASH mice. (See groups and treatment details in Fig. 1 legend). (a) NO; (b) MDA. Data are means±SD. a,b,c The differences between treatments with different letters are significant (P<0.05).

Sesame oil contains hepatoprotective active phenolics such as sesamin, sesamol, and sesamolin. Sesamin attenuates hepatic ischemia-reperfusion injury by inducing both antioxidant and anti-inflammatory activity (Utsumomiya et al., 2003), and it prevents steatosis (Akimoto et al., 1993). Sesamol protects against acetaminophen-induced liver damage by maintaining glutathione levels and inhibiting lipid peroxidation (Chandrasekaran et al., 2011). Sesamolin reduces serum and liver lipid levels, and increases hepatic fatty acid oxidation (Lim et al., 2007). Therefore, phenolics of sesame oil may contribute to the overall therapeutic action against fibrosing steatohepatitis.

The clinical implication of the present study is that a single dose of sesame oil has the potential to inhibit early NASH in patients by attenuating liver injury and steatosis. According to the World Health Organization global estimates from 2008, more than 1.4 billion adults are overweight and at least 500 million adults are obese (WHO, 2008). Due to dramatic increases in childhood obesity, NAFLD is an emerging clinical concern in adolescent populations (Schwimmer et al., 2006). Obesity and insulin resistance have NAFLD as part of metabolic syndrome (Cohen et al., 2011). Presently, there are no effective drug therapies for NAFLD, a risk factor for Type II diabetes (Anstee et al., 2013). The United States Food and Drug Administration do not regulate dietary supplements in the same manner as pharmacological agents (Hurt and Wilson, 2012; Pittler et al., 2005). Alt-
hough the use of dietary supplements for weight loss becomes common (Pillitteri et al., 2008), the optimal dose and safety profiles of many dietary supplements are poorly studied. However, sesame oil is non-toxic natural product, acts as nutritional supplement, and effective against various diseases (Fukuda et al., 1994; 1985). It also protects against multi-organ failure (Hsu and Liu, 2002).

Figure 6: Effect of single doses of sesame oil on proinflammatory cytokines in MCD-fed NASH mice. (See groups and treatment details in Fig. 1 legend). (a) TNF-α; (b) IL-6. Data are means±SD. a,b,c The differences between treatments with different letters are significant (P<0.05).

Sesame oil’s protective interventions against MCD-induced NASH are the events that lead to inhibiting steatosis, oxidative stress, and inflammation, but activating PPAR-α expression. However, more investigation is needed, before sesame oil can be used clinically to treat steatohepatitis in patients.

CONFLICT OF INTERESTS
The authors have declared no conflict of interests.

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