



In vitro antioxidant activity and inhibitory effect, on oleic acid-induced hepatic steatosis, of fractions and subfractions from oat (*Avena sativa* L.) ethanol extract

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ABSTRACT

Oats (*Avena sativa* L.) were extracted with 80% aqueous ethanol and the extract was successively isolated by liquid–liquid partition to yield *n*-hexane, ethyl acetate, *n*-butanol and water layers. Among them, the ethyl acetate (EA) layer exhibited the highest total phenolic content (TPC), the strongest DPPH radical-scavenging activity and an inhibitory effect on an oleic acid-induced (OA-induced) fatty liver model *in vitro*. Thus, it was further fractionated by a Sephadex LH-20 column into three subfractions (SF1–SF3). SF3 was the most active subfraction in all the assays above, the yield being 1.70% of the dry weight of the EA fraction. The major components in SF3 were identified as avenanthramides Bc, Bp and Bf by HPLC analysis, with contents of about 5.20%, 9.19% and 8.06% of the dry weight of SF3, respectively. Also, the avenanthramides Bc, Bp and Bf all had significant inhibitory effects on oleic acid-induced (OA-induced) fatty liver.

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1. Introduction

Calorie-enriched diet intake and lack of exercise have been causing a world-wide surge of obesity, insulin resistance and lipid accretion in liver, which can lead to non-alcoholic steatohepatitis (NASH) (Begrliche, Igoudjil, Pessayre, & Fromenty, 2006). NASH, the inflammatory form of non-alcoholic fatty liver disease (NAFLD), is a chronic liver disease that occurs in individuals without significant alcohol consumption (Baskol, Baskol, & Kocer, 2007). It was histologically characterised by macrovesicular steatosis and lobular hepatitis with necrosis or ballooning degeneration and fibrosis (Malaguarnera, Madeddu, Palio, Werena, & Malaguarnera, 2005). NASH is a potentially progressive hepatic disorder that can lead to end-stage liver disease and hepatocellular carcinoma (Petta, Muratore, & Craxi, 2009). However, most patients with NASH are asymptomatic, and the disease has been detected by chance (Malaguarnera et al., 2005). The prevalence of NASH is 2.1–6.3% in the general population (Slighte et al., 2004). NASH has become, by far, the most frequent cause of liver disease in the US (Begrliche, Knockaert, Massart, Robin, & Fromenty, 2009).

The pathogenesis of NASH is complicated, and the prevailing theory is the “two hits” hypothesis proposed by Day and James (1998). The “first hit” is the deposition of liver free fatty acid and triglyceride in hepatocytes (steatosis). In the “second hit”, steatosis

progresses to NASH and this progress is associated with factors such as oxidative stress, mitochondrial dysfunction, and inflammatory cytokines (Ramirez-Tortosa et al., 2009). Slighte et al. (2004) found that hepatic inflammation and fibrosis had the potential to augment steatosis, oxidative stress and cytokines induction, generating a vicious circle that ultimately leads to cirrhosis and hepatocellular carcinoma.

It has been proved by several lines of studies that oxidative stress may play an important role in the pathogenesis of NASH (Sheth, Gordon, & Chopra, 1997). Oxidative stress results from an imbalance between pro-oxidant and antioxidant chemical species that leads to oxidative damage of cellular macromolecules (Brown-ing & Horton, 2004). It may be secondary to the release of inflammatory mediators which are the prime mediator of cell injury (Robertson, Leclercq, & Farrell, 2001). Reactive oxygen species (ROS), including oxygen ions, free radicals, and peroxides, are the main pro-oxidants in the body. The ROS are generated physiologically during oxidative phosphorylation (Rahman, Biswas, & Kode, 2006). Dietary antioxidants have been proposed as therapeutic agents to counteract liver damage (Lima, Fernandes-Ferreira, & Pereira-Wilson, 2006). Cereals, vegetables and fruits, which contain many kinds of phenolic compounds, may have high free radical-scavenging ability (Yang et al., 2010).

Oat (*Avena sativa* L.) has been recognised as a healthy food for a long time, it contains antioxidants, such as vitamin E and many kinds of phenolic compounds, which have been proved to have strong antioxidant activity *in vitro* and *in vivo* (Peterson, Hahn,

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& Emmons, 2002). Avenanthramides are unique components of oats which exhibit free radical-scavenging ability *in vitro* (Fagerlund, Sunnerheim, & Dimberg, 2009; Peterson et al., 2002). Furthermore, it has been proved that avenanthramides have the potential to inhibit vascular smooth muscle cell proliferation and decrease the risk of atherosclerosis (Liu, Zubik, Collins, Marko, & Meydani, 2004; Nie, Wise, Peterson, & Meydani, 2006).

