



Protective effect of oat bran extracts on human dermal fibroblast injury induced by hydrogen peroxide

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Abstract: Oat contains different components that possess antioxidant properties; no study to date has addressed the antioxidant effect of the extract of oat bran on the cellular level. Therefore, the present study focuses on the investigation of the protective effect of oat bran extract by enzymatic hydrolysates on human dermal fibroblast injury induced by hydrogen peroxide (H₂O₂). Kjeldahl determination, phenol-sulfuric acid method, and high-performance liquid chromatography (HPLC) analysis indicated that the enzymatic products of oat bran contain a protein amount of 71.93%, of which 97.43% are peptides with a molecular range from 438.56 to 1301.01 Da. Assays for 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity indicate that oat peptide-rich extract has a direct and concentration-dependent antioxidant activity. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) colorimetric assay and the TdT-mediated digoxigenin-dUTP nick-end labeling (TUNEL) assay for apoptosis showed that administration of H₂O₂ in human dermal fibroblasts caused cell damage and apoptosis. Pre-incubation of human dermal fibroblasts with the Oatp for 24 h markedly inhibited human dermal fibroblast injury induced by H₂O₂, but application oat peptides with H₂O₂ at same time did not. Pre-treatment of human dermal fibroblasts with Oatp significantly reversed the H₂O₂-induced decrease of superoxide dismutase (SOD) and the inhibition of malondialdehyde (MDA). The results demonstrate that oat peptides possess antioxidant activity and are effective against H₂O₂-induced human dermal fibroblast injury by the enhanced activity of SOD and decrease in MDA level. Our results suggest that oat bran will have the potential to be further explored as an antioxidant functional food in the prevention of aging-related skin injury.

Key words: Oat bran, Extraction, Antioxidant, Human dermal fibroblasts, Cell injury

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1 Introduction

Avena sativa (oat) is usually consumed as a whole-grain cereal and is distinct among the cereals due to its multifunctional feature and nutritional profile (Masood *et al.*, 2008). Recent advances in food and nutrition have revealed that oat contains many types of phytochemicals that have antioxidant properties, such as tocotrienols, flavonoids, phenolic acids,

sterols, and phytic acid (Collins *et al.*, 1991; Peterson, 2001) and that oat and its products have proven helpful in the treatment of diabetes and cardiovascular disorders. In particular, oat bran is a good source of B-complex vitamins, protein, fat, and minerals, as well as the heart-healthy soluble fiber β -glucan (Beck *et al.*, 2009). Recently, Ren *et al.* (2011) reported the effect of avenanthramide-rich extract (ARE) from oat bran processing on the activity of antioxidant enzymes in D-galactose-induced oxidative-stressed mice. Administration of D-galactose markedly lowered not only the activity of superoxide dismutase

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(SOD) and glutathione peroxidase (GPx) but also the gene expression of manganese SOD, copper-zinc SOD, GPx, and lipoprotein lipase (LPL) messenger RNA (mRNA) in mice. Although there are reports that oat bran contains at least 17.1% protein (Marlett, 1993), and our previous study has presented a method of extracting polypeptides from oat bran using enzymatic hydrolysates (Feng *et al.*, 2007; Wang *et al.*, 2008), no study has addressed the effect of oat peptide-rich extract (Oatp) from oat bran on cell injury protection or antioxidative enzymes, particularly at the cellular level. So that, investigation of the antioxidant effect of the protein component in oat bran using enzymatic hydrolysates could increase the understanding of the efficacy of oat bran.

Fibroblasts are essential cellular components of human skin and are the major cell type in the dermis. They play a pivotal role in the regulation of skin physiology and pathology because they generate and compose the extracellular matrix of the dermis. Additionally, fibroblasts carry out communications with each other and with other cell types in the skin (Gillitzer and Goebeler, 2001). Because fibroblasts release multiple cytokines and growth factors through paracrine and autocrine interplay, they also play a significant role in inducing cell apoptosis or stimulating cell proliferation. In addition, they are involved in wound repair, immune processes, and inflammatory responses in the dermis (Niu *et al.*, 2008; Wiegand and Hipler, 2009). Therefore, an investigation of the mechanisms by which fibroblasts modulate cell proliferation or apoptosis is critical to our cognition of dermal physiology and for skin's pharmacological and clinical studies.

The normal cellular environment with oxidation-reduction is important for cell physiological function. Oxidative stress has the capacity to cause cellular damage or death via diverse mechanisms, which depend on the nature of the oxidant and the environment by which it was produced (Klamt and Shacter, 2005; Klamt *et al.*, 2009). Although oxidative stress products include radicals, such as hydroxyl radicals and superoxide anions, it has been proposed that cell damage induced by oxidative stress is mainly associated to the production of hydrogen peroxide (H_2O_2), because H_2O_2 is more stable and, thus, more able to diffuse throughout a larger tissue area. In the past few years, the cytotoxic effects of H_2O_2 on human fibro-

blasts have been studied (Yuan *et al.*, 2003; Ohshima, 2004). H_2O_2 -induced oxidative injury provides a model for studying the effects of cellular protection of antioxidants.

