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Research Article

Role Of Sericin And Its Major Amino Acids As Scavengers Of Hydrogen Peroxide Induced Oxidative Stress In A549 Cells

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ABSTRACT

Free radicals mediate oxidative damage and thereby cause oxidation of proteins, lipids and damage to DNA. Sericin is a fractional protein in natural silk fibre and is removed during silk processing. This waste product is known for its antitumoral, antimicrobial and antioxidant properties. The aim of the present study was to characterize the major constituents of sericin that are providing cytoprotective effect against hydrogen peroxide-induced cell damage in human lung epithelial (A549) cells. Incubation of A549 cells with suboptimal concentration of hydrogen peroxide induced an increase in antioxidant enzyme activity and oxidative stress markers. Sericin as well as its major constituent amino acid mixture inhibited the hydrogen peroxide effects. It also increased the antioxidant capacity and free radical scavenging ability in the cells treated. These results suggest that major constituent amino acids of sericin defend A549 cells against oxidative damage by scavenging reactive oxygen species rather than activating antioxidant enzyme system thereby inhibiting cell damage.

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INTRODUCTION

Oxidative stress is the imbalance between oxidant production and the antioxidant capacity of the cell that usually inflict cell injury. Disturbance in the balance can lead to the toxic effects through the accumulation of peroxyl and free radicals, within the cell and its components [1]. Unchecked or increased levels of free radicals has the potential to cause damage to various cellular compartments, including lipids, proteins and nucleic acids [2]. Hydrogen peroxide is widely regarded as a cytotoxic free radical whose level must be minimized by the action of antioxidant defense system. It is stable and can easily penetrate lipid membrane [3]. The LD_{50} values and the mode of cell death depends upon multiple parameters including the cell type used, its iron content, length of exposure to hydrogen peroxide and its concentration used [4]. Antioxidant defence is primarily contributed by antioxidant enzymes (AOEs) such as peroxidase (POX, E.C. 1.11.1.7), superoxide dismutase (SOD, E.C. 1.15.1.1) and catalase (CAT, E.C. 1. 11. 1.6) [5]. GPx reduces hydrogen peroxide and hydroperoxides, thereby scavenging oxidative radicals in cell membranes, whereas SOD converts O_2^- to molecular O_2 and hydrogen peroxide [6, 7]. Hydrogen peroxide is subsequently scavenged by CAT, resulting in the production of water and molecular oxygen [8].

Macromolecular proteins [9], peptides along with free amino acids [10], sulphur containing amino acids [11], aromatic amino acids [12], hydroxyl amino acids [13, 14, 15] and several naturally occurring macromolecules [16] are known to be effective as antioxidants. Silk protein sericin is a natural polypeptide with a commendable antioxidant property to combat oxidative stress due to the presence of polyphenols and flavanoids [17, 18]. Sericin is a water soluble globular protein which constitutes 20-30% of the silk fibre and is specifically synthesized in the middle silk gland of the silkworm. Sericin is sticky layer that envelopes the filamentous fibroin and together form silk filament and is often discarded as a waste product during silk processing [19]. It consist of 18 amino acids, most of which have strong polar side groups [20] and is especially rich in serine (~32%) [21]. The oxidative potential of amino acids to interact with free radical is dictated in large by its functional R group and also if the of the radical insult is high [22]. The antioxidant activity of proteins in radical mediated oxidation reactions may be due to their ability to act as radical trapping devices [23].

Sericin is an active biomolecule with several implications as a therapeutic agent [24, 25, 26, 27] and component of cosmetics [28, 29]. It also exhibits several pharmacological effects, such as supressing colon carcinogenesis in mice [30, 31, 32, 33] cryoprotection [34] and also acts as an anticoagulant upon sulphonation [35]. Sericin peptide also exhibited a protective effect against alcohol induced gastric injury in mice [26]. Dietary sericin improves lipid metabolism and ameliorates glucose tolerance in rats fed on high fat diet [36].

The aim of this study was to assess the effect of sericin as therapeutic agent in A549 cells against hydrogen peroxide-induced oxidative stress. Also, to explore the possible components of sericin with antioxidant activity that alleviate oxidative injury caused by transient exposure to hydrogen peroxide. We have demonstrated that the sericin and its major amino acids acts as scavenger of free radicals in hydrogen peroxide induced oxidative stress.

MATERIALS AND METHODS

Chemicals

Thiobarbituric acid (TBA), glutathione reductase, horseradish peroxidase and dinitrophenylhydrazine (DNPH) were from Sigma-Aldrich, (St. Louis, MO, USA). H_2DCFDA was from Molecular Probes (Eugene, USA). Reduced glutathione (GSH), NADPH, t-butylhydroperoxide, hydrogen peroxide (H_2O_2), Triton X- 100, epinephrine, sodium dodecyl sulphate (SDS), acetic acid, butanol, pyridine, tetra methoxy propane (TMP) and 2,4,6-tripyridyl-S-triazine (TPTZ)

were from Sisco Research Laboratory (Mumbai, India). L-Aspartic acid and L- Serine from Spectochem, (Mumbai, India).

