Silybum marianum oil attenuates oxidative stress and ameliorates mitochondrial dysfunction in mice treated with D-galactose

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ABSTRACT

Background: *Silybum marianum* has been used as herbal medicine for the treatment of liver disease, liver cirrhosis, and to prevent liver cancer in Europe and Asia since ancient times. *Silybum marianum* oil (SMO), a by-product of silymarin production, is rich in essential fatty acids, phospholipids, sterols, and vitamin E. However, it has not been very good development and use. **Objective:** In the present study, we used olive oil as a control to investigate the antioxidant and anti-aging effect of SMO in D-galactose (D-gal)-induced aging mice. **Materials and Methods:** D-gal was injected intraperitoneally (500 mg/kg body weight daily) for 7 weeks while SMO was simultaneously administered orally. The triglycerides (TRIG) and cholesterol (CHOL) levels were estimated in the serum. Superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), total antioxidant capacity (T-AOC), monoamine oxidase (MAO), malondialdehyde (MDA), caspase-3, and Bcl-2 were determined in the liver and brain. The activities of Na^+-K^+-adenosine triphosphatase (ATPase), Ca^{2+}-Mg^{2+}-ATPase, membrane potential ($\Delta\Psi m$), and membrane fluidity of the liver mitochondrial were estimated. **Results:** SMO decreased levels of TRIG and CHOL in aging mice. SMO administration elevated the activities of SOD, GSH-Px, and T-AOC, which are suppressed by aging. The levels of MAO and MDA in the liver and brain were reduced by SMO administration in aging mice. Enzyme linked immunosorbent assay showed that SMO significantly decreased the concentration of caspase-3 and improved the activity of Bcl-2 in the liver and brain of aging mice. Furthermore, SMO significantly attenuated the D-gal induced liver mitochondrial dysfunction by improving the activities of Na^+-K^+-ATPase, Ca^{2+}-Mg^{2+}-ATPase, membrane potential ($\Delta\Psi m$), and membrane fluidity. **Conclusion:** These results indicate that SMO effectively attenuated oxidative damage and improved apoptosis related factors as well as liver mitochondrial dysfunction in aging mice.

Key words: Aging, D-galactose, mitochondrial dysfunction, oxidative damage, *Silybum marianum* oil

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INTRODUCTION

Aging is an inevitable biological process, and anti-aging has become an important public issue with the increasing elderly population in the world. Oxidative stress is considered to be significant in the pathophysiology of various diseases, including the aging process. Reactive oxygen species (ROS) damage cellular lipids, proteins, and DNA, inhibiting their normal functions. An excess of ROS can result in cell aging and death.[1-3] Indeed, many researchers have discovered that oxidative stress occurs during the pathogenesis of age-associated or neurodegenerative diseases.[4-7]

D-galactose (D-gal), a reducing sugar, is a naturally-occurring substance in the body, which is completely metabolized at normal concentrations. However, at higher concentrations it is converted to aldose, hydrogen peroxide, and galactose oxidase; thus, speeding up the generation of superoxide anion and oxygen-derived free radicals that impair the function of macromolecules and cells.[8] Various studies indicates that D-gal accelerates aging in mice, rats, houseflies, and human fetal lung fibroblast and has been used as an aging model since 1985.[9] Studies with D-gal-induced mice have shown that D-gal induced oxidative stress

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causing cognitive impairment, neurotoxicity, tissue injury, and inflammation.[10,11] Other reports[12,13] demonstrate that mice that are continuously exposed to high doses of D-gal showed increased levels of oxidative stress biomarkers and a decline in antioxidant defense systems. In addition, long-term injection of D-gal in mice can result in mitochondrial dysfunction.[14,15] The mitochondrion is a major organelle that produces ROS. Electrons leaking from the electron transfer chain (ETC) reduce molecular oxygen to ROS, which include some free radicals. The ensuing state of oxidative stress causes damage to ETC components, lipid, and mitochondrial DNA, further increasing the production of ROS. Ultimately, this "vicious cycle" leads to a physiological decline in mitochondrial and cell function.[16-18] Mitochondrial decay has been proposed and also shown to be a major contributor to aging and age-associated degeneration. Mitochondrial dysfunction is a major factor in the mechanism of D-gal-induced aging acceleration. Antioxidants and mitochondrial nutrients have been shown to be able to delay aging and ameliorate age-associated diseases in different aging models.[19,20]