In this study, we first obtained the peptide-rich extract from oat bran by enzymatic hydrolysates. We then tested its effect on H_2O_2 -induced dermal human fibroblast injury. The results demonstrated that Oatp possess antioxidant activity and is effective against H_2O_2 -induced human dermal fibroblast injury through the enhanced activity of SOD and a decrease in the malondialdehyde (MDA) level. In addition to β -glucan which is known to have antioxidant effect, oat bran contains a variety of components whose role has not been fully understood. Our results revealed, for the first time, that the antioxidants effect of oat bran might be derived from Oatp, not only by β -glucan. Therefore, oat bran has the potential for further exploration as an antioxidant-functional food in the prevention of aging-related skin injuries. Moreover, Oatp may be used as additives in skin creams for anti-aging purposes and to help prevent skin damage induced by ultraviolet (UV).

2 Materials and methods

2.1 Oatp preparation and analysis

The oat bran was prepared from naked oats provided by the Institute of Oats, Chinese Academy of Agricultural Science (Hebei, China). It was sieved through a 20–60 mesh screen. The oat bran (10 g) was diluted by 1:15 with 150 ml of 1 mol/L sodium hydroxide for 1 h at 55 °C and then reacted for 4 h with an alcalase enzyme at the temperature recommended by the enzyme manufacturer (55 °C). The enzyme to substrate ratio was 5%. After hydrolyzing, the enzyme in the hydrolysate was inactivated in a water bath at 100 °C for 20 min. Next, the solution was added to 1% β -glucan enzyme (0.01 g/ml; Novozymes, Denmark) and 1% saccharifying enzyme (0.01 g/ml; Novozymes, Denmark) and incubated for 1 h. The suspension was centrifuged at 4000 r/min for 10 min in order to recover the soluble proteins and peptides.

The protein and polysaccharide contents of Oatp from oat bran were quantified using the Kjeldahl method. The distribution of the molecular weight of

Oatp was analyzed using high-performance liquid chromatography (HPLC) with electrochemical detection (ECD), as described by Lahogue *et al.* (2010).

2.2 Human dermal fibroblast culture

Primary cultures of skin fibroblasts from a healthy boy's foreskin left over from surgery were grown in plastic flasks under standard conditions, as described previously (Feng *et al.*, 2012). Cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (0.1 g/ml) calf serum (CS), 100 U/ml penicillin, 100 U/ml streptomycin, and 0.25 mg/ml amphotericin at 37 °C in a humidified atmosphere with 5% CO₂. Isolated cells were plated onto 35-mm-diameter petri dishes, with a seeding density of 5×10^4 cells/well and were grown to confluence over 24 h. The skin tissue was approved by Shanghai TCM-integrated Hospital with the written informed consent of the patient's parents.

2.3 MTT assay

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay, a commonly used method to assess cell viability, measured mitochondrial activity in viable cells as described by Hansen *et al.* (1989). The cells were plated at a density of 5×10^3 cells/well in 96-well plates. After 24 h of incubation, the cells were treated with different concentrations of oat extract (0.1–5.0 mg/ml) and exposed to H₂O₂ (0.8 mmol/L). The MTT was at a concentration of 5 mg/ml in phosphate buffered saline (PBS). MTT was applied to the culture medium at a terminal concentration of 1 mg/ml and incubated for 4 h again at 37 °C. Finally, 150 µl of dimethyl sulfoxide (DMSO) was added and its absorbance at 570 nm wavelength (A_{570}) was measured by an enzyme-labeling instrument.

2.4 Propidium iodide (PI) staining

Cell viability was evaluated by counting live cells (phase-bright cells) under phase-contrast microscopy, and the number of dead cells (PI-labeled nuclei) was counted under a fluorescence microscope. The number of surviving or dead cells was assessed by averaging counts from 32 fields of view in four independent experiments. The viability was calculated as the ratio of the live cells to total cells—that is, phase-bright+PI-stained—and related to values ob-

tained from the untreated control group.

2.5 TUNEL assay evaluation of apoptosis

The TdT-mediated digoxigenin-dUTP nick-end labeling (TUNEL) was used to detect the apoptosis of human dermal fibroblasts with a commercially available kit (Diagnostics GmbH, Germany). The cells were fixed in 4% (40 g/L) paraformaldehyde for 10 min and washed twice with PBS for 5 min each at 23–25 °C. The staining technique of the TUNEL was performed in accordance with the manufacturer's protocol. TUNEL-positive cells were marked using diaminobenzidine, a substrate for the kit's horseradish peroxidase. The stained cells were viewed with a microscope. The images were transferred using a digital camera. The proportion of apoptotic cells was calculated by counting the number of TUNEL-positive cells based on five randomly selected areas across the horizon, and comparing it with the total number of cells.

2.6 Assay for DPPH radical scavenging activity

A 1 ml sample at different concentrations was added to 1 ml of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical solution (12 mg/100 ml). Ultrapure water was added to 4 ml. The mixture was shaken energetically for 30 s and left to stand for 5 min in a dark room at 23–25 °C. Its absorbance was observed at 517 nm (A_{517}). The effect of DPPH radical scavenging of the sample was based on the following formula:

$$\text{DPPH scavenging effect} = [1 - (T - T_0) / (C - C_0)] \times 100\%$$

where T_0 is the absorbance of the contrast sample and T is the absorbance of the sample. C is the absorbance of the positive control and C_0 is the absorbance of the negative control.

