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Lowering blood lipid and hepatoprotective activity of amentoflavone from *Selaginella tamariscina* in vivo

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Amentoflavone (AF) isolated from *Selaginella tamariscina* was screened for antioxidant activities *in vitro*, and lowering blood lipid and hepatoprotective activity *in vivo*. AF had no antioxidant activity in DPPH and ABTS assay and poor reducing power in FRAP assay. Intragastric administration of AF (75 mg/kg body weight per day), ethyl acetate fraction of *S. tamariscina* (STEA) (150 mg/kg) and acidic extract of *S. tamariscina* (STAE) (150 mg/kg) to groups of hyperlipidemia mice for 21 days, they all significantly decreased the level of blood triglyceride (TG), total cholesterol (TC), and low-density lipoprotein cholesterol (LDL-C) in serum. The level of blood high-density lipoprotein cholesterol (HDL-C) significantly increased ($P < 0.001$). Intragastric administration of AF (200 mg/kg body weight per day), AF (100 mg/kg), and AF (50 mg/kg) to mice injected with carbon tetrachloride to induce acute hepatic injury for 8 days, the level of glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) in group of AF (200 mg/kg) significantly decreased ($P < 0.001$). The level of hepatic malondialdehyde (MDA) in groups of AF (200, 100, and 50 mg/kg, respectively) significantly decreased ($P < 0.001$), and the level of hepatic SOD only in group of AF (200 mg/kg) significantly increased ($P < 0.01$). The result showed that AF had a high hypolipidemic activity and hepatoprotective effect *in vivo*.

Key words: Amentoflavone, *Selaginella tamariscina*, hypolipidemic activity, hepatoprotective.

INTRODUCTION

Selaginella tamariscina (Beauv.) Spring., is an important Chinese Traditional Medicine, which has been used to treat diabetes (Li et al., 1999), immunity suppression (Wang et al., 2003), hemostases (Chen et al., 1996), lung cancer (Bi et al., 2003; Yang et al., 2007) and suppress apoptosis of thymus gland and spleen cells (Zheng et al., 2008). In addition, *S. tamariscina* also showed activities of cardiovascular protection, hepatitis, skin diseases, anti-infections (Lin et al., 1994; Lin and Kan, 1990; Mac and Sama, 1983; Wink, 1986). Phytochemical study showed that flavonoids were the main compounds in *S. tamariscina* including amentoflavone, hinokiflavone, sotetsuflavone and apogenin (Cheong et al., 1998; Zheng et al., 1998). AF can promote mutagenic activity (Cardoso et al., 2006), inhibit human cathepsin β (Pan et al., 2005), interact at GABA_A receptors (Reena et al., 2005), exhibit strong neuroprotection against cytotoxic insults induced by oxidative stress and amyloid β (Kang

et al., 2005), inhibit NF-KB activation in macrophages (Woo et al., 2005), phospholipase C1 (Lee et al., 1996) and cAMP-dependent phosphodiesterase (Saponara et al., 1998), release Ca²⁺ in skeletal muscle sarcoplasmic reticulum (Suzuki et al., 1999), and exhibit anti-inflammatory (Gambhir et al., 1987), antioxidant activity (Huguet et al., 1990), antiplasmodial and leishmanicidal activities (Junert et al., 2008). To the best of our knowledge, there is no research about the hypolipidemic and hepatoprotective activity of AF.

Fed high-fat diet is a method of establishing hyperlipemia model (Jun and Adam, 2007; Wan and Zhang, 2004), and hypolipidemic the level of index including the low-density lipoprotein cholesterol (LDL-C), the high-density lipoprotein cholesterol (HDL-C), triglyceride (TG) and total cholesterol (TC) in serum and the level of malondialdehyde (MDA) were assayed (Ikeda and Long, 1990). In our study, high TC type hyperlipemia (Liang and Chen, 2004) was established and the level of TC, TG, LDL-C, HDL-C, SOD and MDA was assayed with Jumingzi tablets as positive control *in vivo*. Carbon tetrachloride (CCl₄), is one of the oldest and widest

