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Inhibitory property of *Piper betel* extract against photosensitization-induced damages to lipids and proteins

S. Bhattacharya [a], S. Mula [b], S. Gamre [b], J.P. Kamat [c], S.K. Bandyopadhyay [a], S. Chattopadhyay [b,*]

[a] *Department of Biochemistry, Dr. B.C. Roy Post Graduate Institute of Basic medical Sciences, 244B, Acharya Jagadish Chandra Bose Road, Kolkata 700 020, India*
[b] *Bio-Organic Division, Bhabha Atomic Research Centre, Mumbai 400 085, India*
[c] *Radiation Biology and Health Science Division, Bhabha Atomic Research Centre, Mumbai 400 085, India*

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Abstract

The protective activity of *Piper betel* ethanolic extract (PE) against the photosensitization-induced damage to lipids and proteins of rat liver mitochondria has been studied. PE could effectively prevent lipid peroxidation, as assessed by measuring thiobarbituric acid reactive substances, lipid hydroperoxide and conjugated diene. In addition, it prevented photo-induced oxidation of proteins in a concentration-dependent manner. Furthermore, its preventive capacity against iron-mediated lipid peroxidation was also confirmed. The protective activity of PE could be attributed to its free radical and singlet oxygen scavenging properties. The activity of PE was primarily due to its phenolic constituents, which were identified as chavibetol and 4-allylpyrocatechol.

Keywords: *Piper betel*; Photosensitization; Lipids; Proteins; Protection

1. Introduction

Photosensitization involving light, a photo-sensitizer and oxygen is a potentially damaging event in biological systems. This generates several reactive oxygen species (ROS) such as singlet oxygen, hydrogen peroxide, superoxide and hydroperoxyl or hydroxyl radicals that are capable of damaging various sub-cellular structures and molecules (Paillous & Fery-Forgues, 1994; Peiette, 1991; Sies, 1993). Among these, singlet oxygen has been identified as one of the major species responsible for biological damage caused by photosensitization (Weishaput, Goomer, & Dougherty, 1976). The generation of free radicals in skin by solar ultraviolet light (UV) accelerates skin cancer and photo-aging (Witt, Motchink, & Packer, 1993). Cellular exposure to UV light also leads to iron release, resulting in excessive production of ROS and ultimately to pathogenesis (Halliwell & Gutterridge, 1989). Therefore, there is a need to develop suitable formulations that can prevent photo-induced biological damage. To this end, exploration of herbs/plants that are credited with various medicinal attributes in Ayurveda might be useful from the point of view of low cost and reduced toxicity. Very recently, we have found (Bhattacharya et al., 2005) that the *Piper betel* ethanolic extract (PE) acts as an excellent radioprotectant, exerting the activity through its superior radical scavenging and immunomodulatory properties. In view of this, it was of interest to investigate the efficacy of PE in protecting biological targets from photo-induced damage. The results are discussed in this paper.

The *P. betel* plant is widely grown in the tropical humid climate of South East Asia, and its leaves, with a strong pungent and aromatic flavour, are widely consumed as a mouth freshener. The leaves are credited with wound heal-

* Corresponding author.
E-mail address: schatt@apsara.barc.ernet.in (S. Chattopadhyay).

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ing property, and as digestive and pancreatic lipase stimulants in traditional medicine (Chatterjee & Pakrashi, 1995). These effects have been demonstrated on experimental animals (Prabhu, Patel, Saraawathi, & Srinivasan, 1995; Santhanam & Nagarjan, 1990). We have also reported the gastrocytoprotective properties of the leaf extract on experimentally-induced gastric lesions and rationalized the activity in terms of its antioxidant property (Majumdar, Roy Chaudhury, Roy, & Bandyopadhyay, 2002, 2003). In addition, its antimicrobial (Ramji, Iyer, & Chandrasekaran, 2002), antifungal and anti-inflammatory (Ambarta, 1986) activities have also been reported.