2.7 Determination of SOD activity and MDA levels

The concentration of MDA and the activities of SOD were determined using commercially available kits purchased from the Jiancheng Biological Engineering Academy (Nanjing, China). All procedures were based on the manufacturer's instructions. The assay for SOD activity was according to its ability to reduce the oxidation of hydroxylamine by O_2^- generated from the xanthine-xanthineoxiase system. One unit of SOD activity was defined as the amount

necessary to reduce the absorbance at 550 nm by 50%. The MDA level was measured at a wavelength of 532 nm by response with thiobarbituric acid to form a stable chromophoric product. The MDA levels are reported in units per milligram of protein.

2.8 Data analysis

SAS 6.0 was used for statistics analysis. Results are expressed as mean±standard error of the mean (SEM). The analysis of variance (ANOVA) test was used for comparison among the multiple groups. The mean values between two groups were compared using Student's *t*-test. A *P*-value<0.05 indicated a statistical significance.

3 Results and discussion

3.1 Determination of the main components of the enzymatic products of oat bran and Oatp's antioxidant capacity

Using DNA samples isolated from 40 barley accessions as templates, polymorphic DNA fragments were amplified from 29 among 35 simple sequence repeat (SSR) primer pairs selected in this study, including three out of five SSRs (60%) located on chromosome 2H, four out of five (80%) on chromosomes 1H, 4H, 5H, and 6H, and all (100%) on chromosomes 3H and 7H, respectively. Using the method described in the materials and methods section, the polypeptide and protein contents of the enzymatic products of oat bran can reach 71.93%, and the polysaccharide content can be reduced to 23% using the Kjeldahl method. The enzymatic products of oat bran were then quantified by HPLC. The results shown in Fig. 1 demonstrate that the distribution of their molecular weights ranged between 438.56 and 1301.01 Da, which accounted for the total distribution of over 97.43% of the enzymatic products of oat bran. Moreover, two major peaks were estimated to correspond to molecular weights of 1301.01 and 921.82 Da based on the calculated calibration curve, which accounted for 54.70% and 31.09% of the total enzymatic products of oat bran, respectively. Because we added β -glucanase to avoid the effect of β -glucan (Beck *et al.*, 2009) during the enzymatic hydrolysis process, the enzymatic products of oat bran are low-molecular-weight peptide-rich extracts (Oatp).

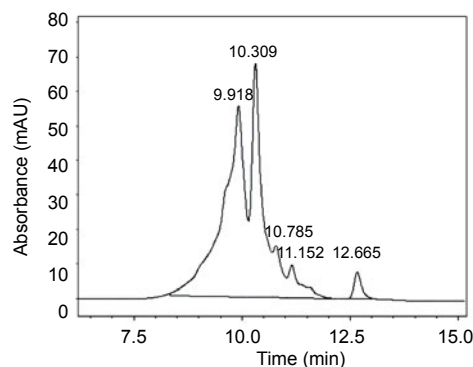


Fig. 1 HPLC chromatogram of the molecular weight distribution in Oatp

Two major peaks with estimated molecular weights of 1301.01 and 921.82 Da were observed

Previous studies in vivo or in vitro reported that oat bran has antioxidant properties (Ren *et al.*, 2011). We first determined whether the oat bran extracts have antioxidant activity using a scavenging DPPH radical assay. (–)-Epigallocatechin-3-gallate (EGCG), which has been reported in previous studies to exhibit certain antioxidant activity (Jung *et al.*, 2008), was used as the positive control. The results shown in Fig. 2a illustrate that Oatp significantly increased the scavenged DPPH radicals in a concentration-dependent manner. When the concentration of Oatp was more than 0.5 mg/ml, 29% to 37% of the DPPH radicals were scavenged during a 20 min treatment. Although the effect of Oatp on DPPH radicals scavenged is lower than that obtained from the EGCG treatment (68%, Fig. 2b), Oatp's potential ability to scavenge DPPH radicals was observed. The results indicate that Oatp possesses antioxidant activity.

3.2 Pretreatment with Oatp to protect human dermal fibroblasts against H₂O₂-induced cell injury

To detect the antioxidant activity of Oatp, we first set up an oxidation damage model for human dermal fibroblasts incubated with different concentrations of H₂O₂ to determine whether Oatp can protect human dermal fibroblasts against injury caused by oxidant stress. Cell viability was assessed using PI staining and MTT colorimetric assay; damaged cell nuclei, which indicated apoptotic cells, were detected with the TUNEL assay. As shown in Fig. 3a, little PI staining and few TUNEL-positive cells were present in untreated control fibroblasts. After exposure to