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toxins, was used to establish the liver injury model. CCl_4 by itself does not have cytotoxic effects on the liver but its metabolic products are responsible for the toxicity. CCl_4 can damage a number of tissues particularly the liver and kidney of many species (Drill, 1952). Administration of CCl_4 can cause cirrhosis (Opoku et al., 2007) and ultimately lead to hepatic carcinoma (Reuber and Glover, 1970). Thus, CCl_4 , a hepatotoxin for evaluating hepatoprotective agents, was commonly used to induce liver injury by producing free radical intermediates (MDA). Hepatic damage induced by CCl_4 resulted in an increase in serum glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) concentrations (Berry et al., 1992; Mitra et al., 1998). The elevation of concentrations of serum enzymes such as GOT and GPT was generally regarded as one of the sensitive markers of hepatic damage (Venkateswaran et al., 1998). Studies on hepatotoxicity induced by CCl_4 indicated that hepatic damage can probably be prevented by some herbal extracts and active compounds isolated from active fraction.

As a part of phytochemical and pharmacological studies on *S. tamariscina* (Kang et al., 2008; Wu et al., 2008; Xu et al., 2008), the antioxidant activity of AF, EtOAC (STEA) and acidic extracts (STAE) was screened by the method of 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2-azino-bis-(3-ethylbenzthiazolin-6-sulfonic acid) (ABTS) radical scavenging activity and ferric-reducing antioxidant power (FRAP) *in vitro* (Kang and Wang, 2009). Subsequently, the levels of TC, TG, LDL-C, HDL-C, MDA and SOD were assayed using Jumingjiangzhi tablets as positive control by the model of hyperlipidemia mice, and the levels of GOT, GPT, MDA and SOD were evaluated to assay the hepatoprotective activity using bifendate as positive control *in vivo*.

MATERIALS AND METHODS

General procedures and reagents

^1H -(400 MHz) and ^{13}C -NMR spectra (100 MHz) were recorded on a Bruker Avance DRX-400 spectrometer. Chemical shifts are expressed in ppm referenced to the residual solvent signals. Coupling constants (J) are reported in Hz. GPT, GOT, SOD, MDA, HDL-C, LDL-C, TG and TC detection kit from Nanjing Jiancheng Chemical Factory (China), bifendate capsule from Sichuan Meidakang Pharmaceutical Co. Ltd. (China, Batch No: 080302), Jumingjiangzhi tablet from Jilin Jichun Pharmaceutical Co. Ltd. (China, Batch No: 080302), DPPH from Tokyo Kasei, 2,4,6-tri-(2-pyridyl)-s-triazine (TPTZ), ABTS from Fluka, 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) from Aldrich, 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium GPT (ABTS) from Fluka. Gallic acid propyl (PG), butyl-*p*-hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and CCl_4 were purchased from Sigma Chemical Co.

Plant materials

S. tamariscina were collected from Guiyang City in Guizhou province of China in April 2007. It was identified by Prof. Fan Liu (Guiyang TCM College).

Animals

Male KM mice weighted 20 ± 2 g and aged 6 weeks were purchased from the Experimental Animal Center of Henan Province of China. The animals were housed in a room maintained at 25°C with a 12 h photoperiod. They were fed a laboratory chow diet and water *ad libitum*. The animals were adapted to laboratory condition for 7 days. All animal procedures were conducted in strict conformation with the 'Institute ethical committee guidelines' for the care and use of laboratory animals.

Extraction and isolation of AF

STEA

Dried powder of the whole plant of *S. tamariscina* (10 kg) was extracted three times with acetone-water (70%) at room temperature. After evaporation of the solvent *in vacuo*, the concentrated extract was suspended in water and extracted with petroleum ether, EtOAC, and *n*-BuOH to yield petroleum ether, EtOAC, and *n*-BuOH extract.

STAE

Dried powder of the whole plant of *S. tamariscina* (1 kg) was extracted with NaOH solution (2 mg/ml) three times at 50°C about 30 min. After filtration, the filtrate was adjusted, the PH value to 3.0 and the precipitate was dried *in vacuo* to yield STAE.