Silybum marianum is an annual or biennial plant indigenous to the Mediterranean area, which has now spread to other regions. In China, it is found mainly in Shaanxi, Qinghai, Hubei, Jiangsu and Jilin provinces. *S. marianum* has been used as herbal medicine for the treatment of liver disease, liver cirrhosis and to prevent liver cancer in Europe and Asia since ancient times. *S. marianum* contains silymarin, which is composed of silybin, silydianin, and silychristine, with silybin being the most biologically active compound.[21-23] The seeds contain a relatively high amount of oil (20-35%) from which silymarin can be extracted. The oil, a by-product of silymarin production, is rich in essential fatty acids, phospholipids, sterols, and vitamin E. It can be used alone in the preparation of food or by mixing it with other vegetable oils.[24]

Most of the beneficial effects of oils that contain essential fatty acids, tocopherols, squalene, and phenolic compounds have been shown to be due to their antioxidant activity and their capacity to prevent cardiovascular diseases and lipid oxidation.[25-28] Olive oil is a typical component of the Mediterranean diet that contains variable amounts of phenolic antioxidants.[29] Olive oil can prevent the cellular oxidative stress and inflammation. It also increases antioxidant capacity, reduces DNA damage, and improves plasma lipid profile.[25,30] At present, no work has been done to investigate whether *Silybum marianum* oil (SMO) is potent against D-gal-induced aging in mice. In the present study, we used olive oil as a control to investigate the antioxidant and anti-aging effect of SMO in ICR (Institute of Cancer Research) mice chronically injected with D-gal.

MATERIALS AND METHODS

Chemicals

SMO was extracted from *S. marianum* seed kernels by AY Mantianxue Food Manufacturing Co. LTD (Anyang, China). Olive oil was purchased from a local supermarket. The kits used for the determination of protein, superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), total anti-oxidation capability (T-AOC), monoamine oxidase (MAO), malondialdehyde (MDA), Na^+-K^+-adenosine triphosphatase (Na^+-K^+-ATPase), and Ca^{2+}-Mg^{2+}-ATPase (Ca^{2+}-Mg^{2+}-ATPase) were purchased from Nanjing Jiancheng Institute of Biological Engineering (Nanjing, China). D-gal was purchased from Amresco Inc., USA. Rhodamin 123 was purchased from Beyotime Institute of Biotechnoligy (Haimen, China) while 1,6-diphenylhexa-1,3,5-triene (DPH) was purchased from Sigma (USA). The enzyme-linked immunosorbent assay (ELISA) kits for Caspase-3 and Bcl-2 were provided by Shanghai Jianglai Bioscience Co., LTD (Shanghai, China). All reagents were of analytical grade.

Determination of chemical composition

The fatty acid compositions of SMO and olive oil were analyzed by gas chromatography-mass spectrometry (GC-MS) after methyl ester, respectively. The GC-MS analysis was carried out on an Agilent HP-5890 gas chromatograph (Agilent Technologies, USA) with a chromatographic column (60 m × 0.25 mm × 0.25 μm). Injector and ion source temperatures were set at 250°C and 200°C, respectively. Oven temperature was held at 180°C for 1 min, increased to 210°C at 10°C/min and held at 210°C for 2 min, then increased to 215°C at 0.2°C/min and held at 215°C for 3 min, and last increased to 240°C at 5°C/min and held at 240°C for 10 min. Each sample (1 μL) was injected into the column at a split ratio of 32:1. Helium was used as a carrier gas at an flow rate of 0.8 mL/min. The mass spectrometer was operated in electron-impact ionization mode by the energy of 70 eV.