The objectives of the present work were to evaluate the inhibitory effect of different oat extracts, together with chromatographically separated fractions and avenanthramide standards, on an OA-induced fatty liver model *in vitro* and to some extent indicate the relationship between radical-scavenging activity and the inhibitory effect. Furthermore, we tentatively identified and quantified the components which may contribute to these functions.

2. Materials and methods

2.1. Reagents and standards

2-Diphenyl-1-picrylhydrazyl (DPPH) radical and oleic acid (OA) were purchased from Sigma Chemicals Co. (St. Louis, MO); foetal bovine serum (FBS), penicillin, streptomycin and Dulbecco's modified Eagle's medium (DMEM) were obtained from Gibco (Grand Island, NY); HPLC grade acetonitrile and methanol were purchased from Mallinckrodt Baker (Phillipsburg, USA). Avenanthramide standards, N-(3',4'-dihydroxy-(E)-cinnamoyl)-5-hydroxyanthranilic acid (Bc), N-(4'-hydroxy-(E)-cinnamoyl)-5-hydroxyanthranilic acid (Bp) and N-(4'-hydroxy-3'-methoxy-(E)-cinnamoyl)-5-hydroxyanthranilic acid (Bf), were kindly provided by Dr. Mitchell L. Wise, United States Department of Agriculture. All the enzymatic kits used in the study were purchased from Beijing Zhongsheng Hightech Bioengineering Company (Beijing, China).

2.2. Plant materials

Oats (harvested in 2007) were purchased from the Chinese Academy of Agricultural Sciences (genotype, G4, Beijing, China) in October 2008. They were stored at 4 °C prior to use.

2.3. Preparation of extracts from oat

Oats (2000 g) were ground into powder under liquid nitrogen with a mortar and pestle, and ultrasonically extracted with 20 l of ethanol/water (80:20 v/v) at 30 °C for 30 min. The mixture was then extracted in a shaking water bath (300 rpm) at 60 °C for 2 h. After cooling to room temperature, the slurries were centrifuged at 4000g for 15 min, and the supernatant was collected. The residue was extracted once more under the same conditions, and the supernatants were combined. The solution was then evaporated under reduced pressure to obtain the ethanol extract, with the yield being 5.85% of the dry weight of oats. The ethanol extract was then dissolved and sequentially extracted with *n*-hexane, ethyl acetate (EA) and water-saturated *n*-butanol, using liquid-liquid partition. After removal of the solvents, four fractions were obtained, as shown in Table 1; they were *n*-hexane, ethyl acetate (EA), *n*-butanol and water fractions, with the extraction yields being 44.57%, 3.86%, 2.66%, and 6.56% of the dry weight of oat ethanol extract, respectively. The TPC, DPPH radical-scavenging activity and the inhibitory effect on an OA-induced fatty liver model *in vitro* were evaluated.

2.4. UV-vis spectrophotometric analysis

EA fraction (1 mg) was dissolved in 10 ml of methanol (100%). The absorbance of sample solution was scanned within the wave-

Table 1

Results of yield, TPC and DPPH radical-scavenging activity for the fractions of ethanol crude extract from oats (*Avena sativa* L.).

Fractions	Yield	TPC	DPPH
<i>n</i> -Hexane fraction	44.6	996 ± 17.3	19.9 ± 1.0
Ethyl acetate fraction	3.86	3764 ± 29.9	2.5 ± 0.4
<i>n</i> -Butanol fraction	2.66	2896 ± 56.7	7.1 ± 0.9
Water fraction	6.56	1746 ± 13.0	17.1 ± 1.1

All measurements are expressed as means ± S.D. ($n = 3$). Yield is expressed as mg/100 mg dry weight of ethanol extract. TPC is expressed as mg gallic acid equivalents/100 g dry weight of fractions. DPPH radical-scavenging activity is expressed as EC50 in mg/ml for fractions.

length range 220–800 nm, using a spectrophotometer (model UV-2450, Shimadzu, Japan). The UV spectrum of the EA fraction is depicted in Fig. 1a, and the characteristic absorption peak of each EA fraction was detected at 330 nm.