AF

STEA (100 g) was separated on a silica gel H with petroleum ether-EtOAC (50:1 to 8:2) and AF was detected by the method of HPLC compared with AF standard. Petroleum ether-EtOAC (10:1-8:2) fraction contained majority of AF. This fraction was further repeatedly chromatographed on Sephadex LH-20 (CHCl_3 -MeOH=1:1) to yield AF.

In vitro antioxidant activity

Scavenging activity against DPPH radical

The stable free radical DPPH \cdot was dissolved in MeOH to give a 60 μM solution. 0.1 ml of a test compound in MeOH (or MeOH itself as control) was added to 3.5 ml of the methanol DPPH solution. For each test compound, different concentrations were tested. After further mixing, the decrease in absorbance was measured at 515 nm after 30 min. The actual decrease in absorption induced by the test compound was calculated by subtracting that of the control. The antioxidant activity of each test sample was expressed as an IC_{50} value, that is the concentration in $\mu\text{g/ml}$ that inhibits DPPH absorption by 50%, and was calculated from the concentration-effect linear regression curve.

Scavenging activity against ABTS radical

The ABTS radical cation (ABTS $^+$) was produced by reacting 7 mM stock solution of ABTS with 2.45 mM potassium persulfate and allowing the mixture to stand in the dark at room temperature for 12 h before use. The ABTS $^+$ solution was diluted with methanol to an absorbance of 0.800 ± 0.05 at 734 nm.

Next, 2.85 ml of this ABTS $^+$ solution was added to 0.15 ml of different concentrations of the methanolic samples and the

decrease in absorbance at 734 nm after mixing up to 10 min. The radical scavenging activity of the tested samples, also expressed with IC_{50} value. The percentage of inhibition of ABTS^{•+} was calculated using the formula: %Inhibition = $[(A_0 - A_1)/A_0] \times 100$, where A_0 was the absorbance of the control and A_1 was the absorbance of sample and the standard compound.

FRAP assay

The stock solutions included 300 mM acetate buffer (pH 3.6), 10 mM TPTZ solution in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$ solution. The fresh working solution was prepared by mixing 25 ml acetate buffer, 2.5 ml TPTZ solution, and 2.5 ml $FeCl_3 \cdot 6H_2O$ solution. Samples (0.2 ml) were allowed to react with 3.8 ml of the FRAP solution for 30 min at 37°C. Readings of the colored product [ferrous tripyridyltriazine complex] were then taken at 593 nm. Trolox was used as a reference standard. The standard curve was linear between 25 and 400 μ mol/L Trolox. Results were expressed in RACT, $RACT_{50} = IC_{50 \text{ Trolox}} / IC_{50 \text{ compound}}$. Additional dilution was needed, if the FRAP value measured was over the linear range of the standard curve.

Hypolipidemic activity *in vivo*

Animal model and experimental design

Sixty KM male mice (20 ± 2 g) were randomly divided into six groups of ten each. Group 1 (normal control) was fed the normal laboratory diet. Group 2 (negative control) was fed the high-cholesterol diet (20% pulverized chow, 10% cholesterol, 2% sodium collate, 1% propacil, 2% propylene glycol, and 1% Tween 80). Group 3 was positive control with Juemingjiangzhi tablet (1400 mg/kg body weight per day).

Groups 4, 5 and 6 were given, in addition to high-cholesterol diet, a daily dose of AF (75 mg/kg), STEA (150 mg/kg), and STAE (150 mg/kg). Groups 1 and 2 were not given the extract but instead received equal volumes of vehicle everyday for the same period. The mice were treated for 21 days. Blood samples were collected from 16 h fasted mice and analyzed for the level of TC, TG, HDL-C, LDL-C, SOD and MDA. Blood was collected from the eyes (venous pool).

Measurement of lipid metabolic parameters

TC, TG, HDL-C, and LDL-C in serum were determined using enzymatic kits (Institute of Biological Engineering of Nanjing Jiancheng, Nanjing, China) according to the manufacturer's instructions.

Measurement of MDA and SOD level in serum

The level of MDA and SOD in serum was determined using enzymatic kits (Institute of Biological Engineering of Nanjing Jiancheng, Nanjing, China) according to the manufacturer's instructions.