Animals and treatments

Forty ICR mice of 3-month-old were housed in an animal house at the Laboratory Animal Research Center of Jiangsu University, Zhenjiang, China. After acclimation for 1 week, the animals were randomly divided into four groups (n = 10/group): (1) Normal control group (NCG) mice were treated with distilled water (0.3 mL/mice) (2) D-gal aging control group (ACG) mice were treated with distilled water (0.3 mL/mice) (3) SMO group mice were treated with SMO (10 mL/kg body weight/day) (4) olive oil positive control group (PCG) mice were treated with olive oil (10 mL/kg body weight/day). The mice of ACG, SMO and PCG received an injection of D-gal intraperitoneally at a dose of 500 mg/kg body weight/day for 7 weeks although NCG

mice were injected with physiological saline intraperitoneally. The mice were housed in polycarbonate cages with controlled temperature (25°C ± 2°C), humidity (60 % ±5 %), and an alternating 12 h light/dark cycle. During the entire experimental period (including acclimation), the mice were fed a standard rodent diet *ad libitum* and water was also available at all times. All procedures were reviewed in advance by the Laboratory Animal Management Committee of Jiangsu University and also met the guidelines of the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

At the end of the experiment, the mice were sacrificed, and the brains and livers were removed promptly, and stored at –80°C until analysis. Blood samples were collected and centrifuged (3000 g for 15 min at 4°C) to separate the serum. Brains and livers were homogenized in cold medium. The homogenate (10%) was centrifuged at 4000 g for 15 min at 4°C and the supernatant was used for biochemical analysis.

Isolation of liver mitochondria

Liver mitochondrial fraction was prepared according to the method described by Tang *et al*.[31] with modifications. Livers were homogenized with ice-cold isolation buffer (0.25 M sucrose, 5 mM Tris-HCl and 1 mM ethylene diamine tetraacetic acid, pH 7.4) and centrifuged at 3500 g for 10 min. Mitochondrial sample was extracted from the resultant supernatant by high-speed centrifuge (10,000 g for 15 min) at 4°C. The mitochondrial pellet was washed and centrifuged (10,000 g) twice before being re-suspended in isolation buffer or specific buffer for various assays.

Serum biochemical indexes

The sera were assayed for glucose (GLUC), triglyceride (TRIG), cholesterol (CHOL), high-density lipoproteins cholesterol (HDLC), and low-density lipoproteins cholesterol (LDLC) levels using an Olympus AU2700 Clinical Chemistry Analyzer (Olympus Inc., Japan).

Biochemical analyses

Commercially available kits were used to determine the levels of protein, SOD, GSH-Px, T-AOC, MDA, MAO, Na^+-K^+-ATPase, Ca^{2+}-Mg^{2+}-ATPase. Caspase-3 and Bcl-2 were determined using ELISA kits. All procedures were carried out according to manufacturers' instructions.

Mitochondrial membrane potential (ΔΨm) assay

Mitochondrial membrane potential was measured using fluorescent rhodamin 123 probe, which is selectively retained in mitochondria with an intact membrane potential. The mitochondria suspension was incubated with rhodamin 123 for 20 min at 37°C. After sedimentation, the mitochondria were washed twice and resuspended in phosphate buffer saline. Fluorescence intensity was recorded using Cary Eclipse fluorescence spectrophotometer (America Varian Technology China Ltd.) at an excitation wavelength of 505 nm and an emission wavelength of 534 nm.

Mitochondrial membrane fluidity

Measurement of membrane fluidity was carried out using a DPH probe in a Cary Eclipse spectrophotofluorimeter following previously described procedures.[32,33]

Statistical analysis

Statistical analysis was performed using the SPSS software package, version 17.0. The values were analyzed by one-way analysis of variance (ANOVA), followed by Duncan's multiple range test. All the results were expressed as mean ± SD. The result were considered statistically significant at $P < 0.05$.

RESULTS

Chemical composition in SMO and olive oil

Table 1 shows fatty acid composition in SMO and olive oil. The percent of the total unsaturated fatty acid were 77.21% and 77.42% in SMO and olive oil, respectively. The major components of fatty acid in SMO were linoleic acid, oleic acid and palmitic acid. Oleic acid was the major component of fatty acid in olive oil.