Cell culture and experimental design

Human lung epithelial cells (A549) were grown in culture flask in RPMI-1640 medium supplemented with 2 mM glutamine, 100 U/ml penicillin, 100 µg / ml streptomycin and with or without 10% fetal bovine serum. The cells were cultured in a humidified atmosphere at 37ºC by passing 5 % CO_2. The 90–100 % confluent cells were sub cultured in 6 wells plate ($3x10^{-5}$ cells/well) for 24h. The subcultures were washed with phosphate buffer pH 7.4 repeatedly to remove the remnants of the media. Cells were exposed to hydrogen peroxide (20 µM) for 10 min to induce oxidative stress. To evaluate the antioxidant nature of sericin, the cells were incubated with 50 ng/ml sericin for 10 min at RT prior to the treatment of hydrogen peroxide. The cells were lifted from the wells with the help of rubber police man. The cells were homogenized in cold phosphate buffer and centrifuged at 3000 rpm for 10 minutes.

Extraction of sericin

Sericin was extracted from silk cocoon according to (Wu et al, 2007) with slight modification. Briefly, multi-voltine cocoons were boiled for several hours in distilled water to extract sericin. The water extract was condensed further by evaporating the water at 50ºC, and the concentrate was spray dried and collected as a powder. The powder was dissolved in distilled water in a 10:1 ratio (w/v), and was chilled overnight at 4ºC. Pure chilled ethanol was added to the sericin solution with constant stirring to obtain a final ethanol concentration of 75% (w/v). The obtained mixture was then kept at ⁻20ºC overnight, followed by centrifugation at 3500 rpm for 20 min. Alcohol evaporation was performed at 40ºC and the samples were lyophilized and stored until use.

LC-MS analysis of sericin protein

LC-MS analysis was performed according to Niessen [37] using an API 3000 LC-MS system fitted with a turbo ion spray source and a quadrupole mass spectrometer (Perkin Elmer Sciex, Thornton, Canada). The instrument was operated in positive ion mode with a spray voltage of 5500 V and a source temperature of 475ºC using a Phenomenex column (2540 x 6.6 mm) with methanol: water (3:1) as the mobile phase. Data were analysed with Analyst software version 1.4.2.

Antioxidant capacity

Antioxidant capacity was analysed by the modified method of Benzie and Strain [38] using ascorbic acid as the standard. FRAP reagent was prepared fresh with acetate buffer (pH 3.6). 10 mM TPTZ diluted with 40 mM HCl and 20 mM ferric chloride solution at a ratio of 10:1:1 (v/v), respectively, were warmed to 37ºC prior to use. 100 µl of cell homogenate and 3 ml of the FRAP reagent were vortexed and absorbance was measured at 593 nm at 0 min. The samples were incubated at 37ºC and absorbance was recorded after 30 min. The antioxidant capacity of the cell homogenate was expressed in µM Fe^{2+} /100 mg cell mass.

Measurement of intracellular ROS

To assess the intracellular ROS levels A549 cells were loaded with an ROS-sensitive indicator, CM-H_2DCFDA of 20 µM for 10 min at RT. The excess stain was washed off with insect Ringer's solution. In cells, esterase cleaves CM-H_2DCFDA to release CM-H_2DCFH, which is converted to the fluorescent product CM-H_2DCF when exposed to ROS [39]. CM-H_2DCFDA was excited at 520 nm and the emitted light was collected at 570 nm using a fluorescent microscope (Olympus IX 71, Japan). ROS was quantified using Image Pro Express version 6.3 software from stored images.

Lipid peroxidation level

Malondialdehyde (MDA), a product of LPO, was determined as described by Ohkawa

et al, [40]. In brief, 200 µl of cell homogenates were added to 8.1% SDS, vortexed and incubated for 10 min. 375 µl of 20% acetic acid and 0.6% thiobarbituric acid were added to the reaction mixture and placed in a boiling water bath for 60 min. The samples were allowed to cool and 1.25 ml of a butanol: pyridine mixture (15:1, v/v) was added and centrifuged at 640 g for 5 min. Absorbance was measured at 532 nm using 1, 3, 3-tetramethoxy propane (TMP) as the standard. The MDA concentration was expressed as nmol/mg protein.

Protein carbonyl level

Protein carbonyl (PrC) was measured according to the method of Uchida and Stadtman [41]. 0.1% DNPH in 2 N HCl was added to 800 µl of cell homogenate. Samples were kept in the dark for 1 h. The protein was precipitated with 20% trichloroacetic acid and centrifuged. The pellets were washed thrice with ethanol and ethyl acetate (1:1, v/v) and were dissolved in 2 ml of 8 M guanidine hydrochloride, and centrifuged. The supernatant was used to measure the absorbance at 365 nm and the PrC level was calculated using a molar absorption coefficient of 22,000 M^{-1} cm^{-1}. The results were expressed as µM/mg protein.

Superoxide dismutase (SOD, E.C. 1.15.1.1) activity

SOD activity was measured according to Misra and Fridovich [42] with slight modifications. Briefly, 100 µl of a 5% cell homogenate was added to 880 µl of carbonate buffer (0.5 M, pH 10.2). 20 µl of epinephrine (30 mM in 0.05% acetic acid) were added to the mixture and measured spectrophotometrically (model: Genova MK3, Jenway, UK) at 480 nm for 4 min. SOD activity was measured as the amount of enzyme that inhibits oxidation of epinephrine by 50%, which is equal to 1 unit.

Catalase (CAT, E.C. 1.11.1.6) activity

Catalase was determined by the method of Aebi [43]. Briefly, 100 µl enzyme samples with 10 µl of absolute alcohol were incubated for 30 min at 0°C followed by the addition of 10 µl Triton X-100. An aliquot of 50 µl was placed in 1.25 ml of 0.066 M H_2O_2 in phosphate buffer and the decrease in absorbance was measured at 240 nm for 60 s in a spectrophotometer. An extinction coefficient of 43.6 M cm^{-1} was used to determine enzyme activity and was expressed as one µmole of H_2O_2 degraded/min/mg protein.