Hepatoprotective activity of AF *in vivo*

Animal model and experimental design

Sixty KM male mice (20 ± 2 g) were randomly divided into six groups of ten each. Group 1 (normal control) was treated with distilled water. Group 2 (negative control) normal mice was treated

with corn oil. Group 3 was given bifendate (200 mg/kg body weight per day) as positive control. Groups 4, 5 and 6 were given AF (200 mg/kg body weight per day), AF (100 mg/kg), and AF (50 mg/kg) respectively. The duration of treatment was 8 days for mice by intragastric administration and given CCl_4 with 0.05 ml/kg b.w. diluted in corn oil by intraperitoneally injecting after 2 h of the last administration except for the group 1. Blood samples for determining the level of GPT and GOT were collected after 16 h. The liver homogenate solution of 10 and 1% for determining the level of MDA and SOD was processed at 0 to 3°C by tissue homogenizer respectively.

Measurement of GPT, GOT, MDA and SOD

The content of protein was determined by the method of Coomassie Brilliant Blue G-250. The level of GPT, GOT, SOD and MDA was determined using enzymatic kits (Institute of Biological Engineering of Nanjing Jiancheng, Nanjing, China) according to the manufacturer's instructions.

Statistical analysis

All the grouped data were statistically evaluated with SPSS 11.00 software. Hypothesis testing methods included one-way analysis of variance followed by least significant difference (LSD) test. $P < 0.05$ was considered to indicate statistical significance. All results are expressed as mean \pm standard deviation (SD) for ten mice in each group.

RESULTS AND DISCUSSION

Structure elucidation of AF

AF was isolated from the STEA, by correlating with melting points and spectral data (1H and ^{13}C NMR) of literature values (Chair et al., 1977). Amentoflavone: Yellow powder, mp 298-300°C. 1H NMR (400 MHz, CD_3OD) δ : 6.55 (1H, s, H-3), 6.34 (1H, s, H-6), 7.48 (2H, d, $J = 8.8$ Hz, H-2', 6'), 6.70 (2H, dd, $J = 2.8, 11.6$ Hz, H-3', 5'), 6.56 (1H, s, H-3''), 6.16 (1H, d, $J = 2.1$ Hz, H-6''), 6.39 (1H, d, $J = 2.1$ Hz, H-8''), 7.92 (1H, d, $J = 2.3$ Hz, H-2''), 7.07 (1H, d, $J = 8.6$ Hz, H-5''), 7.83 (1H, dd, $J = 2.3, 8.7$ Hz, H-6''). ^{13}C NMR (100 MHz, CD_3OD) δ : 165.8 (C-2), 103.5 (C-3), 184.2 (C-4), 162.4 (C-5), 100.2 (C-6), 163.1 (C-7), 105.6 (C-8), 156.5 (C-9), 105.5 (C-10), 123.5 (C-1'), 129.3 (C-2', 6'), 116.9 (C-3', 5'), 162.5 (C-4'), 166.0 (C-2''), 104.2 (C-3''), 183.8 (C-4''), 163.4 (C-5''), 100.3 (C-6''), 166.2 (C-7''), 95.2 (C-8''), 159.4 (C-9''), 105.3 (C-10''), 123.3 (C-1'''), 132.8 (C-2'''), 12115 (C-3'''), 160.8 (C-4'''), 117.5 (C-5'''), 128.9 (C-6''').

In vitro antioxidant activity

The result of antioxidant activity was listed in Table 1 with BHT, BHA and PG as positive control. In DPPH assay, only bifendate ($IC_{50} = 23.06 \pm 0.37$ μ g/ml) showed antioxidant activity and was lower than that of PG, BHA and BHT. Others did not have scavenging activity against DPPH radical antioxidant. In ABTS assay, the stronger ABTS radical scavengers were STAE ($IC_{50} = 2.53 \pm 0.02$

Table 1. Antioxidant activity of STEA, STAE and AF *in vitro*.