Serum biochemistry

There were no significant ($P > 0.05$) differences in the levels of HDLC, GLUC, and LDLC among the four groups [Table 2]. Compared to NCG, the levels of CHOL and TRIG were significantly ($P < 0.05$) higher in ACG. Both SMO and olive oil significantly decreased the level of TRIG while only SMO significantly decreased the level of CHOL in aging mice.

Levels of SOD, GSH-Px, T-AOC and MDA in the livers

To determine whether SMO can attenuate the increased oxidative damage in the livers of aging mice, we measured

Table 1: Fatty acid composition in *Silybum marianum* oil and olive oil

Fatty acid	*Silybum marianum* oil	Olive oil
Myristiv acid (C14:0)	0.12	-
Palmitic acid (C16:0)	10.54	12.86
Palmtoleic acid (C16:1)	-	0.94
Stearic acid (C18:0)	5.38	4.12
Oleic acid (C18:1)	27.54	69.36
Linoleic acid (C18:2)	48.20	6.44
Linolenic acid (C18:3)	0.51	0.68
Arachidic acid (C20:0)	3.18	-
11-eicosenoic acid (C20:1)	0.96	-
Tetracosanoic acid (C24:0)	-	2.92

the activities of SOD, GSH-Px, T-AOC, and the level of MDA in livers. The results showed that SMO increased the activities of these antioxidant enzymes and decreased D-gal-induced lipid peroxidation level in the livers of D-gal-induced mice [Table 3].

Compared with NCG, The levels of SOD, GSH-Px and T-AOC were significantly lower in ACG compared to NCG mice while the opposite was true for MDA level. Supplementation with SMO or olive oil significantly increased levels of SOD, GSH-Px and T-AOC. SMO and olive oil treatment significantly decreased the level of MDA in livers.

Levels of SOD, GSH-Px, T-AOC, MAO and MDA in the brains

Levels of SOD, GSH-Px, and T-AOC were significantly lower while the levels of MAO and MDA were significantly higher in ACG mice compared to NCG mice [Table 4]. Mice treated with SMO, and olive oil had significantly higher levels of GSH-Px and significantly lower levels of MAO and MDA than ACG mice. There were no significant differences in the levels of SOD and T-AOC among ACG, SMO and PCG mice. Interestingly, no significant differences were observed in the levels of GSH-Px, MAO, and MDA in the brains of NCG mice and those treated with SMO or PCG.

Table 2: Levels of GLUC, CHOL, TRIG, HDLC, and LDLC in the serum of normal and aging Mice

Groups	GLUC (mmol/L)	CHOL (mmol/L)	TRIG (mmol/L)	HDLC (mmol/L)	LDLC (mmol/L)
NCG	4.32±0.79^{a}	2.06±0.22^{b}	2.28±0.71^{a}	1.95±0.22^{a}	0.24±0.04^{a}
ACG	4.87±0.71^{a}	2.46±0.58^{a}	2.99±1.08^{b}	2.11±0.43^{a}	0.21±0.06^{a}
SMO	4.45±0.57^{a}	2.06±0.28^{b}	1.10±0.13^{c}	2.09±0.44^{a}	0.28±0.07^{a}
PCG	4.64±0.52^{a}	2.12±0.22^{ab}	1.10±0.32^{c}	1.98±0.29^{a}	0.26±0.03^{a}

Data expressed as mean±SD (n=10);^{a,b,c} Means in the same column with common superscript are not significantly different, whereas values with different superscript are significantly different at P<0.05. GLUC: Glucose; TRIG: Triglyceride; CHOL: Cholesterol; HDLC: High-density lipoproteins cholesterol; LDLC: Low-density lipoproteins cholesterol; NCG: Normal control group; ACG: Aging control group; SMO: *Silybum marianum* oil; PCG: Positive control group

Table 3: Activities of SOD, GSH-Px, T-AOC, and level of MDA in the livers of normal and aging mice