2. Materials and methods

2.1. Materials

Ascorbic acid and 2-thiobarbituric acid (TBA) (both from Himedia Lab. Pvt. Ltd., India), ethylenediaminetetraacetic acid (EDTA) (E. Merck, India), trichloroacetic acid (TCA) (Thomas Baker, India) and potassium phosphate, sodium azide, KOH and HCl (all from SRL, India) were used. Tetraethoxypropane, guanidine hydrochloride, glutathione, α-tocopherol, superoxide dismutase (SOD) (type I, from bovine liver, specific activity 26 000 U/mg protein), and haematoporphyrin were obtained from Sigma Chemicals, USA. All other reagents were of analytical grade. Leaves of *P. betel* were collected locally during March to May and identified by the Botanical Survey of India as *P. betel* Linn., of family *Piperaceae*.

Stock solutions of ascorbic acid and EDTA were prepared in deaerated water just prior to use. The test extract was used as an aqueous solution. All solutions were made with triply distilled water.

2.2. Animals

The rats were bred in the Bhabha Atomic Research Centre (BARC) Laboratory Animal House Facility and procured after obtaining clearance from the BARC Animal Ethics Committee. All the experiments were conducted with strict adherence to the ethical guidelines laid down by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), constituted by the Animal Welfare Division of the Government of India on the use of animals in scientific research.

2.3. Preparation of the plant extract and its chemical composition

Finely chopped *P. betel* leaves were soaked in 95% ethanol for seven days and the supernatant decanted. The extract was filtered through a nylon mesh. The entire process was repeated three times, the combined alcoholic extracts were evaporated in vacuo and finally dried in a lyophilizer to obtain an amorphous yellowish brown solid in 2.2% w/w yield. This was designated as *P. betel* ethanolic extract (PE) and was stored in a vacuum desiccator. The chemical constituents of PE were analyzed by HPTLC (Camag instrument) using silica gel plates ($60F_{254}$, E. Merck, Germany) and different mobile phases. The compounds were detected under UV light (254 nm).

2.4. Preparation of mitochondria

Mitochondria were isolated from male Wistar rats weighing 250 ± 20 g as described earlier (Bhattacharya et al., 2005).

2.5. Lipid peroxidation assay

2.5.1. Photosensitization induced

The system for exposing mitochondria to photosensitization was simple, physiologically relevant and similar to that described earlier (Kamat & Devasagayam, 1996). In brief, the reaction mixture (final volume 1.0 ml) containing mitochondrial protein (final concentration 0.5 mg/ml), methylene blue (50 μg/ml) and PE (0–100 μg/ml) in KH_2PO_4–KOH buffer (pH 7.4) was kept in a 'trap' maintained at 37 °C and irradiated for 30 min with a 100 W tungsten lamp. The distance from the light source and the trap was 15 cm. The light intensity of the system, as measured by a Lux meter, was calculated to be 32.45 W m^{-2}.

The UV light-induced lipid peroxidation of the rat liver mitochondria was assayed by the thiobarbituric acid (TBA) method described elsewhere (Patro et al., 2002). For estimating the thiobarbituric acid reactive substances (TBARS), the reactants were heated for 15 min on a boiling water bath with TBA reagent (0.5% TBA/10% TCA/6 mM EDTA/0.63 M HCl). After cooling, the precipitate formed was removed by centrifugation at 1000*g* for 10 min. The absorbance of the sample was determined at 532 nm against a blank, that contained all the reagents except the mitochondria. Malonaldehyde standard was prepared by the acidic hydrolysis of tetraethoxypropane. For correction of endogenous TBARS, fresh samples were boiled without photo-irradiation, and the values were subtracted. Various antioxidants were used as the positive controls.

The assays for the conjugated diene (CD) and lipid hydroperoxide (LOOH) were carried out as described earlier (Bhattacharya et al., 2005).

2.5.2. Fe(II)-induced

The mitochondrial lipid peroxidation was initiated by the addition of Fe(II) (50 μM) and ascorbic acid (200 μM) to the reaction mixture containing the following components at the final concentrations stated: mitochondrial fraction (4.0 mg protein/ ml) and PE (50 μg/ml) in 50 mM potassium phosphate buffer pH 7.4. The mixture was incubated at 37 °C for 30 min, TCA–TBA–HCl (2 ml, 15% TCA, 0.375% TBA, 0.25 M HCl) solution was added, and the amount of TBARS measured as above.