Test material	DPPH IC ₅₀ (µg/ml)	ABTS IC ₅₀ (µg/ml)	FRAP RACT ₅₀ (µmol/g)
STEA	NA ^c	37.33±0.15	111.99±2.06
STAE	NA ^c	2.53±0.02	85.02±0.01
AF	NA ^c	NA ^c	112.14±0.06
Juemingjiangzhi ^a	NA ^c	46.81±0.51	193.14±0.01
Bifendate ^a	23.06±0.37	9.56±0.72	601.55±2.19
PG ^b	0.61±0.01	0.91±0.02	11554.78±501.34
BHA ^b	2.16±0.03	1.78±0.01	6633.04±114.04
BHT ^b	8.76±0.20	1.54±0.05	1581.68±97.41

^aJuemingjiangzhi tablet and Bifendate is one of the Chinese patent drugs, not include adjuvant and have been detected in antioxidant capability. ^bThree positive controls (PG, BHA and BHT) are the finest antioxidants, have strong toxicity and often are used in antioxidant experiments *in vitro*. ^cNA is no activity, the suppression ratio of samples are below 50%.

Table 2. Effect of STEA, STAE and AF on TC, TG, HDL-C and LDL-C in nutritive hyperlipidemia mice.

Group	TC (mmol/L)	TG (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)
1	3.9650±0.7858	2.6008±0.7783	0.7106±0.1281	1.7146±0.3196
2	5.6711±1.4984 ^{###}	1.7645±0.1812 ^{###}	0.3812±0.0724 ^{##}	3.0498±1.5870 ^{###}
3	3.7332±0.8868 ^{***}	0.9170±0.2185 ^{***}	0.3629±0.1525	0.6276±0.3332 ^{***}
4	4.0814±1.3110 ^{***}	1.2804±0.658 [*]	1.7427±0.2002 ^{***}	1.5979±0.8311 ^{**}
5	4.3587±1.3839 [*]	1.0956±0.3368 ^{**}	1.4164±0.4319 ^{***}	2.0875±1.0011 [*]
6	4.1421±0.9537 ^{**}	1.2710±0.2629 [*]	1.5653±0.3701 ^{***}	2.0385±1.1073 [*]

Values are mean ± SD for 10 mice. [#]P<0.05, ^{##}P<0.01, ^{###}P<0.001 compared with group 1; ^{*}P<0.05, ^{**}P<0.01, ^{***}P<0.001 compared with group 2 (negative group). Group 1 = normal group, group 2 = negative group, group 3 = Jueming tablets, group 4 = AF, group 5 = STEA, group 6 = STAE.

µg/ml), bifendate (IC₅₀=9.56±0.72 µg/ml), STEA (IC₅₀=37.33 ±0.15 µg/ml) and Jueming tablet (IC₅₀=46.81±0.51 µg/ml) respectively; but they all were lower than that of PG, BHA and BHT. In FRAP assay, STAE, bifendate, STEA and Jueming tablet showed poor antioxidant activity in ferric reducing power compared with positive control. It indicated that these samples had poor ability to donate electrons. AF did not show the activity in DPPH and ABTS assay.

Hypolipidemic activity *in vivo*

Effect of AF, STEA and STAE on serum lipid profile

The effect of intragastric administration of Jueming tablet (1400 mg/kg, positive control), AF (75 mg/kg), STEA (150 mg/kg), and STAE (150 mg/kg) on serum lipid profiles is summarized in Table 2 and Figures 1 and 2. In Table 2, the level of TG, TC and LDL-C in group 2 showed a significant increase compared with group 1 (P<0.001), and the level of HDL-C also significantly decreased compared with group 1 (P<0.01). It indicated that the hypolipidemic model was established in the level

of TG, TC, HDL-C and LDL-C. Compared with group 2, the level of TC, TG and LDL-C in groups 3, 4, 5, and 6 significantly decreased in serum. The level of HDL-C in groups 4, 5, and 6 was significantly increased (P<0.001) compared with group 2, except that the level of HDL-C in group 3 was similar to group 2. Compared with positive control of Jueming tablet, the result showed that intragastric administration of AF to nutritive hyperlipidemia mice may increase the level of HDL-C (Figure 2).