2.5. Sephadex LH-20 column chromatography

Because of its strong antioxidant activity and inhibitory effect on an OA-induced fatty liver model *in vitro*, the EA fraction (2.0 g) was further fractionated by a Sephadex LH-20 column (40 cm × 2.5 cm i.d., GE Healthcare Bio-Sciences AB, Sweden). The column was previously equilibrated with methanol and then eluted with methanol at a flow rate of 1.0 ml/min. The eluent was collected (5 ml/tube), using a fraction collector (model DBS-

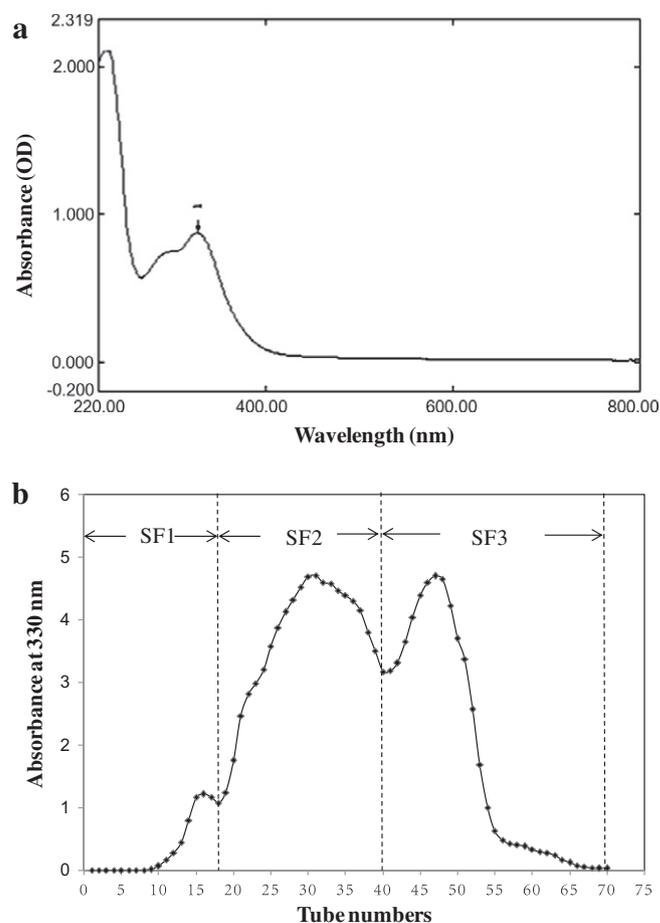


Fig. 1. UV-vis spectra of EA fraction. Peak 1 was the characteristic absorption peak detected at the wavelength of 330 nm (a), and chromatographic profiles and selected chromatographic subfractions of EA fraction through Sephadex LH-20 column detected by absorbance at the wavelength of 330 nm (b).

100, Shanghai Hu Xi Analysis Instrument Factory Co. Ltd., Shanghai, China) and the absorbance was detected at 330 nm, using a UV spectrophotometer. As shown in Fig. 1b, three main subfractions (SF1–SF3) possessing high absorbance were obtained, and these were lyophilised using a freeze dryer (model LGJ-18, PLA Academy of Military Sciences, Beijing, China) after removing the organic solvent under reduced pressure. According to the data presented in Table 2, the yields of SF1–SF3 were 40.95%, 36.17% and 1.70% of the dry weight of the EA fraction, respectively. The TPC, DPPH radical-scavenging activity and the inhibitory effect on OA-induced fatty liver model *in vitro* of SF1–SF3 were evaluated.

2.6. Determination of DPPH radical-scavenging activity

The DPPH radical-scavenging activity was assayed according to the method described by Kondo, Tsuda, Muto, and Ueda (2002) with some modifications. Briefly, an aliquot of 4.0 ml of 0.06 mM DPPH radical solution, dissolved in ethanol, was mixed with 0.2 ml of various concentrations of samples, and the mixture was kept at room temperature in the dark for 30 min. The absorbance of the resulting solution was measured at 516 nm. Ethanol was used instead of the sample solution as the control.

The scavenging activity of sample was expressed as 50% effective concentration (EC₅₀) which represented the concentration of sample having a 50% of DPPH radical-scavenging effect (Lo & Cheung, 2005). Lower EC₅₀ value indicated higher free radical-scavenging activity of a sample.