Groups	SOD (U/mgprot)	GSH-P$_x$ (U/mgprot)	T-AOC (U/mgprot)	MDA (nmol/mgprot)
NCG	33.27±4.09^{b}	415.91±27.60^{a}	1.02±0.15^{b}	7.14±0.53^{a}
ACG	24.37±6.05^{a}	273.66±43.57^{b}	0.62±0.13^{a}	11.52±0.29^{b}
SMO	38.96±3.95^{c}	539.93±20.45^{c}	0.97±0.09^{b}	8.98±1.09^{c}
PCG	38.13±3.66^{bc}	492.35±29.69^{d}	0.92±0.12^{b}	8.83±1.03^{c}

Data expressed as mean±SD (n=10);^{a,b,c,d} Means in the same column with common superscript are not significantly different, whereas values with different superscript are significantly different at P<0.05. SOD: Superoxide dismutase; GSH-Px: Glutathione peroxidase; T-AOC: Total anti-oxidation capability; MDA: Malondialdehyde; NCG: Normal control group; ACG: Aging control group; SMO: *Silybum marianum* oil; PCG: Positive control group

Concentration of Bcl-2 and caspase-3 in the livers and brains

The level of Bcl-2 was significantly lower while the level of caspase-3 was significantly higher in ACG mice compared to NCG mice [Figures 1 and 2]. SMO or olive oil supplemented mice had significantly higher levels of Bcl-2, but lower levels of caspase-3. There were no significant differences in the level of Bcl-2 in SMO and PCG mice. No significant difference in caspase-3 level was observed in the livers of mice treated with SMO and those treated with olive oil.

Liver mitochondrial membrane fluidity and potential (ΔΨm)

The effect of SMO treatment on membrane fluidity was demonstrated by a reduction in microviscosity (η) and fluorescence polarization (P) in SMO mice relative to ACG mice [Table 5]. Compared to NCG microviscosity (η) and

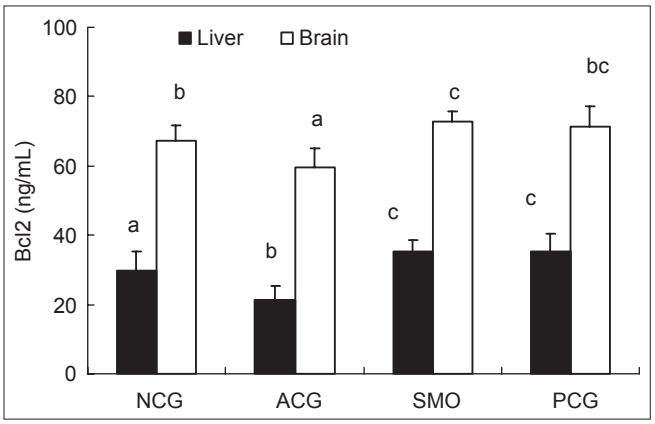

Figure 1: Concentration of Bcl-2 in the livers and brains of normal and aging mice. Data expressed as mean ± SD (n = 10); a, b, c Means in the same column with common superscript are not significantly different whereas values with different superscript are significantly different at P < 0.05

Table 4: Activities of SOD, GSH-Px, T-AOC and levels of MAO, MDA in the brains of normal and aging mice

Groups	SOD (U/mgprot)	GSH-P$_x$ (U/mgprot)	T-AOC (U/mgprot)	MAO (U/mgprot)	MDA (nmol/mgprot)
NCG	65.53±9.16^{a}	16.17±4.11^{a}	0.40±0.08^{a}	1.20±0.27^{a}	3.52±0.25^{a}
ACG	41.06±2.64^{b}	7.33±3.14^{b}	0.23±0.07^{b}	1.94±0.31^{b}	5.06±0.64^{b}
SMO	45.48±7.93^{b}	17.74±1.19^{a}	0.22±0.04^{b}	1.05±0.27^{a}	3.89±0.36^{a}
PCG	46.76±5.88^{b}	15.74±2.96^{a}	0.25±0.03^{b}	1.15±0.36^{a}	3.97±0.46^{a}