Glutathione peroxidase enzyme (GPx, E. C. 1.11.1.9) activity

GPx enzyme activity was analysed by the method of Flohe and Gunzler [44]. 50 µl of 0.1 M phosphate buffer (pH 7.0), 100 µl of the enzyme sample, 100µl glutathione reductase (0.24 units) and 100 µl of 10 mM GSH were mixed. The mixture was pre incubated for 10 min at 37°C followed by the addition of 100 µl of 1.5 mM NADPH in 0.1% $NaHCO_3$. 50 µl of 12 mM t–butylhydroperoxide was added to monitor the hydrogen peroxide independent concentration of NADPH for 3 min. The overall reaction was started by adding 100 µl of pre-warmed H_2O_2, and the decrease in absorption at 340 nm was monitored for 5 min. GPx activity was expressed as µm NADPH oxidized/min/mg protein.

Statistical analysis

Data are shown as the mean ± SD of six observations. Changes between the groups were analysed by MANOVA and further tested by the Bonferroni post-hoc test using Statistical Package for Social Sciences (SPSS) software [45] and $p<0.05$ was considered significant. Statistically significant data are presented in the text.

RESULTS

Composition of silk protein sericin

A flow rate of 0.3 ml/min through the Phenomenex column was used in the LC-MS studies to provide information on the retention times and the endogenous concentrations of various amino acids in the silk protein sericin. The retention times from the samples were compared with synthetic standards. Among the

amino acids, serine was the most abundant, followed by glycine, aspartic acid, threonine and glutamic acid (Table 1).

Amino acids	g/100g sample
Serine	11.18
Aspartic acid	3.27
Glycine	3.21
Threonine	1.04
Glutamic acid	0.23

Table 1. *Prominent amino acid Composition of sericin protein in B. mori.*

Antioxidant capacity

A549 cells incubated with sericin and also with serine and aspartic acid mixture prior to hydrogen peroxide treatment showed a significant increase in antioxidant capacity compared to that of the residual antioxidant capacity of untreated A549 cells. The hydrogen peroxide treated A549 cells were not different to control with respect to antioxidant capacity (Fig. 1).

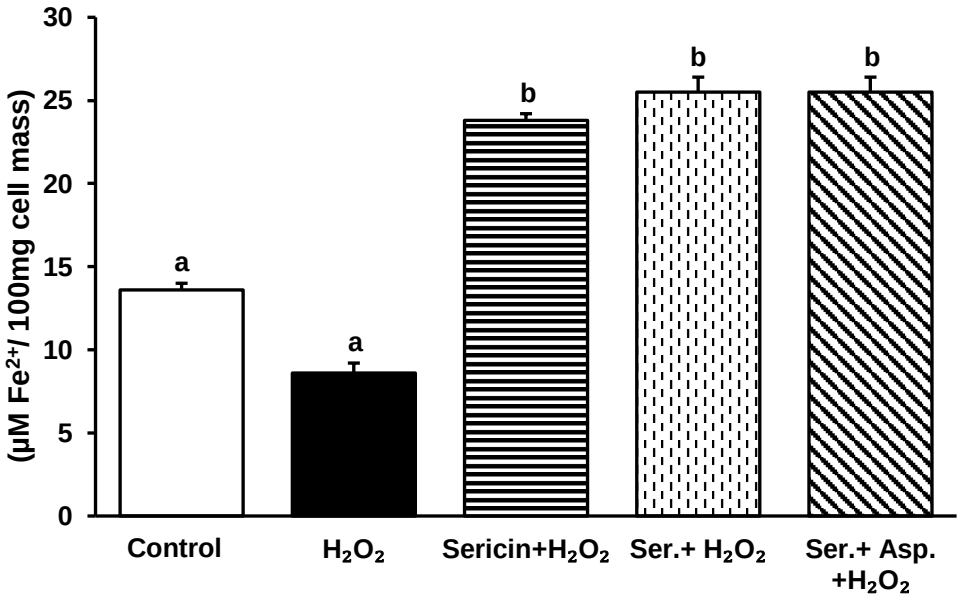

Fig.1 *Effect of sericin, amino acid serine and a mixture of serine and aspartic acid on antioxidant capacity in human lung epithelial cells treated with or without hydrogen peroxide. Data are means ± SE (n = 6). P< 0.05 was considered significant. Values between the treatments are represented in lower cases (a, b). Those not sharing the same letters are significant.*

Hydrogen peroxide induced an increase in ROS

H_2DCFDA probe was used to study the H_2O_2-induced change in reactive oxygen species in A549 cells under a fluorescent microscope. Under phase contrast, blebbing of the plasma membrane and swelling of the cells were observed in cells treated with H_2O_2 at concentrations above 20 µM. However, pre-incubation of cells with sericin or amino acid mixture of serine and aspartic acid inhibited

the hydrogen peroxide induced morphological changes in A549 cells (Fig. 2). H_2DCFDA treated cells revealed a significant increase in the intensity of fluorescence, indicating an increase in ROS upon exposure to hydrogen peroxide. Incubation of cells with sericin or amino acid mixture prior to the treatment of hydrogen peroxide did not show any significant difference from control cells (Fig. 3).