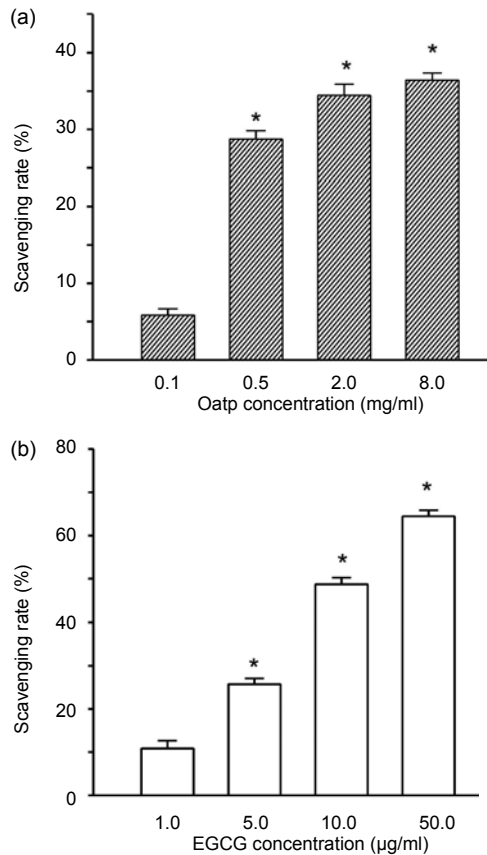


Fig. 2 Antioxidant activity of oat bran extracts detected by a DPPH radical scavenging assay

(a) Oatp significantly increased the scavenged DPPH radicals in a concentration-dependent manner. (b) EGCG that exhibited certain antioxidant activity in previous research was used as the positive control. * $P < 0.05$ when comparing different concentrations of Oatp or EGCG by one-way ANOVA. Data are presented as the mean \pm SEM obtained from three independent experiments

H_2O_2 for 6 h, the cells staining with PI and the numbers of TUNEL-positive nuclei were significantly enhanced in a dose-dependent manner. The statistical analysis indicated that 0.4, 0.6, and 0.8 mmol/L of H_2O_2 reduced the cell survival rate to (75.2 \pm 9.3)%, (71.8 \pm 8.5)%, and (45.4 \pm 15.2)% of the control, respectively (Fig. 3b). In contrast, the number of TUNEL-positive nuclei was significantly enhanced to (10.7 \pm 0.8)%, (16.2 \pm 1.1)%, and (32.3 \pm 2.9)% of the control (Fig. 3c). This result indicates that approximately 30% of the injured cells were apoptotic when the cells were treated with 0.8 mmol/L H_2O_2 for 6 h. In addition, a concentration-dependent reduction in cell viability was examined by the MTT assay. The result shown in Fig. 3d indicate that H_2O_2 in a 0.1 to

1.0 mmol/L concentration range significantly reduced cell viability; when the H_2O_2 concentration was higher than 1.0 mmol/L, only a few viable cells were observed. Moreover, the MTT assay result showed that the concentration that induced 50% cell death was 0.8 mmol/L H_2O_2 for 6 h.

We then determined the effect of oat bran extracts on H_2O_2 -induced dermal fibroblast injury using MTT assay and TUNEL detection. The concentration of Oatp was between 0.1 and 5.0 mg/ml (Fig. 4a). Pre-incubation of human dermal fibroblasts in Oatp for 3–12 h before H_2O_2 injury did not significantly increase cell viability in human dermal fibroblasts exposed to 0.8 mmol/L H_2O_2 for 6 h, except for a slight increase (8%) in cell viability observed in human dermal fibroblasts pre-incubated in 1.0 mg/ml of Oatp for 12 h (Figs. 4b and 4c). When the pre-incubation time with Oatp was increased to 24 h before H_2O_2 injury, oat bran extracts exhibited a significant effect against H_2O_2 -induced cell damage (Fig. 4d). The cell viability rate increased from (34.9 \pm 0.19)% in the absence of H_2O_2 to (51.8 \pm 2.12)%, (69.1 \pm 1.61)%, and (78.0 \pm 1.79)% in the presence of 0.1, 0.5, and 1.0 mg/ml oat bran extracts, respectively. Interestingly, when Oatp and H_2O_2 treatments were given simultaneously without pre-incubation, oat bran extract's protective effect against cell injury did not occur (Fig. 4a), suggesting that the protection mechanism of oat bran extract against H_2O_2 -induced cellular injury did not directly affect the chemical reaction of H_2O_2 or the free radical absorption, although Oatp showed the effect on DPPH radicals scavenged like EGCG. However, we noticed that when the concentration of Oatp was higher than 2.5 mg/ml, cell viability decreased instead. This result suggests that in a concentration range of 0.1 to 1.0 mg/ml, pre-incubation of human dermal fibroblasts with Oatp for 24 h reduced cell injury induced by H_2O_2 treatment. An Oatp concentration higher than 2.5 mg/ml may lead to cell toxicity.

Because H_2O_2 -induced dermal fibroblast injury includes cell apoptosis, we also examined the effect of Oatp on H_2O_2 -induced apoptosis with the TUNEL assay. In the control group, few morphological signs of nuclear damage or chromatin condensation were observed (Fig. 4e). Once injured by H_2O_2 , cells exhibited condensed nuclei. Pre-incubation with 1.0 mg/ml oat bran extracts for 24 h before H_2O_2