Data expressed as mean±SD (n=10);^{a,b} Means in the same column with common superscript are not significantly different whereas values with different superscript are significantly different at P<0.05. SOD: Superoxide dismutase; GSH-Px: Glutathione peroxidase; T-AOC: Total anti-oxidation capability; MDA: Malondialdehyde; NCG: Normal control group; ACG: Aging control group; SMO: *Silybum marianum* oil; PCG: Positive control group; MAO: Monoamine oxidase

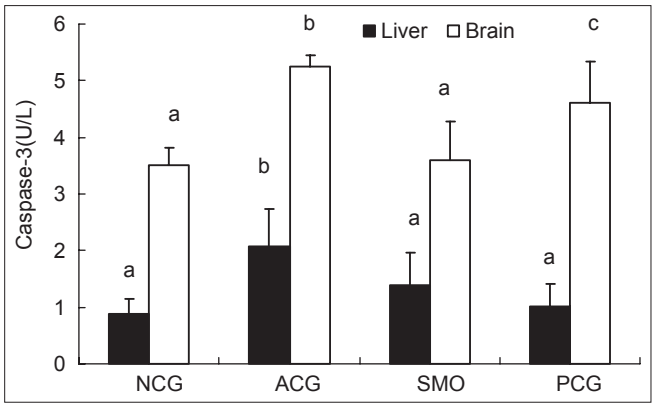

Figure 2: Concentration of caspase-3 in the livers and brains of normal and aging mice. Data expressed as mean ± SD (n = 10);a,b,c Means in the same column with common superscript are not significantly different whereas values with different superscript are significantly different at P < 0.05

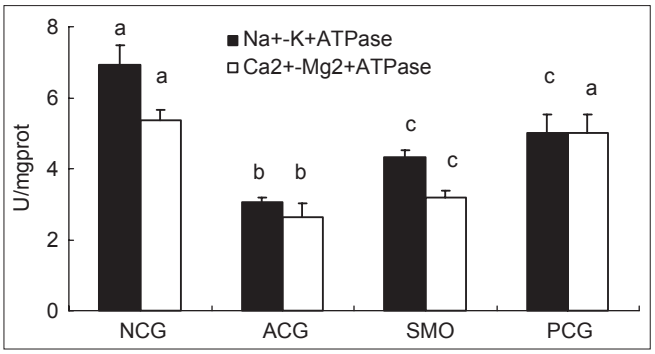

Figure 3: Activities of Na+-K+-adenosine triphosphatase (ATPase) and Ca2+-Mg2+-ATPase in the liver mitochondria of normal and aging mice. Data expressed as mean ± SD (n = 10);a,b,c Means in the same column with common superscript are not significantly different whereas values with different superscript are significantly different at P < 0.05

Table 5: Membrane potential (ΔΨm) and fluidity in liver mitochondria of normal and aging mice

Groups	ΔΨm	Fluorescence polarization (*P*)	Microviscosity (η)
NCG	44.46±0.48^{a}	0.16±0.02^{a}	1.05±0.15^{a}
ACG	24.94±1.54^{b}	0.32±0.02^{b}	4.49±0.69^{b}
SMO	41.19±1.03^{c}	0.23±0.01^{c}	1.97±0.24^{c}
PCG	47.04±1.08^{d}	0.24±0.02^{c}	1.96±0.27^{c}

Data expressed as mean±SD (n=10);$^{a, b, c}$ Means in the same column with common superscript are not significantly different, whereas values with different superscript are significantly different at P<0.05. NCG: Normal control group; ACG: Aging control group; SMO: *Silybum marianum* oil; PCG: Positive control group

fluorescence polarization (*P*) in the liver mitochondrial membrane were significantly higher in ACG. SMO or olive oil significantly decreased average microviscosity and fluorescence polarization (*P*) of the liver mitochondrial membrane in D-gal-induced aging mice. Compared with NCG, the level of membrane potential (ΔΨm) in the liver mitochondrial membrane was significantly lower in ACG. Supplementation with SMO or olive oil significantly increased the level of membrane potential (ΔΨm).