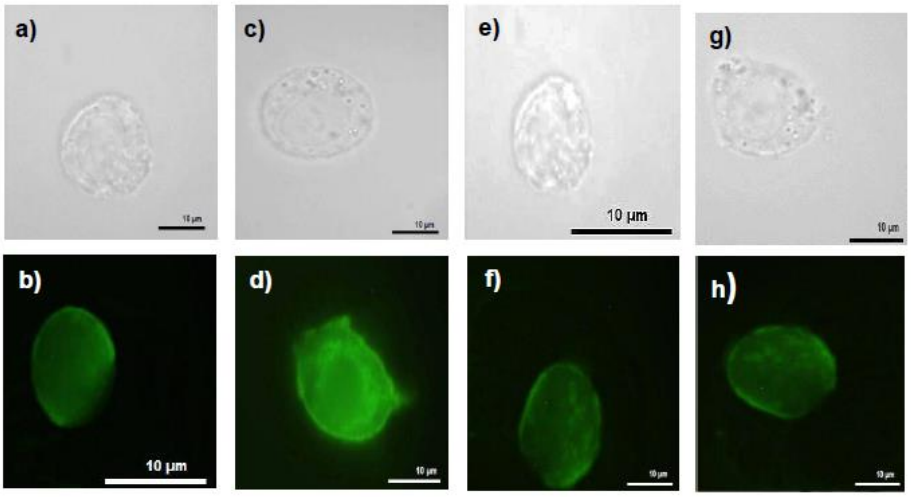

Fig. 2 *Effect of sericin on the hydrogen peroxide-induced an increase in reactive oxygen species in the human lung epithelial cells (A549). (a-b) Normal human lung epithelial cells (c-d) hydrogen peroxide treated cells, (e-f) cells incubated with sericin prior to hydrogen peroxide treatment and (g-h) cells incubated with a mixture of serine and aspartic acid prior to hydrogen peroxide treatment.*

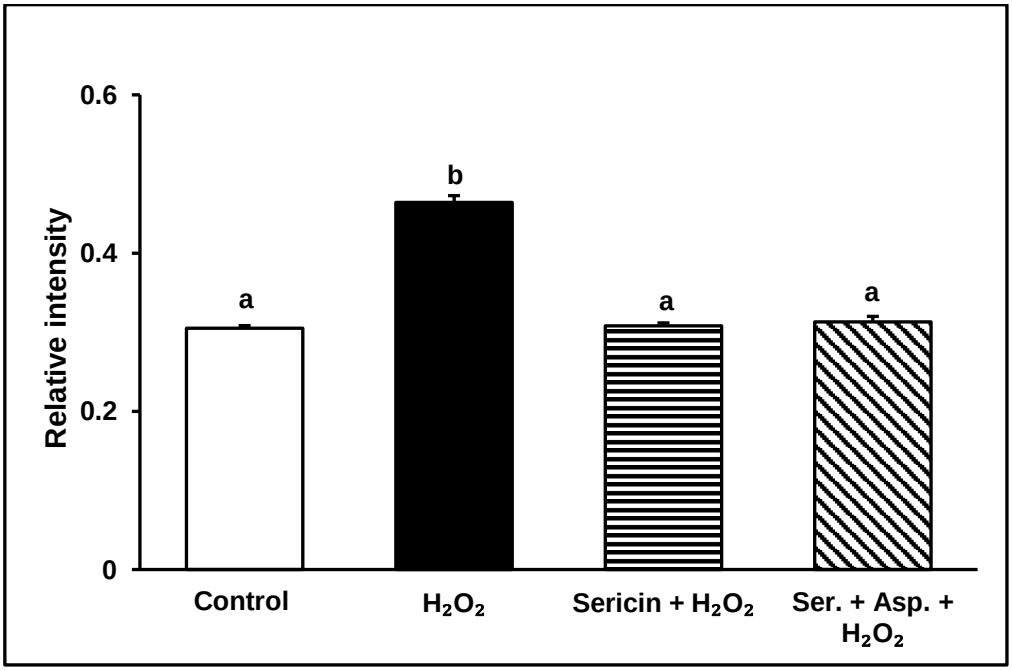

Fig. 3 *Effect of sericin and its amino acids on the hydrogen peroxide-induced increase of ROS in A549 cells. Data are shown as mean ± SE (n=3). p< 0.05 was considered significant. Values between the treatments are represented by lower case letters (a, b). Those not sharing the same letters are significant.*

Oxidative stress markers

Malondialdehyde, a product of lipid peroxidation was significantly higher in A549 cells on treatment with hydrogen peroxide. However, cells on pre incubation with silk protein sericin and amino acid serine or serine/ aspartic acid mixture prevented hydrogen peroxide induced increase of MDA level in cells (Table 2).

Nature of the treatment	MDA (nmol/ mg protein)	Protein carbonyl (µmol/ mg protein)
Control	1.23 [a]	17.45 [a]
	±0.23	±2.41
H_2O_2 treatment	2.29 [b]	47.87 [b]
	±0.33	±2.10
Incubation with sericin	0.96 [a]	15.87 [a]
	±0.16	±4.91
Incubation with Ser.	1.08 [a]	18.55 [a]
	±0.03	±2.32
Incubation with Ser. and Asp.	1.01 [a]	16.97 [a]
	±0.04	±2.74

Table 2. *MDA and PrC level in human lung epithelial cells (A549) treated with hydrogen peroxide and pre-incubated with sericin, amino acid serine or amino acid mixture prior to hydrogen peroxide treatment. Data are means ± SE (n = 6). P< 0.05 was considered significant. Values between the treatments are represented in lower cases (a, b). Those not sharing the same letters are significant.*

A significant increase in protein carbonyl as a result of oxidative stress was evident following treatment with hydrogen peroxide in A549 cells. Prior incubation with sericin or amino acid serine or a mixture of serine and aspartic acid significantly inhibited hydrogen peroxide induced protein oxidation in A549 cells (Table 2).