2.7. Determination of total phenolic content (TPC)

The TPC in samples was determined according to the Folin–Ciocalteu method described by Singleton and Rossi (1965). In short, 1 ml of sample solution was mixed with 1 ml of Folin–Ciocalteu reagent. After 1 min of incubation at room temperature, 1.5 ml of Na₂CO₃ solution (20% aqueous solution) were added to the mixture, followed by the addition of 7.5 ml of distilled water. The mixture was then kept in a constant-temperature water bath at 70 °C for 10 min. After cooling to room temperature, absorbance was measured at 765 nm. The TPC was expressed as gallic acid equivalents (GAE) by comparing with a gallic acid calibration curve. All tests were performed in triplicate.

2.8. Cell culture

HepG2 cells were purchased from the Peking Union Medical College Hospital (Beijing, China). They were cultured in DMEM supplemented with 10% foetal bovine serum (FBS), penicillin (100 units/ml), and streptomycin (100 µg/ml) in a humidified incubator (model MCO-20AIC, Sanyo Electric Biomedical Co., Japan) with 5% CO₂ in air at 37 °C. The cells were subcultured every 3–4 days at 1:3 split ratios. Cells with 10–20 passages were used in this study. Medium was changed every other day.

Table 2
Results of yield, TPC and EC₅₀ value for the subfractions from the EA fraction.

Subfractions	Yield	TPC	DPPH
SF1	41.0	190 ± 1.8	22.2 ± 4.4
SF2	36.2	4536 ± 52.0	1.27 ± 0.4
SF3	1.7	41,079 ± 689.0	0.23 ± 0.1

All measurements are expressed as means ± S.D. (n = 3). Yield is expressed as mg/100 mg dry weight of ethyl acetate fraction. TPC is expressed as mg gallic acid equivalents/100 g dry weight of subfractions. DPPH radical-scavenging activity is expressed as EC₅₀ in mg/ml for subfractions.

2.9. Testing the cytotoxicity of samples

Four fractions extracted from oat ethanol extract, and three subfractions from the EA fraction, were dissolved in DMSO and diluted with DMEM medium to different concentrations and incubated with HepG2 cells for 24 h. The cytotoxicity of samples in 0.1% DMSO (final DMSO concentration in medium) was tested, using 3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide (MTT) assay, as described by Mossman (1983) with some modifications. In brief, cells were washed once with PBS, carefully, and incubated with 0.2 ml of serum-free DMEM medium containing 0.05% MTT for 4 h. After that, the culture medium was removed and 0.15 ml of DMSO was added to solubilise the formed formazan. The absorbance of each well was measured at 570 nm with a microplate reader. We compared the absorbance of the treated cells with the controls, which were considered as the 100% viability value.

2.10. Inhibitory effect on OA-induced fatty liver model *in vitro*

After 24 h in culture, FBS-containing medium was removed from wells and cells were washed twice with PBS. Then the PBS was changed to the medium containing 0.75 mM oleic acid–bovine serum albumin (OA–BSA) complex (molar ratio of OA/BSA was 4/1), with or without a 50 µg/ml concentration of samples and avenanthramide standards. Then the cells were incubated for a further 24 h (Lin et al., 1995). The medium with only BSA was selected as the control. Finally, we collected the supernatants of different groups to determine the TG levels in the cell lysates with Multiskan MK3 (Thermo Electron Co., USA).

2.11. Qualitative–quantitative chromatographic analysis

The compounds of SF3 were investigated with an HPLC system consisting of a Shimadzu HPLC (Model LC-10ATvp Pumps and DGU-12A Degasser) equipped with a diode array detector (Model SPD-M10Avp) (Shimadzu, Kyoto, Japan). The separation was performed on a Shim-Pack VP-ODS column (250 mm × 4.6 mm i.d., particle size 5 µm) with a guard column (Shim-pack G VP-ODS, 10 mm × 4.6 mm i.d., particle size 5 µm) (Shimadzu, Kyoto, Japan). Two solvents were used for the mobile phase: (A) acetonitrile, (B) 0.1% formic acid. The gradient elution used for SF3 was: 0–5 min, 18% A; 5–50 min, 18–35% A; 50–51 min, 35–60% A; 51–65 min, 60% A; 65–66 min, 60–18% A. SF3 was injected to the column, using a 20 µl loop valve. Flow rate was 1.0 ml/min, and the detection was performed at 330 nm. Components were tentatively identified by comparison of their retention times with those of authentic standards under identical analysis conditions at 330 nm. All standard and sample solutions were injected triplicate.