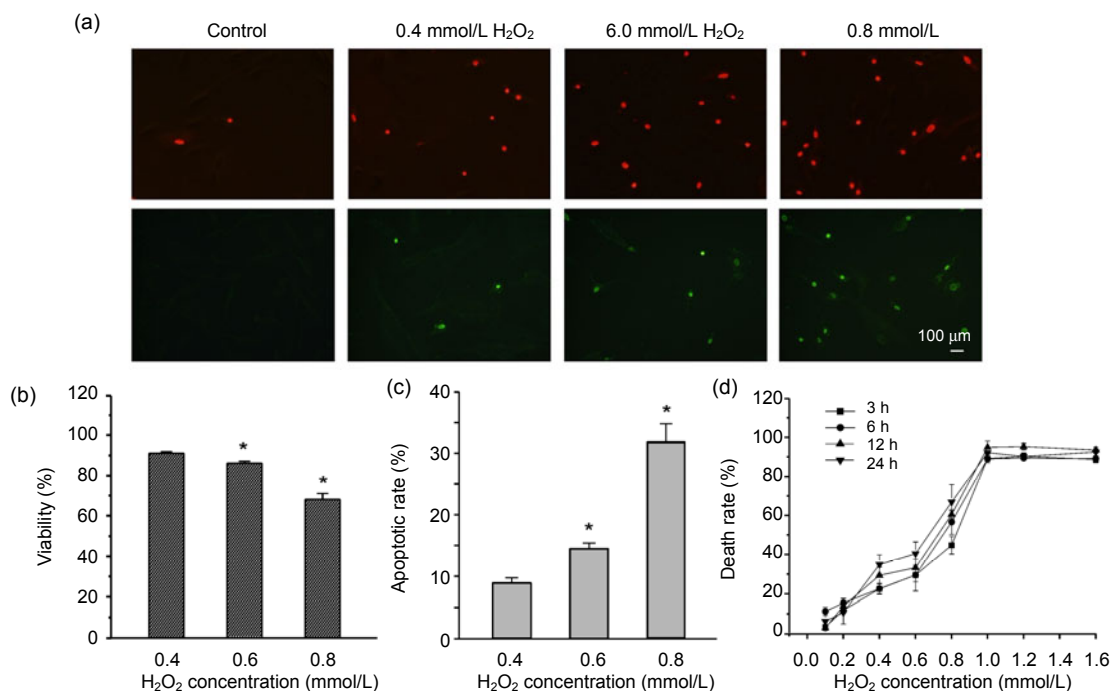


Fig. 3 Oxidation damage to human dermal fibroblast induced by different concentrations of H₂O₂

(a) Cell viability and damaged cell nuclei detected by PI staining and TUNEL assay after exposure of human dermal fibroblasts to different concentrations of H₂O₂. (b, c) The statistical analyses of the reduced survival rates and TUNEL-positive cells were resulting from treatments with different concentrations of H₂O₂. (d) Statistical analysis of cell death rate measured by an MTT colorimetric assay with different concentrations of H₂O₂ and treatment time. **P*<0.05 according to one-way ANOVA. Data (mean±SEM) are obtained from five independent experiments

injury significantly reduced the number of TUNEL-positive nuclei of fibroblasts exposed to 0.8 mmol/L H₂O₂ for 6 h. Statistical analysis indicated that the number of TUNEL-positive nuclei was reduced from (8.9±0.7)% to (14.5±0.9)% of the control (Fig. 4f).

3.3 Oatp intervention in H₂O₂-induced fibroblast damage with increased SOD activity and lower MDA formation

Lipid peroxidation is one of the main events in free radical-induced cell injury. MDA is a byproduct of lipid peroxidation and is widely used as a biomarker of oxidative stress (Cini *et al.*, 1994). However, antioxidant enzymes, such as SOD and glutathione (GSH)-Px, are considered to effectively augment cells' antioxidant defenses (Luo and Xia, 2006). In our study, Oatp's ability to protect fibroblasts against H₂O₂-induced cell damage required a longer pre-incubation time before the H₂O₂ injury. Therefore, we determined the SOD and GSH-Px activities and the level of MDA formation to address whether Oatp prevented cell apoptosis due to

increased antioxidant enzymatic activity and decreased intracellular MDA. The results show that exposing human dermal fibroblasts to 0.8 mmol/L H₂O₂ for 6 h significantly decreased SOD activity from (25.4±1.35) U/mg protein of the control to (14.8±1.17) U/mg proteins, resulting in a reduction of (41.7±1.31)% compared to the control (Fig. 5a). Consistent with the pretreatment of human dermal fibroblasts with Oatp for 24 h before H₂O₂ injury, SOD activity exhibited a concentration-dependent recovery. When 1.0 mg/ml of Oatp was used in the pre-incubation medium, SOD activity reached (25.0±0.88) U/mg protein, close to the level of the control. In contrast, Oatp significantly reduced the level of intracellular MDA. Exposing the human dermal fibroblasts to 0.8 mmol/L H₂O₂ for 6 h significantly increased the level of intracellular MDA from (10.3±0.77) nmol/mg protein of the control to (14.4±1.02) nmol/mg proteins, resulting a raise of (28.5±1.21)% compared to the control (Fig. 5a). Pretreatment of human dermal fibroblasts with Oatp for 24 h before H₂O₂ injury significantly inhibited