Antioxidant enzymes

Superoxide dismutase activity was significantly increased on exposure to hydrogen peroxide in A549 cells. Incubation of cells with sericin or serine or serine/aspartic acid mixture prior to hydrogen peroxide treatment inhibited the cells from hydrogen peroxide induced increase in SOD activity (Fig. 4a). Incubation with 20 µM hydrogen peroxide significantly increased CAT activity in A549 cells. Cells treated with sericin or amino acid serine or mixture of serine and aspartic acid before the induction of oxidative stress with hydrogen peroxide did not elicit a rise in CAT activity under stress (Fig. 4b). A549 cells exhibited a significant increase in GPx activity on exposure to hydrogen peroxide. However, prior incubation of cells with sericin or serine or serine and aspartic acid mixture obliterated the hydrogen peroxide induced increase in GPx activity (Fig. 4c).

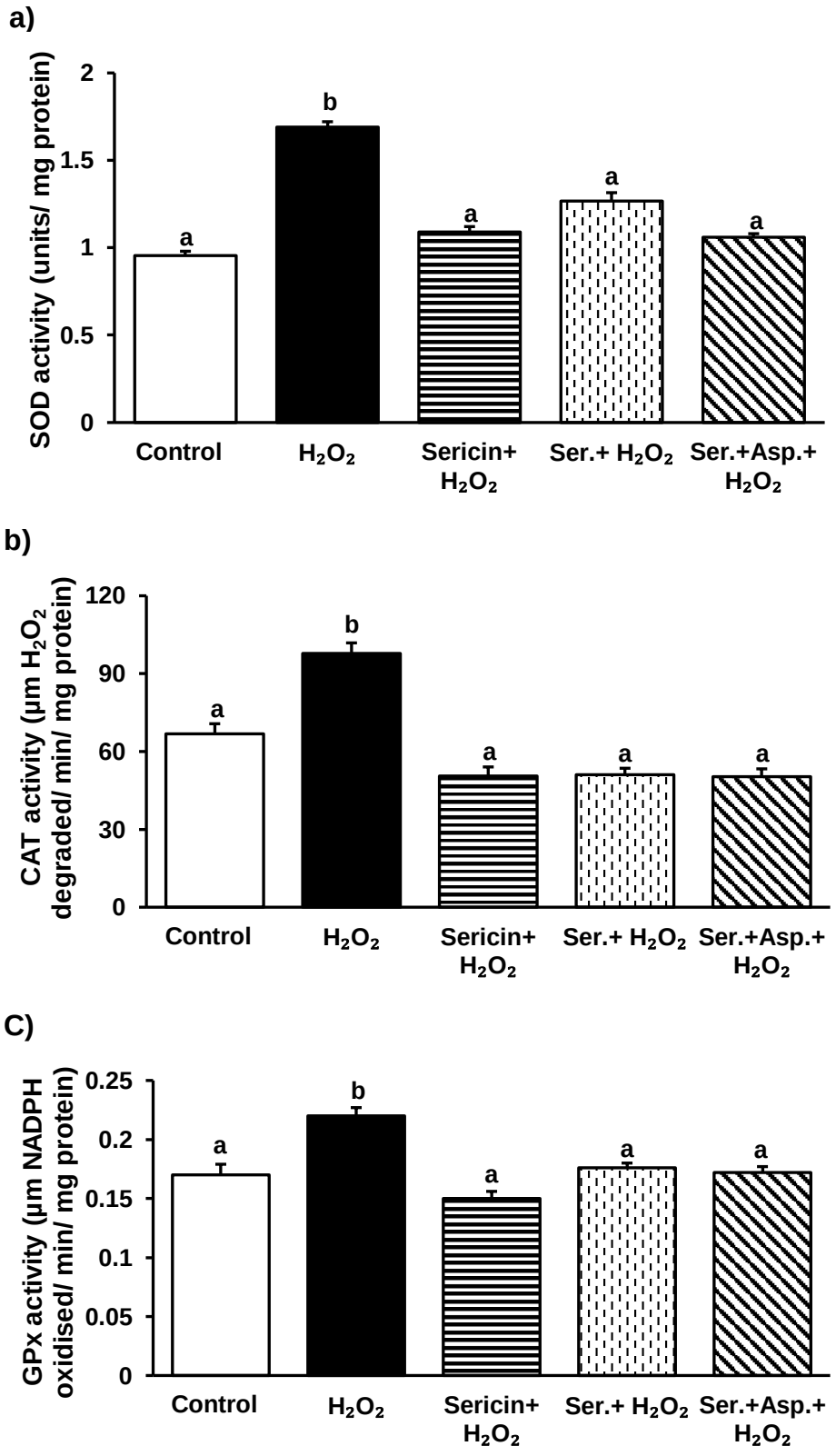

Fig. 4 *(a) SOD activity, (b) CAT activity and (c) GPx activity in human lung epithelial cells (A549) treated with hydrogen peroxide and incubated with sericin, amino acid serine or amino acid mixture prior to hydrogen peroxide treatment. Data are means ± SE (n = 6). P< 0.05 was considered significant. Values between the treatments are represented in lower cases (a, b). Those not sharing the same letters are significant.*