2.6. Protein carbonyl assay

Mitochondrial proteins (final concentration 0.5 mg/ml) in 50 mM potassium phosphate buffer pH 7.4 were treated with 20% aqueous TCA solution (1 ml) and the mixture centrifuged at 2000 rpm for 20 min to get pellets. The pellets were reconstituted in the same buffer and treated with 2,4-dinitrophenylhydrozine (DNPH) (21% in 2 M HCl, 2 ml). The blank samples were prepared by adding 2 M HCl without DNPH. After incubating for 1 h at room temperature in the dark, 20% TCA solution (2 ml) was added to each tube, the samples were centrifuged for 20 min at 2000 rpm. The final pellets of proteins were washed with a 1:1 ethanol–ethyl acetate mixture, dissolved in 6 M guanidine hydrochloride and the absorbance at 370 nm measured (Palamanda & Kehrer, 1992). Various antioxidants were used as the positive controls.

2.7. Superoxide dismustase (SOD) assay

The assay of SOD activity was based (Sun & Zigman, 1978) on the spectrophotometric detection (absorbance at 320 nm) of adrenochrome formed by autoxidation of epinephrine in 50 mM sodium carbonate buffer at pH 10. Appropriate sample blanks were taken to ensure that PE did not interfere with the assay. Data were subjected to statistical analysis by the Student's *t*-test.

2.8. Assay of singlet oxygen scavenging by PE

Singlet oxygen was produced by photosensitization of haematoporphyrin (120 mM, optical density at 532 nm $\sim$ 0.5) in acetonitrile and its luminescence at 1270 nm, in the absence and presence of various concentrations of PE, was detected with a transient luminescence spectrometer model TL 900 (Edinburgh Instr., UK).

3. Results

3.1. Chemical composition of PE

Given that the constituents of PE were of different polarities, its HPTLC analyses were carried out using various mobile phases and the results are presented in Table 1. The principal chemical constituents of PE were found to be the phenolics, chavibetol and 4-allylpyrocatechol (Fig. 1) along with their respective glycosides. However, contrary to a previous report (Evans, Bowers, & Funk, 1984), the corresponding acetates and chavicol were not present in PE.

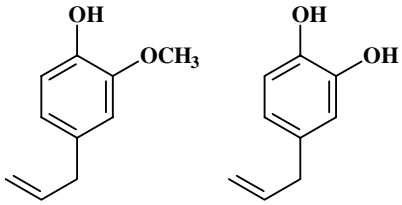

Fig. 1. Chemical structures of chavibetol and allylpyrocatechol, the major phenolic compounds of PE.

3.2. Prevention of photosensitization-induced lipid peroxidation by PE

The protective capacity of PE against UV photo-induced peroxidation of rat liver mitochondria was studied by assaying the TBARS, LOOH and CD formed during the lipid peroxidation. Our studies showed that exposure of mitochondria to methylene blue plus light-induced photosensitization enhanced formation of TBARS as a function of illumination time (data not shown). Exposure of mitochondria to UV irradiation for 0.5 h showed significant increase in TBARS (12.44 ± 0.1 nmol/mg protein) as against the control value (2.95 ± 0.04 nmol/mg protein) without photo-exposure.

When the mitochondria were irradiated for 0.5 h in the presence of various concentrations of PE, significant protection was observed. Fig. 2a demonstrates photosensitization-induced formation of TBARS in rat liver mitochondria and its possible prevention by PE. A replot of the data (Fig. 2b) reveals the percentage of protection offered by PE against photo-induced mitochondrial lipid peroxidation. At a low concentration (50 μg/ml), PE showed measurable ($\sim$17%) prevention in TBARS formation, which increased in a concentration-dependent manner. For example, the protection offered by 75 and 100 μg/ml of PE was $\sim$24% and 40%, respectively. The protection offered (for a 30 min photo-exposure) by various antioxidants under similar conditions is shown in Table 2. Typically, the protection offered by sodium azide (10 mM), a singlet oxygen ($^1O_2$)quencher, was 76%.

Photo-irradiation of the rat liver mitochondria expectedly led to the increased formation of LOOH and CD,

Table 1
Chemical composition of PE

Mobile phase	Compound (R_f)	Concentration (%)[a]
2:8 ethyl acetate:hexane	Chavibetol (0.55)	0.09
2:8 ethyl acetate:hexane	Allylpyrocatechol (0.24)	0.51
3:3:4 toluene:acetone:formic acid	Chavibetol glycoside (0.24)	0.23
3:3:4 toluene:acetone:formic acid	Allylpyrocatechol glycoside (0.24)	0.52

[a] Based on the weight of *Piper betel* leaves.