2.12. Statistical analysis

Data were expressed as the mean values ± standard deviation for each measurement. The data were also analysed by one-way analysis of variance (one-way ANOVA). Tukey's procedure was used for significance of difference (*p* < 0.05). Analysis was done with SPSS 13.0 (SPSS, Inc., Chicago, IL, USA).

3. Results and discussion

3.1. Total phenolic content (TPC) and DPPH radical-scavenging activity of four fractions from oat ethanol extract

The TPC of different fractions of oat ethanol extract are shown in Table 1. Among the four fractions, the EA fraction had the

highest value ($p < 0.01$), followed by the *n*-butanol and water fractions, while the *n*-hexane fraction had the lowest GAE value ($p < 0.01$). The results indicate that phenolic compounds in the oat ethanol extract were much more soluble in organic solvents (especially EA) than in water.

The antioxidant activities of the four fractions of oat ethanol extract were evaluated by DPPH radical-scavenging assay, based on the electron transfer mechanism (Foti, Daquino, Mackie, DiLabio, & Ingold, 2004). Then DPPH radical is a stable free radical that shows a maximum absorption at 516 nm. When it encounters an antioxidant, the DPPH radical will be scavenged, and the absorbance at 516 nm will disappear (Jayasinghe, Gotoh, Aoki, & Wada, 2003). The more the absorbance decreases, the more potent is the antioxidant activity of the extract in terms of its electron donating capacity (Alasalvar et al., 2009).

As shown in Table 1, the EA fraction had the strongest DPPH radical-scavenging activity, followed by the *n*-butanol fraction; the scavenging activities of water and *n*-hexane fractions were the weakest. Compared with the EA fraction, the EC_{50} value of the *n*-butanol fraction was 2.84-fold higher while the water fraction and the *n*-hexane fraction were 7.91-fold and 6.84-fold higher, respectively. Antioxidant compounds were mainly soluble in EA. Furthermore, the statistical analysis revealed that there was a significant negative correlation ($r = -0.991$, $p < 0.01$) between EC_{50} value and the TPC, suggesting that phenolic compounds were likely significant contributors to antioxidant activity in the oat extracts. Mahattanatawee et al. (2006) reached a similar conclusion in their study on tropical fruits.

3.2. Cell cytotoxicity test

The four fractions extracted from oat ethanol extract, and the three subfractions of EA fraction dissolved in 0.1% DMSO, all showed no toxicity on HepG2, as tested up to the 50 $\mu\text{g}/\text{ml}$ concentration; the 0.1% DMSO also showed no toxicity to HepG2 during a 24 h incubation. Therefore, a 50 $\mu\text{g}/\text{ml}$ concentration of sample can be used in our study.

3.3. Inhibitory effect of four fractions from oat ethanol extract on an OA-induced fatty liver model *in vitro*

The accumulation of triglyceride (TG) in the liver is a hallmark of hepatic steatosis, as it reflects an imbalance between hepatic

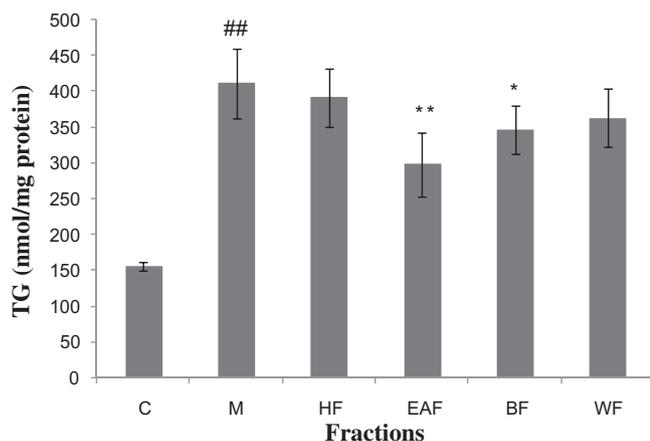


Fig. 2. Effect of 0.75 mM OA-BSA complex, with or without fractions extracted from oat ethanol extract, on the TG level of HepG2 cells. The concentration of fraction was 50 $\mu\text{g}/\text{ml}$. Data are expressed as the means \pm standard deviation ($n = 4$). * $p < 0.05$, ** $p < 0.01$ means significantly different from the model. # $p < 0.05$, ## $p < 0.01$ means significantly different from the control.