DISCUSSION

Hydrogen peroxide is cytotoxic to a wide range of animal cells however, LD_{50} values and the mode of cell death depends upon multiple parameters including the cell type used, length of exposure and the concentration used [46]. The toxic effect of hydrogen peroxide largely relates of its conversion to its in-discriminatively reactive hydroxyl radical ($OH^{\cdot}$), either by exposure to UV light [47] or an interaction with metal ions [48]. Indeed our results suggest that on exposure to 23µM hydrogen peroxide, caused close to 50% mortality in human lung epithelial cell (A549) with a concomitant increase in ROS, as indicated by fluorescent microscopy studies. Studies on skin fibroblast cells (AH927) subjected to hydrogen peroxide, exhibited cell shrinkage and also revealed nuclear condensation [49]. Whereas, in mouse gingivial fibroblast challenged with hydrogen peroxide induced morphological changes such as enlarged nuclei and expanded cytoplasm [50]. In the current study a decrease in cell volume was observed in A549 cells subjected to hydrogen peroxide treatment. Sericin is known to promote cell viability in mouse fibroblasts cell (L929) [51]. Amino acids such as glycine and alanine declined the maitotoxin induced endothelial cell injury in bovine aorta [52]. In the present study, we have demonstrated that A549 cells subjected to hydrogen peroxide accumulated ROS as indicated by H_2DCFDA fluorescence and prior incubation with sericin or its constitutive amino acids obliterated the accumulation of peroxyl radicals. Fan et al, [53] have also demonstrated antioxidative properties of sericin against multiple radicals. The ability of a compound to reduce Fe^{3+} and Fe^{2+} serve as an important indicator of its potential antioxidant power [38, 54]. Iron sequestering activity of sericin prevent chronic skin photoageing in female Skh/HR-1 hairless mice [55]. In the present study, prior incubation with sericin and its amino acids showed an increased ferric reducing capacity in the cells subjected to oxidative stress.

Oxidative modification in proteins [56, 57] and peroxidation of unsaturated lipids in the cell membrane [58] could occur due to increased hydrogen peroxide level. Sericin exert a protective effect against alcohol mediated hepatic injury in mice by preventing the peroxidative detioration of structural lipids in membranous organelles [26]. Several amino acids have the property of reducing LPO [14, 15] and protecting ability of amino acids against peroxidation is because of its capacity to chelate copper [10]. There has been no evidence of sericin as inhibitor of protein oxidation. However, protein thiols were found to inhibit protein hydroperoxide formation [59]. In our study, sericin and its integral amino acids such as serine alone or in combination with aspartic acid lessened hydrogen peroxide induced LPO and protein oxidation. We presume that amino acid components of sericin might have functioned as scavengers of ROS thereby reducing LPO and protein oxidation.

Protection against hydrogen peroxide induced ROS was afforded by the up-regulation of antioxidant enzymes [60]. Hydrogen peroxide induced an increase in the activity of SOD, CAT and GPx [61, 49, 62] in fibroblast cells. Role of sericin in reduced SOD and GPx activity and unaltered CAT activity was demonstrated in rat mucosal tissue treated with alcohol [26]. Amino acid glycine also afforded protection to hepatic tissue against alcohol induced damage in rats [15]. Sericin has antioxidative and cytoprotective effects through elevation of some crucial antioxidant factors in human endothelial cells (HUVEC) [63]. In our study, a significant increase in AOEs were observed on hydrogen peroxide treatment while such an elevation of AOEs were not observed in cells prior treated with sericin or its amino acids.

CONCLUSION

A possible explanation for the present scenario is that the hydrogen peroxide induced ROS would have triggered an increase in the AOEs as a defense mechanism under stress. The notable point in our study is that, sericin and its amino acids protect the cell through scavenging

the free radicals rather than enhancing the AOEs.

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REFERENCES

[1] Burton GJ, Jauniaux E. Oxidative stress. Best Prac. Res. Cl. Ob. 2011; 25: 287-299.

[2] Thannickal VJ, Fanburg BL. Reactive oxygen species in cell signaling. Am. J. Physiol. Lung Cell Mol. Physiol. 2000; 279 (L): 1005-1028.

[3] Nakamura H, Nakamura K, Yodoi J. Redox regulation of cellular activation. Annu. Rev. Immunol. 1997; 15: 351-369.

[4] Halliwell B. Cell culture, oxidative stress, and antioxidants: avoiding pitfalls. Biomed. J. 2014; 37: 99-105.

[5] Felton GW, Summers CB. Antioxidant systems in insects. Arch. Insect. Biochem. Physiol. 1995; 29: 187-197.

[6] Maiorino M, Scapi M, Ursini F, Biasolo M, Bosello V, Flohe L. Distinct promoters determine alternative transcription of *gpx-4* into phospholipid-hydroperoxide glutathione peroxidase variants. J. Biol. Chem. 2003; 278: 34286-34290.

[7] Imlay JA. Cellular defense against superoxide and hydrogen peroxide. Annu. Rev. Biochem. 2008; 77: 755-776.

[8] Kashiwagi A, Kashiwagi K, Takase M, Hanada H, Nakamura M. Comparison of catalase in diploids and haploid *Ranarugosa* using heat and chemical inactivation techniques. Comp. Biochem. Physiol. 1997; 118: B499-B503.

[9] Medina-Navarro R, Duran-Reye G, Diaz-Flores M, Vilar-Rojas C. Protein antioxidant response to the stress and the relationship between molecular structure and antioxidant function. PLOS One. 2010; 5: e8971.

[10] Wu HC, Chen HM, Shiau CY. Free amino acids and peptides as related to antioxidant properties in protein hydrolysates of mackerel (*Scomber austriasicus*). Food Res. Int. 2003; 36: 949–957.