Effect of AF, STEA and STAE on liver oxidative status

Compared with group 1, the level of SOD in group 2 was significantly decreased (P<0.001), and the level of MDA was significantly increased (P<0.001). Compared with group 2, the level of MDA in groups 3, 4, and 5 was significantly decreased, and the level of SOD was significantly increased in serum. The level of MDA in group 6 was significantly decreased, whereas the level of SOD was similar to group 2 (Table 3). Compared with positive control of Jueming tablet (group 3), the results showed that intragastric administration of STEA (group 5)

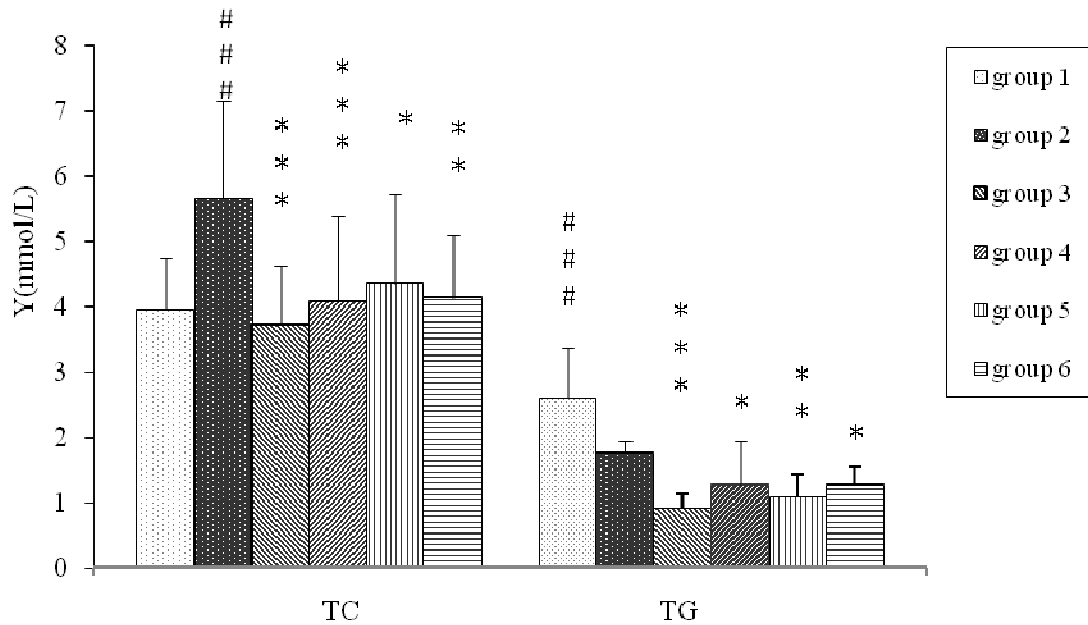


Figure 1. Effect of groups 1, 2, 3, 4, 5 and 6 on serum TC and TG in mice.