TG synthesis and secretion (Fong, Nehra, Lindor, & Buchman, 2000). Fig. 2 shows the intracellular TG levels of samples in the model group (column M), control group (column C) and administration groups. The intracellular TG levels were increased significantly ($p < 0.01$) by the addition of 0.75 mM OA-BSA complex. Compared to the model group, the addition of the EA fraction (50 $\mu\text{g}/\text{ml}$) showed the strongest inhibitory effect ($p < 0.01$) on the increase of TG levels in the OA-BSA treated cells among the four administration groups, with the inhibition percentage being 27.6%. Addition of the *n*-butanol fraction (50 $\mu\text{g}/\text{ml}$) also exhibited a significant inhibitory effect ($p < 0.05$), and the inhibition percentage was up to 11.7%, while the *n*-hexane and the water fractions showed no inhibitory effect on the increase of TG levels at such concentration. It was concluded that the active components which could inhibit the increase of TG in the HepG2 cells were richest in the EA fraction. The statistical analysis revealed that there was a significant correlation between the TG levels and the TPC ($r = -0.968$, $p < 0.05$), as the EC_{50} value ($r = 0.936$), indicating that phenolic compounds, which showed high antioxidant activity, may have great inhibitory effects on the increase of TG levels.

3.4. Yield, TPC and DPPH radical-scavenging activity of subfractions from EA fraction

Since the EA fraction exhibited the highest TPC, the strongest DPPH radical-scavenging activity and the greatest inhibitory effect on the OA-induced fatty liver model *in vitro*, it was further separated by a Sephadex LH-20 column into three subfractions, with methanol used as the mobile phase. The yields of the three subfractions decreased from SF1 to SF3 (Table 2), showing that the earlier eluted subfractions had relatively higher yield. According to the normal-phase and size-exclusion chromatographic separation mechanism, SF3 may have relatively stronger polarity than fractions with low polarity and high molecular weight. Lo and Cheung (2005) reached a similar conclusion in their study.

The TPC and DPPH radical-scavenging ability are also shown in Table 2. The TPC of SF3 was almost 216-fold and 9-fold higher than SF1 and SF2, respectively. In the evaluation of DPPH radical-scavenging ability, SF3 exhibited the strongest DPPH radical-scavenging ability. The EC_{50} values of SF1 and SF2 were almost 97-fold and 6-fold higher than SF3, respectively.

3.5. Inhibitory effect on an OA-induced fatty liver model *in vitro* of subfractions from EA fraction

The intracellular TG levels are shown in Fig. 3. Both of the SF2- and SF3-treated groups (50 $\mu\text{g}/\text{ml}$) showed significant ($p < 0.01$) inhibitory effects on the increase of TG level. Compared with the model group, the TG levels of the SF2- and SF3-treated group decreased by 37% and 50%, respectively. This demonstrated that SF2 and SF3, especially the SF3, could protect the hepatocytes and effectively inhibit the fat deposition. However, SF1 exhibited no inhibitory effect on the OA-induced fatty liver model *in vitro* at such concentration. From the results of the TG level inhibitory effects and DPPH radical-scavenging abilities (Section 3.4) of subfractions from the EA fraction, it appears that antioxidant activity may have a close relationship with the inhibitory effect of the TG levels increase. This needs a further systematic study in future work.

3.6. HPLC analysis of SF3

Among the three subfractions, since SF3 showed the highest TPC, the strongest DPPH radical-scavenging activity and the greatest inhibitory effect on the OA-induced fatty liver model *in vitro*,