[11] Atmaca G. Antioxidant effects of sulfur-containing amino acids. Yonsei. Med. J. 2004; 45: 776-788.

[12] Nimalaratne C, Lopes-Lutz D, Schieber A, Wu J. Free aromatic amino acids in egg yolk show antioxidant properties. Food Chem. 2011; 129: 155–161.

[13] Liu DZ, Lin YS, Hou WC. Monohydrates of aspartic acid and glutamic acid exhibit antioxidant and angiotensin converting enzyme inhibitory activities. J. Agric. Food. Chem. 2004; 52: 2386- 2390.

[14] Movahedian A, Naderi GA, Dashti GR, Asgary S, Zadhoosh F. Antioxidant effects of L-serine against fatty streak formation in hypercholestrolemic animals. ARYA. Atherosclerosis J. 2006; 2: 126-129.

[15] Selvaraju R, Subbashinidevi K. Impact of glycine on antioxidant defence system in rats with alcohol induced liver injury. Int. J. Res. Pharm. Biomed. Sci. 2011; 2: 1314-1320.

[16] Balsano C, Alisi A. Antioxidant effect of natural bioactive compounds. Curr. Pharm. Des. 2009; 15: 3063-3073.

[17] Devi R, Deori M, Devi D. Evaluation of antioxidant activities of silk protein sericin secreted by silkworm *Antheraea assamensis* (Lepidoptera: Saturniidae). J. Pharm. Res. 2011; 4: 4688-4691.

[18] Prasong S. Screening of antioxidant activity of some *Samia ricini* (Eri) silks: comparison with *Bombyx mori*. J. Biol. Sci. 2011; 11: 336-339.

[19] Wei T, Li MZ, Xie RJ. Preparation and structure of porous silk sericin materials. Macr. Mat. Eng. 2005; 290: 188–194.

[20] Wu JH, Wang Z, Xu SY. Preparation and characterization of sericin powder extracted from silk industry waste water. Food Chem. 2007; 103: 1255-1262.

[21] Kwang YC, Jae YM, Yong WL, Kwang GL, Joo HY, Hae YK. Preparation of self-

assembled silk sericin nanoparticles. Int. J. Biol. Macromol. 2003; 32: 36-42.

[22] Elias RJ, Kellerby S, Decker A. Antioxidant activity of proteins and peptides. Crit. Rev. Food Sci. Nutr. 2008; 48: 430-441.

[23] Ostdal H, Davies MJ, Andersen HJ. Reaction between protein radicals and other biomolecules. Free Radic. Biol. Med. 2002; 33: 201-209.

[24] Khudaiderdier HA. Synthesis of copolymer processing an anticoagulant action. Chem. Nat. Compd. 1997; 33: 603-604.

[25] Aramwit P, Sangcakul A. The effects of sericin cream on wound healing in rats. Biosci. Biotechnol. Biochem. 2007; 71: 2473-2477.

[26] Li Y-G, Ji D-F, Chen S, Hu G-Y. Protective effects of sericin protein on alcohol mediated liver damage in mice. Alcohol Alcoholism. 2008; 43: 246-253.

[27] Senakoon V, Nuchadomrong S, Sirimungkararat S, Senawong T, Kitikoon P. Antibacterial action of eri (*Samia ricini*) sericin against *Escherichia coli* and *Staphylococcus aureus*. As. J. Food Agro-Ind. 2009; S: 222-228.

[28] Padamwar MN, Pawar AP. Silk sericin and its application: a review. J. Sci. Ind. Res. 2004; 63: 323-329.

[29] Patel RJ, Modasiya MK. Sericin: Pharmaceutical applications. Int. J. Res. Pharm. Biomed. Sci. 2011; 2: 913-917.

[30] Sasaki M, Kato N, Watanabe H, Yamada H. Silk protein, sericin, suppresses colon carcinogenesis induced by 1, 2-dimethylhydrazine in mice. Oncol. Rep. 2000; 7: 1049-1101.

[31] Zhaorigetu S, Sasaki M, Watanbe H, Kato N. Supplemental silk protein, sericin, suppresses colon tumorigenesis in 1,2-dimethylhydrazine treated mice by reducing oxidative stress and cell proliferation. Biosci. Biotechnol. Biochem. 2001; 65: 2181-2186.

[32] Zhaorigetu S, Yanaka N, Sasaki M, Watanbe H, Kato N. Inhibitory effects of silk protein, sericin on UVB-induced acute damage and tumor promotion by reducing oxidative stress in the skin of hairless mouse. J. Photochem. Photobiol. 2003; 71: 11-17.

[33] Zhaorigetu S, Sasaki M, Kato N. Consumption of sericin suppress colon oxidative stress and aberrant crypt foci in 1,2-dimethylhydrazine-treated rats by colon undigested sericin. J. Nutr. Sci. Vitaminol. 2007; 53: 297-300.

[34] Tsujimoto K, Takagi H, Takahashi M, Yamada H, Nakamori S. Cryoprotective effects of the serine rich repetitive sequence in silk protein sericin. J. Biol. Chem. 2001; 129: 979-986.

[35] Tamada Y, Sano M, Niwa K, Imai T, Yoshino G. Sulfation of silk sericin and anticoagulant activity of sulfated sericin. J. Biomater. Sci. Polym. Ed. 2004; 15 971–980.

[36] Okazaki Y, Kakehi S, Xu Y, Tsujimoto K, Sasaki M, Ogawa H, Kato N. Consumption of sericin reduces serum lipids, ameliorates glucose tolerance and elevates serum adiponectin in rats fed on a high fat diet. Biosci. Biotechnol. Biochem. 2010; 74: 1534-1538.