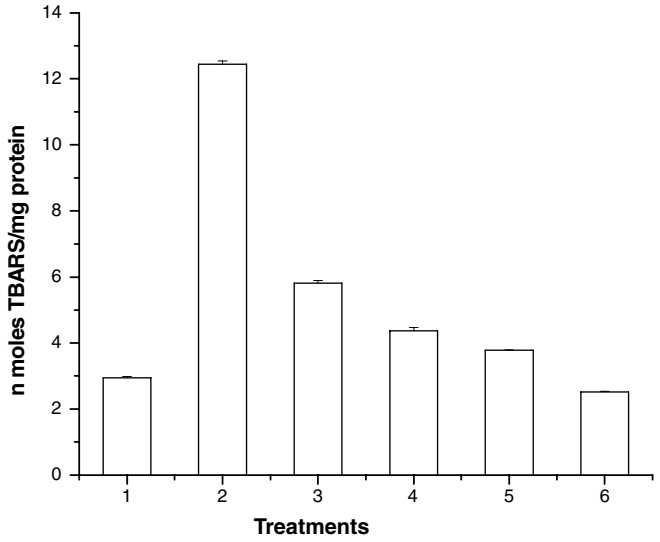

Fig. 2a. Photosensitization-induced formation of TBARS in rat liver mitochondria and prevention by PE and sodium azide. A mixture of mitochondrial protein (0.5 mg/ml), methylene blue (50 μg/ml) without and with PE (50–100 μg/ml) was incubated for 30 min and the amounts of TBARS were estimated. 1: control, 2: without PE, 3–5: in the presence of PE (50, 75 and 100 μg/ml respectively), 6: sodium azide (10 mM). The values are mean ± SE ($n = 5$).

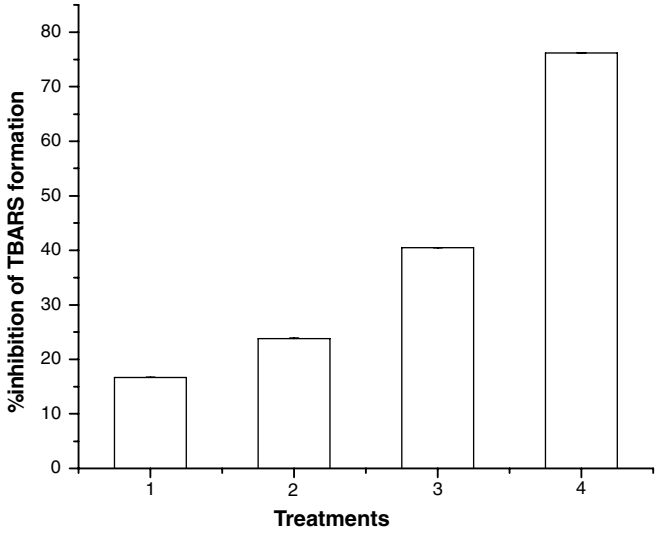

Fig. 2b. Concentration dependent protective activity of PE against photosensitization-induced formation of TBARS in rat liver mitochondria. A mixture of mitochondrial protein (0.5 mg/ml), methylene blue (50 μg/ml) without and with PE (50–100 μg/ml) was incubated for 30 min and the amounts of TBARS were estimated. 1: PE (50 μg/ml), 2: PE (75 μg/ml), 3: PE (100 μg/ml), 4: sodium azide (10 mM). The values are mean ± SE ($n = 5$).

which, however, were prevented by PE. Following photo-irradiation for 0.5 h, the LOOH concentration increased more than two-fold. This was revealed from the increase in the absorbance at 560 nm from 0.034 ± 0.002 (unirradiated control) to 0.07 ± 0.004 (after irradiation). Addition of PE (50 μg/ml) to the mitochondria prior to photo-irradiation could inhibit the formation of LOOH by

Table 2
Relative protective activities[a] of PE and other antioxidants against photo-induced oxidation lipids and proteins

Antioxidant (concentration)	% Protection[b] against lipid peroxidation	% Protection[b] against protein carbonyl formation
PE (50 μg/ml)	16.8 ± 0.08	63.9 ± 3.22
Mannitol (100 mM)	19.9 ± 1.06	1.5 ± 0.01
Glutathione (10 mM)	31.8 ± 3.21	52.3 ± 5.21
Ascorbic acid (10 mM)	27.1 ± 3.10	56.5 ± 4.88
Sodium azide (10 mM)	76.0 ± 2.14	53.4 ± 5.09
SOD (1000 unit/assay)	8.1 ± 0.05	25.1 ± 4.12

[a] The values are for photo-irradiation carried out for 30 min.
[b] The values are mean ± SE ($n = 5$).