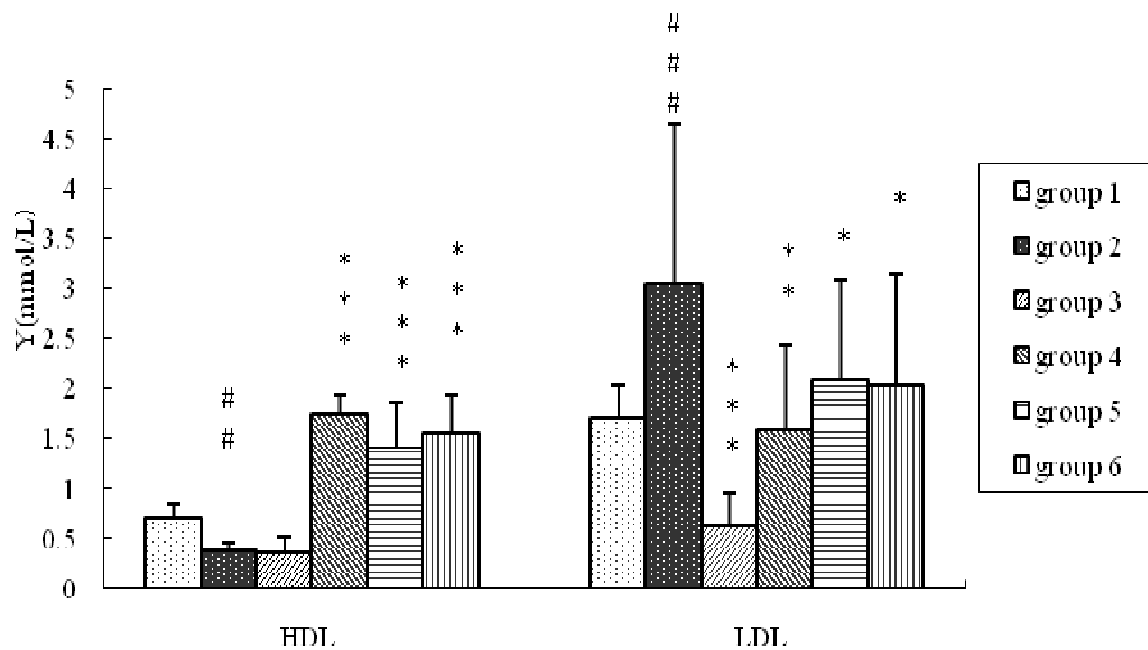


Figure 2. Effect of groups 1, 2, 3, 4, 5 and 6 on serum HDL and LDL in mice.

and STAE (group 6) to nutritive hyperlipidemia mice may decreased the level of MDA.

Hepatoprotective activity of AF *in vivo*

Effect of AF on liver GPT and GOT

The level of hepatic GPT and GOT is presented in Table

4. The level of GPT and GOT in group 2 was significantly increased ($P < 0.001$) compared with group 1 after CCl_4 injection. The level of GPT and GOT in groups 3 and 4 was significantly decreased ($P < 0.001$) with group 2, group 5 whereas only the level of GOT significantly decreased ($P < 0.001$) compared with group 2. Compared with positive control of bifendate (200 mg/kg), the result showed that intragastric administration of AF (200 mg/kg)

Table 3. Effect of STEA, STAE and AF on SOD and MDA in nutritive hyperlipidemia mice.

Group	SOD (U/ ml)	MDA (nmol/ ml)
1	268.4516±24.3778	5.6970±1.0421
2	150.3047±34.4318 ^{###}	7.0023±0.7676 ^{###}
3	272.4355±38.7828 ^{***}	5.7143±0.6588 ^{**}
4	182.8515±36.9608 [*]	6.9189±0.2599 [*]
5	193.5743±35.6970 [*]	5.5631±1.9554 ^{**}
6	159.5248±35.0334	4.8348±1.4877 ^{***}

Values are mean ± SD for 10 mice. [#]P<0.05, ^{##}P<0.01, ^{###}P<0.001 compared with group 1; ^{*}P<0.05, ^{**}P<0.01, ^{***}P<0.001 compared with group 2 (negative group). Group 1 = normal group, group 2 = negative group, group 3 = Jueming tablets, group 4 = AF, group 5 = STEA, group 6 = STAE.

Table 4. Effect of AF on GPT and GOT in liver damage mice.

Group	GPT (IU/L)	GOT (IU/L)
1	28.5059±6.9612	57.2750±5.1400
2	5335.504±2176.9994 ^{###}	3190.8340±1568.9852 ^{###}
3	425.8067±149.3341 ^{***}	449.0662±237.4615 ^{***}
4	439.1177±365.0718 ^{***}	526.0301±349.7313 ^{***}
5	3671.0440±1323.2118	965.1698±195.7928 ^{***}
6	8060.7420±1557.4852	1967.1970±697.2690

Values are mean ± SD for 10 mice. [#]P<0.05, ^{##}P<0.01, ^{###}P<0.001 compared with group 1; ^{*}P<0.05, ^{**}P<0.01, ^{***}P<0.001 compared with group 2 (negative group). Group 1 = normal group, group 2 = negative group, group 3 = Bifendate (200 mg/kg) as positive control, group 4 = AF (200 mg/kg), group 5 = AF (100 mg/kg), group 6 = AF (50 mg/kg).