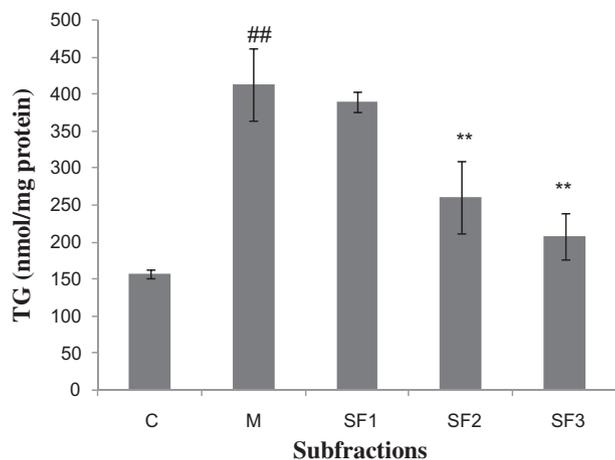


Fig. 3. Effect of 0.75 mM OA–BSA complex, with or without subfractions extracted from EA fraction, on the TG level of HepG2 cells. The concentration of subfraction was 50 $\mu\text{g/ml}$. Data are expressed as the means \pm standard deviation ($n = 4$). * $p < 0.05$, ** $p < 0.01$ means significantly different from the model. # $p < 0.05$, ## $p < 0.01$ means significantly different from the control.

the major components of SF3 were tentatively identified and quantified by HPLC analysis.

As shown in Fig. 4, the HPLC chromatogram of SF3 was complicated. Peaks 1, 2 and 3 were the three major peaks, which had identical retention times with the avenanthramide standards of Bc, Bp and Bf under the same HPLC conditions, respectively (Fig. 5). It is proposed that these three peaks were avenanthramides Bc, Bp and Bf, respectively. Quantification was carried out by the external standard method. Pure standards of Bc, Bp and Bf, at five different concentrations in methanol, were injected into the HPLC system, and the peak areas were calculated. The calibration curves were prepared and response factors were calculated under the same conditions. Based on the comparison of peak areas of each peak with those of authentic samples and from calibration curves, Bc, Bp and Bf in SF3 were quantified to be about 5.20%, 9.19% and 8.06%, respectively.

3.7. Inhibitory effect, on the OA-induced fatty liver model in vitro, of avenanthramide standards (Bc, Bp, Bf)

It is proposed that the three peaks of SF3 were avenanthramides Bc, Bp and Bf, respectively, and SF3 could protect the hepatocytes and effectively inhibit the fat deposition. So we tested the inhibitory effects of the TG level increases of three avenanthramide standards, Bc, Bp and Bf. The result are shown in Fig. 6. Avenanthramide

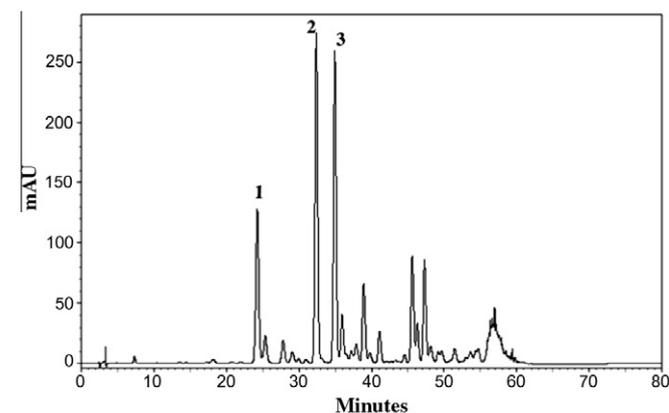


Fig. 4. Analytical HPLC chromatogram of SF3 isolated from EA fraction.

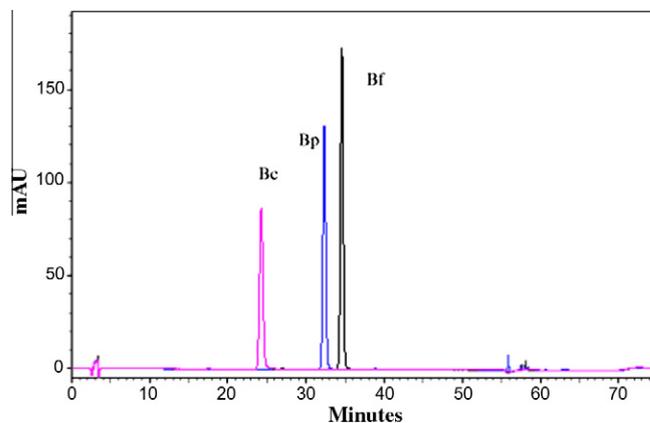


Fig. 5. HPLC profile of the three avenanthramides, Bc (Peak 1), Bp (Peak 2), and Bf (Peak 3).