70.2 ± 0.46%. Similarly, the level of CD was also significantly enhanced by photo-irradiation (0.5 h), as evident from the increase in absorbance at 330 nm from 0.04 ± 0.01 to 0.3 ± 0.04. This was, however, reduced by 36 ± 1.01% in the presence of PE (50 μg/ml). Under similar conditions, the positive control, sodium azide, (10 mM) offered 52.53 ± 0.9% and 16 ± 0.02% protection against the formation of LOOH and CD respectively.

3.3. Prevention of photosensitization-induced SOD depletion by PE

Fig. 3 shows data on the inactivation of SOD in rat liver mitochondria by photosensitization and its prevention by PE. The damaging effect was rapid and time-dependent. For example, compared to the control value (6.85 ± 0.31 units/mg protein) the mitochondrial SOD activity was reduced to 1.77 ± 0.11 and 0.85 ± 0.015 units/mg protein on photo-irradiation for 30 and 60 min, respectively. Thus,

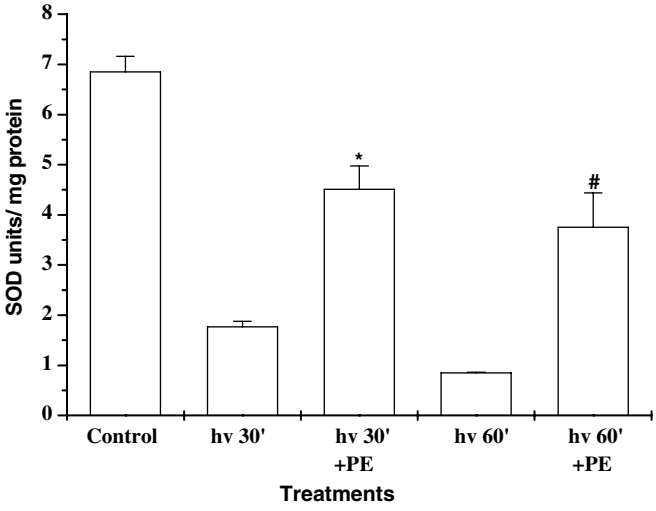

Fig. 3. Photosensitization-induced depletion of superoxide dismutase (SOD) in rat liver mitochondria and its restoration by PE. Mitochondria (0.5 mg protein/ml) were exposed to light (100 W) in the presence of methylene blue and oxygen for 30 and 60 min without and with PE (50 μg/ml), and the activity of SOD was measured. The values are mean ± SE ($n = 5$) $^{*}P < 0.001$, as compared to photosensitization at 30 min. $^{\#}P < 0.01$, as compared to photosensitization at 60 min.

the percentages of inactivation observed at 30 and 60 min were significantly high (74.2 and 87.6 respectively). Addition of PE (50 μg/ml) to the mitochondria prior to its photo-irradiation could restore the SOD activity substantially. The SOD activities of the PE-treated mitochondria after photo-irradiation for 0.5 and 1.0 h were 4.51 ± 0.47 (66% restoration) and 3.75 ± 0.69 (55% restoration) units/mg protein, respectively.

3.4. Prevention of photosensitization-induced protein carbonyl formation by PE

Fig. 4 demonstrates the photosensitization-induced formation of mitochondrial protein carbonyls and its inhibition by PE. Our studies revealed that photosensitization also induced formation of protein carbonyls, an index of protein oxidation, in a time-dependent manner. Thus, photosensitization for 0.5 h increased (~5 times) the amounts of protein carbonyls from the control value 1.31 ± 0.39 to 6.7 ± 0.35 nmol/mg protein, which was reduced significantly (78%) to 2.5 ± 0.45 by PE. Likewise, the photosensitization for 1.0 h increased the amounts of protein carbonyls by ~7.2 times, which was considerably reduced (46%) by PE. The extents of reduction in protein carbonyls formation (for a 30 min photo-exposure) in the presence of various antioxidants are shown in Table 2. Typically, the positive control ascorbic acid (10 mM) showed 56.5% prevention of protein carbonyls formation for a photo-exposure of 30 min.