Activities of Na^{+}-K^{+}-ATPase and Ca^{2+}-Mg^{2+}-ATPase in liver mitochondria

The effect of SMO on the activities of Na^{+}-K^{+}-ATPase and Ca^{2+}-Mg^{2+}-ATPase are presented in Figure 3. The results show that the activities of Na^{+}-K^{+}-ATPase and Ca^{2+}-Mg^{2+}-ATPase were significantly decreased in ACG mice as compared to NCG mice. Mice treated with SMO or olive oil had significantly higher levels of Na^{+}-K^{+}-ATPase and Ca^{2+}-Mg^{2+}-ATPase than ACG mice.

DISCUSSION

Aging is a biological process characterized by a general and progressive decline in physiological functions, especially of the brain and liver.[34] D-gal has caused age-related changes in many animal models. It has been reported that the level of free radicals increases in animals that receive D-gal. The free radical theory of aging suggests that increased free radicals might contribute to the incidence of age-related degenerative diseases.[35-37]

The free radical damage hypothesis states that the generation of ROS or free radicals can lead to cell and tissue damage as well as alterations in the function of the genetic apparatus, resulting in aging and untimely cell death. SOD, GSH-Px, and Catalase (CAT) are the most important antioxidant enzymes that inhibit free radical formation. These enzymes also act as the primary defense system against ROS that are generated *in vivo* during oxidative stress.[38] Both SOD and GSH-Px are principally localized in the cytosolic and mitochondrial compartments of cells, whereas CAT is localized in the peroxisomes of cells. Under normal physiological conditions, these enzymes can efficiently counteract oxidative damages induced by free radicals in normal cells. In contrast, there are not enough endogenous antioxidant enzymes against excessive free radicals under abnormal physiological conditions.[39] In the present study, the mean activities of SOD and GSH-Px were found to be significantly lower in the liver and brain of D-gal-treated mice than in normal mice. Similar low levels of SOD and GSH-Px have been noted in tissues of D-gal-treated mice.[12] However, SMO significantly increased the activities of SOD and GSH-Px in the liver and the activity of GSH-Px in the brain. T-AOC reflects the capacity of the non-enzymatic antioxidant defense system. In the present study, FRAP (ferric-reducing antioxidant power) assay was used to evaluate the T-AOC of antioxidants. As shown in Table 2, SMO treatment increased the T-AOC in the liver indicating that the non-enzymatic antioxidant defense system of aging mice had been enhanced.

Lipid peroxidation is the process that involves chain reactions of free radicals with polyunsaturated fatty acids. These reactions lead to hydroperoxide generation and lipid breakdown into lower molecular weight fragments. Therefore, the inhibition of lipid peroxidation is of great importance in disease processes that involves free radicals.[40] MDA is a major reactive aldehyde resulting from the peroxidation of biological membrane-polyunsaturated fatty acids. It is therefore, used as an indicator of tissue damage involving a series of chain reactions. The levels of MDA were significantly higher in the liver and brain of D-gal-induced aging mice than in normal mice [Tables 2 and 3]. Similar findings have been reported in tissues of D-gal-treated mice.[41] Treatment with SMO or olive oil significantly reduced levels of MDA in both the liver and the brain of mice. These results indicate that the administration of SMO was effective in inhibiting the effects on lipid peroxidation in aging mice.

MAO is the major enzyme that catalyzes the degradation of neuroactive and vasoactive amines. Products of MAO-catalyzed reaction are the compelling inductors of lipid peroxidation. The activation of MAO is associated with age-related disturbances, homeostasis, and the generation of free radicals that involves the nervous tissue.[42] Brain tissues from mice injected with D-gal showed an increase in the activity of MAO. However, the administration of SMO or olive oil could significantly prevent this increase. The inhibitory effect of SMO or olive oil may correct the maladjusted metabolism of monoamine transmitter.

Apoptosis, also known as programmed cell death, is a biological process that plays a crucial role in the normal development and tissue homeostasis.[43] In this study, we found that chronic treatment with D-gal induced a significant increase in apoptosis in the liver and brain. The results also indicated that SMO or olive oil treatment significantly reduced apoptosis in the liver and brain of D-gal-treated mice [Figures 1 and 2].