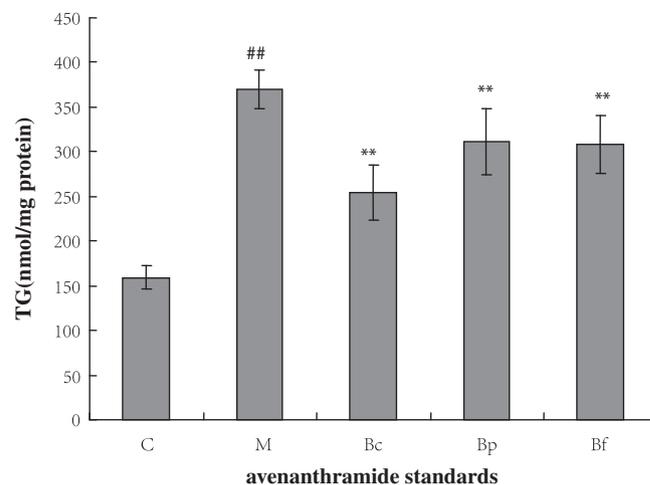


Fig. 6. Effect of 0.75 mM OA–BSA complex, with or without avenanthramide standards, on the TG level of HepG2 cells. The concentration of avenanthramide standards was 50 $\mu\text{g/ml}$. Data are expressed as the means \pm standard deviation ($n = 4$). * $p < 0.05$, ** $p < 0.01$ means significantly different from the model. # $p < 0.05$, ## $p < 0.01$ means significantly different from the control.

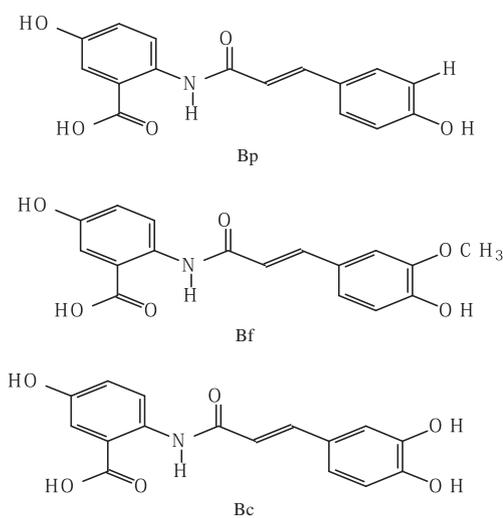


Fig. 7. The structures of the three avenanthramides, Bc Bp and Bf.

standards had great inhibitory effects on OA-induced fatty liver model *in vitro*. Bc, Bp, Bf-treated groups (50 µg/ml) all showed significant ($p < 0.01$) inhibitory effects on the increase of TG level compared with the model group (column M), especially the Bc compound, which showed 31% decrease, and no significant difference ($p < 0.05$) from Bp and Bf which showed no significant difference ($p > 0.05$) from each other. Compound Bc also showed better DPPH radical-scavenging abilities than did compounds Bp and Bf (Peterson et al., 2002), which may be related to *ortho*-dihydroxy substitutions in the benzene ring of its molecular structure (Fig. 7).

Avenanthramides have been proved to be beneficial in decreasing the expression of endothelial pro-inflammatory cytokines (Guo, Wise, Collins, & Meydani, 2008) and inhibiting the development of atherosclerosis (Liu et al., 2004). Bc, Bp and Bf were the most abundant avenanthramides in oat (Skoglund, Peterson, Andersson, Nilsson, & Dimberg, 2008). In the present work, the avenanthramides also showed strong inhibitory effects on the OA-induced fatty liver model *in vitro*.

4. Conclusions

We examined the TPC, DPPH radical-scavenging activity and inhibitory effect on OA-induced hepatic steatosis *in vitro* of fractions from the oat ethanol extract. The EA fraction exhibited the strongest activities. Therefore, it was further fractionated into three subfractions (SF1–SF3). SF3 was proved to be the most active subfraction by the three assays performed above. The major components in SF3 were tentatively identified to be avenanthramides Bc, Bp and Bf by HPLC analysis, with the contents being 5.20%, 9.19% and 8.06% of the dry weight of SF3, respectively. Through examining the inhibitory effect of avenanthramides Bc, Bp and Bf on OA-induced hepatic steatosis *in vitro*, we found that all avenanthramide standards also have significant inhibitory effects. Further experiments are needed to elucidate the specific relation between the inhibitory effect and antioxidant activity.

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