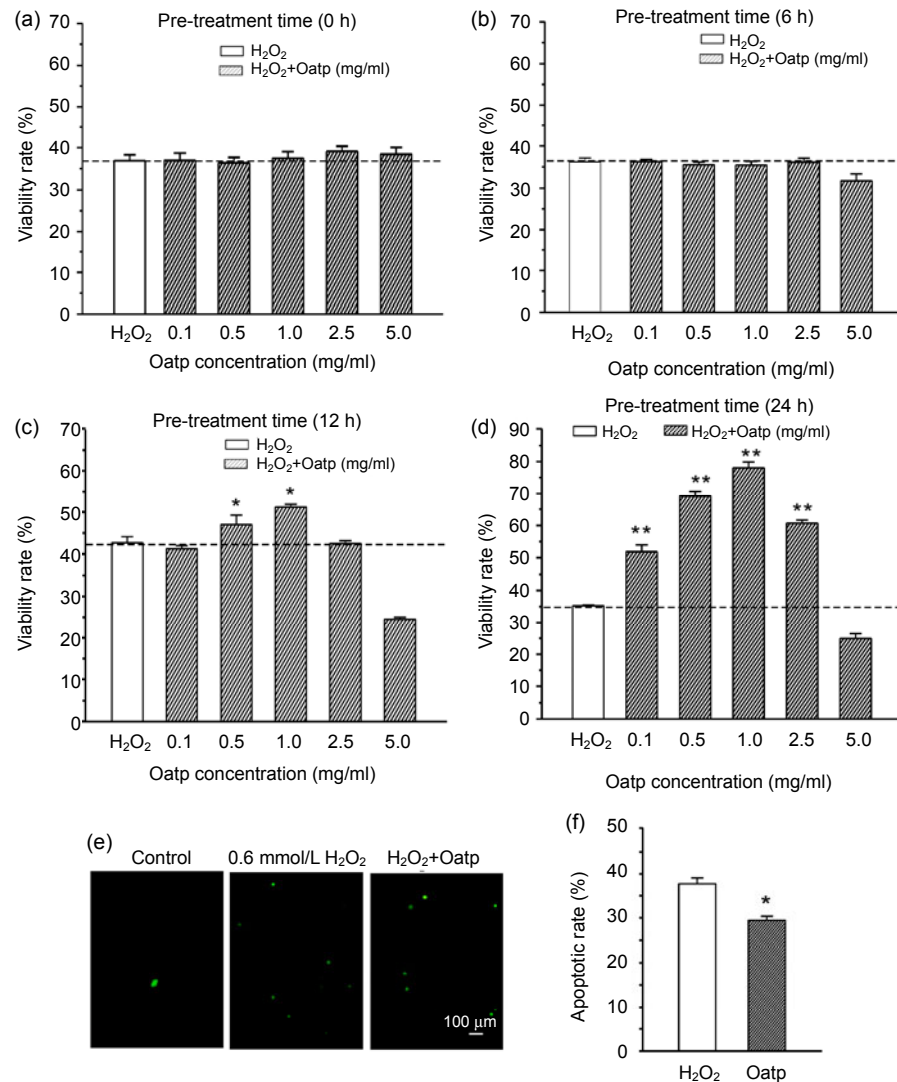


Fig. 4 Effect of oat bran extracts on H_2O_2 -induced dermal fibroblast injury

(a–d) Statistical analyses of the effect of oat bran extracts on viability rates of human dermal fibroblasts undergoing pre-incubation time of 0–24 h before H_2O_2 injury. The viability rates were measured with an MTT colorimetric assay. * $P < 0.05$; ** $P < 0.01$ according to the Student's *t*-test, compared with H_2O_2 alone. Data (mean \pm SEM) are obtained from five independent experiments. (e) Effect of oat bran extracts on H_2O_2 -induced apoptosis detected by the TUNEL assay. (f) Statistical analysis of the effect of oat bran extracts on the apoptotic rate induced by H_2O_2 . * $P < 0.05$ according to Student's *t*-test, compared with H_2O_2 alone

the level of intracellular MDA (Fig. 5b). Moreover, the experimental results of the SOD activity and the level of intracellular MDA are consistent with the results observed for cell survival: the effect of Oatp on SOD activity and the level of intracellular MDA occurred only after pretreatment with Oatp for 24 h before H_2O_2 injury.

It is notable that the activity of another antioxidant enzyme, GSH-Px, was not significantly affected by Oatp (Fig. 5c). Exposing the human dermal

fibroblasts to 0.8 mmol/L H_2O_2 for 6 h significantly decreased the level of intracellular GSH-Px from (101.7 \pm 2.11) nmol/mg protein of the control to (51.4 \pm 1.91) nmol/mg protein, a decrease of (49.5 \pm 1.97)% compared to the control. However, pretreatment of human dermal fibroblasts with Oatp for 24 h before H_2O_2 injury did not lead to a significant recovery of the level of intracellular GSH-Px, whether low or high concentrations of Oatp were used (Fig. 5c).