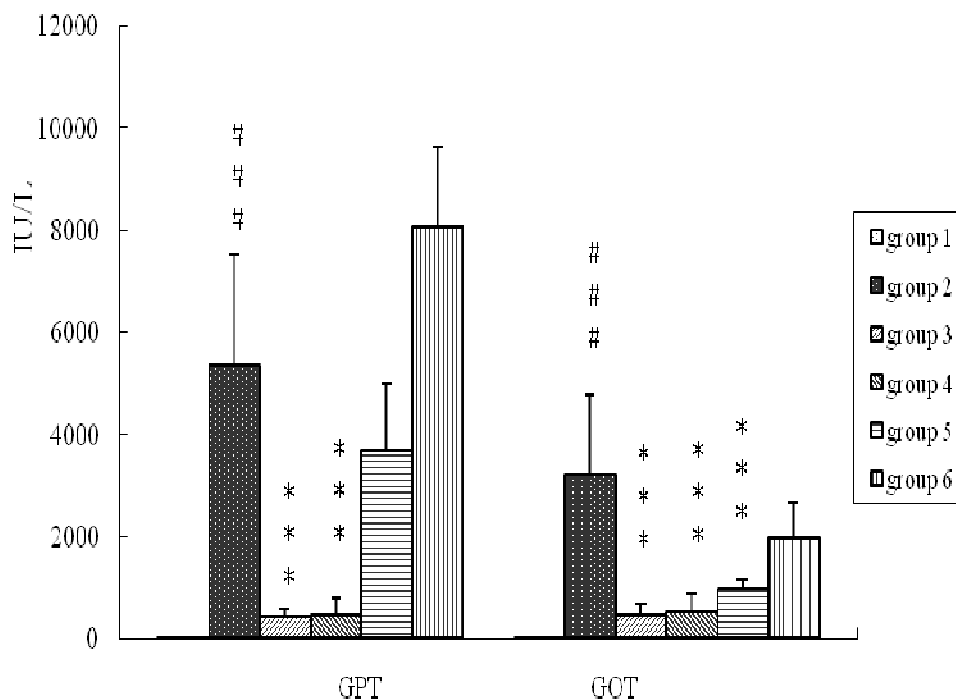
**Figure 3.** Effect of groups 1, 2, 3, 4, 5 and 6 on serum GPT and GOT.

Table 5. Effect of AF on hepatic SOD and MDA in liver damage mice.

Group	MDA (nmol/ mgprot)	SOD (U/ mgprot)
1	0.4801±0.3017	149.8931±22.5606
2	1.5829± 0.8818 ^{###}	124.7190±2.2146 ^{###}
3	0.3471±0.0801 ^{***}	136.8188±21.1332
4	0.38172±0.0246 ^{***}	207.5105±71.4256 ^{**}
5	0.4349±0.0542 ^{***}	130.9980±30.2643
6	0.4567±0.1918 ^{***}	129.7856±40.7032

Values are mean ± SD for 10 mice. [#]P<0.05, ^{##}P<0.01, ^{###}P<0.001 compared with group 1; *P<0.05, **P<0.01, ***P<0.001 compared with group 2 (negative group). Group 1 = normal group, group 2 = negative group, group 3 = Bifendate (200 mg/kg) as positive control, group 4 = AF (200 mg/kg), group 5 = AF (100 mg/kg), group 6 = AF (50 mg/kg).

was similar to bifendate and showed dose dependence (Figure 3).

Effect of AF on liver oxidative status

Compared with groups 1 and 2, the level of hepatic MDA was significantly decreased in groups 3, 4, 5, and 6 (P<0.001), and the level of hepatic SOD was significantly increased only in group 4 (P<0.01) Table 5.

Conclusion

AF as the majority constituent in STEA had no scavenging activity in DPPH and ABTS radicals, as well as poor reducing power in FRAP assay *in vitro*. AF, STEA, and STAE significantly decreased the level of TG, TC, and LDL-C in serum *in vivo* and showed strong lowering blood lipid activity. Furthermore, they significantly increased the level of HDL-C and SOD in serum, and significantly decreased the level of MDA. The result suggested that AF, STEA, and STAE might be capable of lowering or slowing oxidative-stress-related lipid AF (200 mg/kg) can significantly decreased the level of MDA in liver, GPT and GOT in serum significantly increased the level of SOD in liver. The result suggested that AF has relatively good and favorable hepatoprotective effect peroxidation.

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