[37] Niessen WMA. Liquid chromatography-mass spectrometry. Third ed., Taylor and Francis Group, Baco Raton, New York, 1999.

[38] Benzie IF, Strain JJ. The Ferric reducing ability of plasma (FRAP) as a measure of "Antioxidant power": The FRAP assay. Anal. Biochem. 1996; 239: 70-76.

[39] Xie Z, Kometiani P, Liu J, Li J, Shapiro JI, Askari A. Intracellular reactive oxygen species mediates the linkage of Na^+/K^+ -ATPase to hypertrophy and its marker genes in cardiomyocytes. J. Biolo. Chem. 1999; 274: 19323-19328.

[40] Ohkawa H, Ohishi M, Yagi K. Assay for lipid peroxidation in animal tissues by thiobarbituric acid reaction. Anal. Biochem. 1979; 95: 351–358.

[41] Uchida K, Stadtman ER. Covalent attachment of 4 – hydrosynoneal to glyceradehyde 3-phosphate dehydrogenase. J. Biol. Chem. 1993; 268: 6388–6393.

[42] Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay of superoxide dismutase. J. Biol. Chem. 1972; 247: 3170-3175.

[43] Aebi H. Catalase *in vitro*. Methods Enzymol. 1984; 105: 121–126.

[44] Flohe L, Gunzler WA. Assays of glutathione peroxidase. Methods Enzymol. 1984; 105: 115-121.

[45] Huberty CJ, Olejnik S. Applied MANOVA and discriminate analysis, New Jersey, John Wiley and Sons, 2006.

[46] Halliwell B. Gutteridge JMC. Free Radical in Biology and Medicine, Third ed., Clarendon Press, Oxford, UK, 1999.

[47] Ueda J, Saito N, Shimazu Y, Ozawa T. A comparison of scavenging abilities of antioxidants against hydroxyl radicals. Arch. Biochem. Biophys. 1996; 333: 377-384.

[48] Halliwell B, Clement MV, Ramalingam J, Long L.H. Hydrogen peroxide. Ubiquitous in cell culture and in vivo? IUBMB. Life. 2000; 50: 251-257.

[49] Dash R, Acharya C, Bindu PC, Kundu SC. Antioxidant potential of silk protein sericin against hydrogen peroxide-induced oxidative stress in skin fibroblasts. BMB. Reports. 2008; 41: 236-241.

[50] Kiyoshima T, Enoki N, Kobayashi I, Sakai T, Nagata K, Wada H, Fujiwara H, Ookuma H. Sakai Y. Oxidative stress caused by a low concentration of hydrogen peroxide induces senescence-like changes in mouse gingival fibroblasts. Int. J. Mol. Med. 2012; 30: 1007-1012.

[51] Aramwit P, Kanokpanont S, Nakpheng T, Srichana T. The effect of sericin from various extraction methods on cell viability and collagen production. Int. J. Mol. Sci. 2010; 11: 2200-2211.

[52] Estacion M**,** Weinberg JS, Sinkins WG, Schilling WP. Blockade of maitotoxin-induced endothelial cell lysis by glycine and L-alanine. Am. J. Physiol. 2003; 284: C1006–C1020.

[53] Fan JB, Wu LP, Mao XY, Ren FZ. Antioxidant activities of silk sericin from silkworm *Bombyx mori*. J. Food Chem. 2009; 33: 74-88.

[54] Yen GC, Chen HY, Lee CE. Measurement of antioxidant activity in metal ion-reduced lipid peroxidation systems. J. Sci. Food Agr. 1999; 79: 1213-1217.

[55] Kitazawa M, Ishitsuka Y, Kobayashi M, Nakano T, Iwasaki K, Sakamoto K. Protective effects of an antioxidant derived from serine and vitamin B6 on skin photoagin g in hairless mice. Photochem. Photobiol. 2005; 81: 970-974.

[56] Dalle-Donne I, Rossi R, Giustarini D, Mizani A, Colombo R. Protein carbonyl groups as biomarkers of oxidative stress. Clin. Chim. Acta. 2003; 329: 23-28.

[57] Stadtman ER. Protein oxidation and ageing. Sci. 1992; 257: 1220-1224.

[58] Fridovich I. Superoxide radical and superoxide dismutases, Annu. Rev. Biochem. 1995; 64: 97-112.

[59] Platt AA, Gieseg SP. Inhibition of protein hydroperoxide formation by protein thiols. Redox Report. 2003; 8: 81-86.

[60] Fulda S, Gorman AM, Hori O, Samali A. Cellular stress responses: cell survival and cell death. Int. J. Cell Biol. 2010; 214074 doi: 10.1155/2010/214074.

[61] Caldini R, Chevanne M, Mocali A, Tombaccini D, Paoletti F. Premature induction of ageing in sub lethally H_2O_2-treated young MRC5 fibroblasts correlates with increased glutathione peroxidase levels and resistance to DNA breakage. Mech. Ageing Dev. 1998; 105: 137-150.

[62] Tsai C-C, Wu S-B, Cheng C-Y, Kao S-C, Kau H-C, Lee S-M, Wei Y-H. Increased

response to oxidative stress challenge in Graves opthalmopathy orbital fibroblast. Mol. Vis. 2011; 17: 2782-2788.

[63] Maralani MN, Movahedian A, Javanmard SH. Antioxidant and cytoprotective effects of L-serine on human endothelial cells. Res. Pharm. Sci. 2012; 7: 209-215.

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