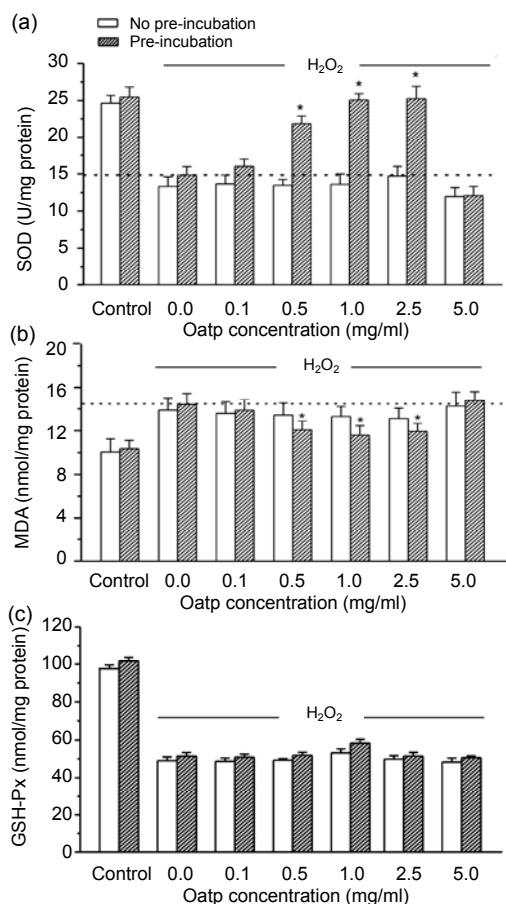


Fig. 5 Effect of oat bran extracts on antioxidant enzymes SOD and GSH-Px, and production of lipid peroxidation MDA

(a) The statistical analyses of the effect of oat bran extracts on antioxidant enzymes SOD of human dermal fibroblasts undergoing pre-incubation time of 0–24 h or no pre-incubation before H_2O_2 injury. (b) The statistical analyses of the effect of oat bran extracts on the production of lipid peroxidation MDA of human dermal fibroblasts undergoing pre-incubation time of 0–24 h or no pre-incubation before H_2O_2 injury. (c) The statistical analyses of the effect of oat bran extracts on antioxidant enzymes GSH-Px of human dermal fibroblasts undergoing pre-incubation time of 0–24 h or no pre-incubation before H_2O_2 injury. The data (mean \pm SEM) for this statistical analysis were obtained from four independent experiments. * $P < 0.05$ by Student's *t*-test, compared with H_2O_2 alone

It is well known that SOD serves as the first gatekeeper in the antioxidant defense system designed to scavenge superoxide anions (Reiter *et al.*, 2000), while GSH-Px is a catalyzer that activates the reaction of lipid hydroperoxides with reduced glutathione to form glutathione disulfide (Venukumar and Latha, 2002). An avenanthramides (AVE)-enriched mixture extracted from oat was reported to increase human plasma GSH-Px activity by

30%–35% (Chen *et al.*, 2007). In this study, both SOD and GSH-Px activities were significantly downregulated in H_2O_2 -treated groups. However, pretreatment with Oatp restored only the activity of SOD, not GSH-Px. Therefore, we presumed that Oatp was able to prevent H_2O_2 -induced oxidative stress in human dermal fibroblasts by selectively enhancing SOD activity, rather than GSH-Px activity.

Previous research has revealed that lipid peroxidation occurs when ROS attacks the membrane polyunsaturated fatty acids, leading to their disintegration and the formation of short-chain alkyl radicals and aldehydes. MDA is a major reactive aldehyde that is formed during the final stages of lipid peroxidation of biological membrane polyunsaturated fatty acid (Cini *et al.*, 1994). Our results demonstrated that the administration of H_2O_2 caused an increase in the MDA concentration of the human dermal fibroblasts, while the application of Oatp decreased the MDA level, suggesting that Oatp was effective in scavenging free radicals and suppressing the production of MDA. However, because the effect of Oatp on SOD and MDA required a longer pre-incubation time, further studies are needed to confirm whether it involves the expression of antioxidant enzymes and the details of its mechanism.

4 Conclusions

In summary, the present study has shown that the extract obtained from oat bran by enzymatic hydrolysates is rich in low-molecular peptides. Administration of H_2O_2 to human dermal fibroblasts caused cell damage and apoptosis, decreased antioxidants' enzyme activity, and increased the MDA level. Pretreatment with Oatp could restore the antioxidant defense system by increasing SOD activity. Therefore, oat bran has the potential to be explored further as an antioxidant-functional food in the prevention of aging-related skin injury. Moreover, Oatp may be used as additives in skin creams for anti-aging purpose and to help prevent skin damage induced by UV.

References

- Beck, E.J., Tosh, S.M., Batterham, M.J., Tapsell, L.C., Huang, X.F., 2009. Oat β -glucan increases postprandial cholecystokinin levels, decreases insulin response and extends