The proto-oncogene Bcl-2 is one of the major regulators of the mitochondrial apoptotic pathway and plays a significant role in the regulation of apoptosis. The stability and expression levels of Bcl-2 protein can be regulated by ROS and NO through various mechanisms.[44] In our study, chronic administration of D-gal led to low levels of Bcl-2 in the liver and brain. But SMO treatment prevented the decline in Bcl-2 levels [Figure 1]. This may be due to the fact that SMO may reduce the level of oxidative stress.

Caspases, a family of cysteine proteases, are integral parts of the apoptotic pathway. Caspase-3, a key executor of apoptosis playing a vital role in programmed cell death, could be activated by ROS, leading to neuronal dysfunction.[45] Studies have shown that an increase in Bcl-2 levels limits the effects of Bax and prevents the mitochondrial release of cytochrome c, thereby inhibiting the activation of caspase cascade and apoptosis.[46] In the present study, we found that SMO reversed the elevation of caspase-3 activity in the liver and brain of D-gal-treated mice [Figure 2]. Similarly, low levels of Bcl-2 and high levels of caspase-3 have been reported in the hippocampus of D-gal-treated mice.[47] These results suggest that SMO probably regulates the expression of apoptosis-related proteins by mitigating oxidative stress to attenuate apoptosis in the mitochondrial pathway. However, the detailed mechanism of action of SMO is unknown and requires further research.

Oxidant-induced mitochondrial damage, resulting in progressive loss of cellular energy (ATP) resources, degeneration, and eventually cell death, is believed to play a key role in the aging process.[48] Mitochondrial oxidative stress should be considered a hallmark of cellular aging and the identification of mitochondria as the major source of ROS led to the mitochondrial theory of aging. ROS can oxidize membrane lipids in close proximity to the respiratory chain; thus, decreasing the fluidity and increasing the permeability of the mitochondrial membrane.[49] Research has shown that mitochondrial dysfunction is a key factor in the mechanism of D-gal-caused aging acceleration.[15] In this study, we found that chronic treatment with D-gal induced mitochondrial dysfunction in the liver of mice. Treatment of mice with SMO was effective in ameliorating D-gal-induced mitochondrial dysfunction [Table 4 and Figure 3].

The mitochondrial membrane is a target for attack by ROS. Membrane fluidity and membrane potential reflect the biophysical and biochemical characteristics of the mitochondrial membrane and are the markers of membrane function, especially the production of energy. In the present study, membrane fluidity and membrane potential of the liver mitochondria in ICR mice were assessed. A significant reduction in the membrane potential and fluidity was observed when mice were treated with D-gal. However, SMO or olive oil significantly increased levels of membrane fluidity and membrane potential of liver mitochondria. This could be due to reduced oxidative damage thereby preventing mitochondrial membrane damage.

Mitochondrial oxidative phosphorylation is the primary source of energy for metabolism. Ion transport across the membranes regulates a number of biochemical reactions in the cell. About 40% of the total ATP is consumed by Na^+-K^+-ATPase and Ca^{2+}-Mg^{2+}-ATPase, which maintain the plasma membrane potential and intracellular Ca^{2+} sequestration. ATPases are very sensitive to peroxidation reactions since they are intimately associated

with the plasma membrane and participate in the energy requiring translocation of Na+, K+, and Ca^{2+} ions.[50] In the present study, the levels of Na^{+}-K^{+}-ATPase and Ca^{2+}-Mg^{2+}-ATPase were lower in the liver mitochondria of D-gal-treated mice compared to normal mice (NCG). Treatment with SMO or olive oil significantly increased levels of ATPases probably by preventing free radical induced membrane damage.

CONCLUSIONS

The results of this study indicate that SMO has a protective effect in D-gal-induced aging mice. SMO can attenuate lipid peroxidation, renew the activities of antioxidant enzymes, modulate the expression of apoptosis-related factors, and alleviate mitochondrial damage. The data suggested that the effect of SMO is similar to that of olive oil. Therefore, SMO has the potential to be further explored as a new vegetable oil.

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