- subjective satiety in overweight subjects. *Mol. Nutr. Food Res.*, **53**(10):1343-1351. [doi:10.1002/mnfr.200800343]
- Chen, C.O., Milbury, P.E., Collins, F.W., Blumberg, J.B., 2007. Avenanthramides are bioavailable and have antioxidant activity in humans after acute consumption of an enriched mixture from oats. *J. Nutr.*, **137**:1375-1382.
- Cini, M., Fariello, R.G., Bianchetti, A., Moretti, A., 1994. Studies on lipid peroxidation in the rat brain. *Neurochem. Res.*, **19**(3):283-288. [doi:10.1007/BF00971576]
- Collins, F.W., McLachlan, D.C., Blackwell, B.A., 1991. Oat phenolics: avenaluminic acids, a new group of bound phenolic acids from oat groats and hulls. *Cereal Chem.*, **68**:184-189.
- Feng, B., Wang, C.T., Lei, F., Dong, Y.M., 2007. Study on hydrolysis conditions of oat bran protein with different proteinase. *Sci. Technol. Food Ind.*, **10**:120-122 (in Chinese).
- Feng, B., Ye, W.L., Ma, L.J., Fang, Y., Mei, Y.A., Wei, S.M., 2012. Hydrogen peroxide enhanced potassium currents and promoted cell injury in human fibroblasts. *Life Sci.*, **90**(11-12):424-431. [doi:10.1016/j.lfs.2011.12.020]
- Gillitzer, R., Goebeler, M., 2001. Chemokines in cutaneous wound healing. *J. Leuk. Biol.*, **69**:513-521.
- Hansen, M.B., Nielsen, S.E., Berg, K., 1989. Re-examination and further development of a precise and rapid dye method for measuring cell growth/cell kill. *J. Immunol. Meth.*, **119**(2):203-210. [doi:10.1016/0022-1759(89)90397-9]
- Jung, S.H., Kang, K.D., Ji, D., Fawcett, R.J., Safa, R., Karmalden, T.A., 2008. The flavonoid baicalin counteracts ischemic and oxidative insults to retinal cells and lipid peroxidation to brain membranes. *Neurochem. Intern.*, **53**(6-8):325-337. [doi:10.1016/j.neuint.2008.09.004]
- Klamt, F., Shacter, E., 2005. Taurine chloramine, an oxidant derived from neutrophils, induces apoptosis in human B lymphoma cells through mitochondrial damage. *J. Biol. Chem.*, **280**(22):21346-21352. [doi:10.1074/jbc.M501170200]
- Klamt, F., Zdanov, S., Levine, R.L., Pariser, A., Zhang, Y., Zhang, B., 2009. Oxidant-induced apoptosis is mediated by oxidation of the actin-regulatory protein cofilin. *Nat. Cell Biol.*, **11**(10):1241-1246. [doi:10.1038/ncb1968]
- Lahogue, V., Réhel, K., Taupin, L., Haras, D., Allaupe, P., 2010. A HPLC-UV method for the determination of angiotensin I-converting enzyme (ACE) inhibitory activity. *Food Chem.*, **118**(3):870-875. [doi:10.1016/j.foodchem.2009.05.080]
- Luo, T., Xia, Z., 2006. A small dose of hydrogen peroxide enhances tumor necrosis factor- α toxicity in inducing human vascular endothelial cell apoptosis: reversal with propofol. *Anesth. Analg.*, **103**(1):110-116. [doi:10.1213/01.ane.0000221183.02244.80]
- Marlett, J.A., 1993. Comparisons of Dietary Fiber and Selected Nutrient Compositions of Oat and Other Grain Fractions. In: Wood, P.J. (Ed.), *Oat Bran*. American Association of Cereal Chemists, p.49-82.
- Masood, S.B., Muhammad, T.N., Muhammad, K.I.K., Rabia, S., Mehmood, S.B., 2008. Oat: unique among the cereals. *European J. Nutr.*, **47**(2):68-79.
- Niu, Y., Xie, T., Ge, K., Lin, Y., Lu, S., 2008. Effects of extracellular matrix glycosylation on proliferation and apoptosis of human dermal fibroblasts via the receptor for advanced glycosylated end products. *Am. J. Dermatopathol.*, **30**(4):344-351. [doi:10.1097/DAD.0b013e31816a8c5b]
- Ohshima, S., 2004. Apoptosis in stress-induced and spontaneously senescent human fibroblasts. *Biochem. Biophys. Res. Commun.*, **324**(1):241-246. [doi:10.1016/j.bbrc.2004.09.044]
- Peterson, D.M., 2001. Oat antioxidants. *J. Cereal. Sci.*, **33**(2):115-129. [doi:10.1006/jcsc.2000.0349]
- Reiter, R.J., Tan, D., Osuna, C., Gitto, E., 2000. Actions of melatonin in the reduction of oxidative stress. *J. Biomed. Sci.*, **7**(6):444-458. [doi:10.1007/BF02253360]
- Ren, Y., Yang, X., Niu, X., Liu, S., Ren, G., 2011. Chemical characterization of the avenanthramide-rich extract from oat and its effect on D-Galactose-induced oxidative stress in mice. *J. Agric. Food Chem.*, **59**(1):206-211. [doi:10.1021/jf103938e]
- Venukumar, M.R., Latha, M.S., 2002. Antioxidant activity of *Curculigo orchioides* in carbon tetrachloride-induced hepatopathy in rats. *Indian J. Clin. Biochem.*, **17**(2):80-87. [doi:10.1007/BF02867976]
- Wang, C.T., Han, Y., Feng, B., Dong, Y.M., 2008. Study on extraction technology of oat peptide. *Sci. Technol. Food Ind.*, **6**:232-234 (in Chinese).
- Wiegand, C., Hipler, U.C., 2009. Evaluation of biocompatibility and cytotoxicity using keratinocyte and fibroblast cultures. *Skin Pharmacol. Physiol.*, **22**(2):74-82. [doi:10.1159/000178866]
- Yuan, J., Murrell, G.A., Trickett, A., Wang, M.X., 2003. Involvement of cytochrome c release and caspase-3 activation in the oxidative stress-induced apoptosis in human tendon fibroblasts. *Biochim. Biophys. Acta*, **1641**(1):35-41. [doi:10.1016/S0167-4889(03)00047-8]