3.5. Prevention of Fe(II)-induced lipid peroxidation by PE

Fig. 5 shows the preventive potential of PE against Fe(II)-induced lipid peroxidation in rat liver mitochondria.

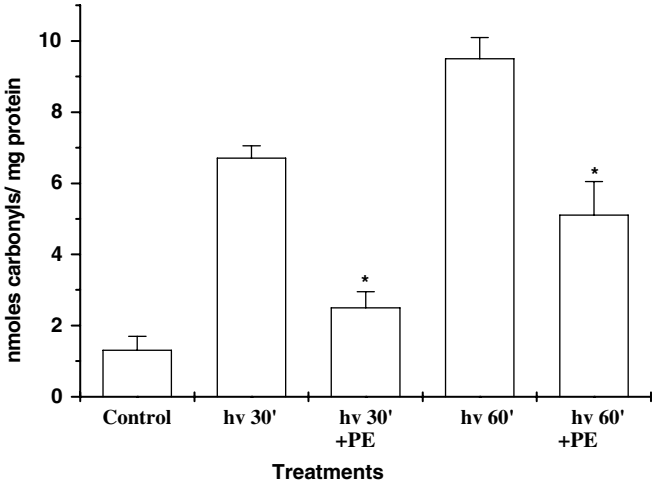

Fig. 4. Photosensitization-induced protein oxidation in rat liver mitochondria and its prevention by PE. Mitochondria (0.5 mg protein/ml) were exposed to light (100 W) in the presence of methylene blue and oxygen for 30 and 60 min without and with PE (50 μg/ml) and formation of protein carbonyls was measured. The values are mean ± SE ($n = 5$). *$P < 0.001$ as compared to photosensitization at 30 and 60 min.

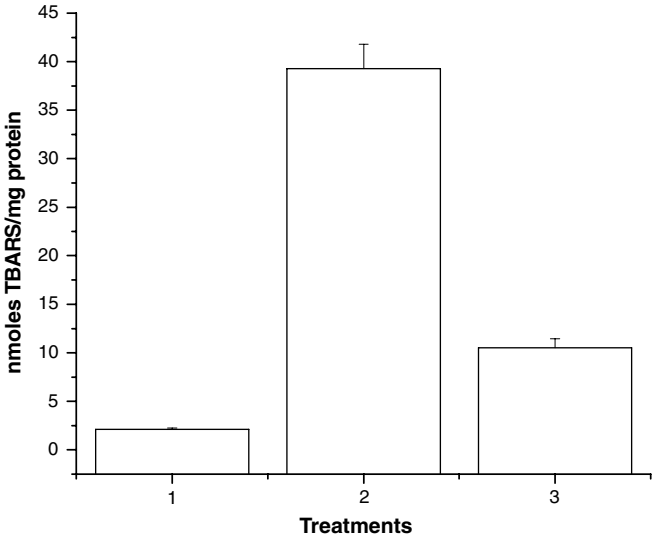

Fig. 5. Fe (II)-ascorbic acid induced formation of TBARS in rat liver mitochondria and its prevention by PE. A mixture of mitochondrial protein (2 mg/ml), Fe(II) (50 μM) and ascorbic acid (200 μM) without and with PE (50 μg/ml) was incubated for 30 min and the amounts of TBARS were estimated. 1: control, 2: in the presence of Fe(II) and ascorbic acid, 3: in the presence of Fe(II), ascorbic acid and PE. The values are mean ± SE ($n = 5$).

Treatment of rat liver mitochondria with Fe(II) (50 μM) and ascorbic acid (200 μM) led to significant lipid peroxidation, as revealed from the TBARS value, which increased from the control value 2.1 ± 0.15 to 39.3 ± 2.5 nmol/mg protein. PE (50 μg/ml) could significantly reduce the formation of TBARS (10.5 ± 0.95 nmol/mg protein) in rat liver mitochondria. Thus, PE also offered significant protection (77.4%) against Fe(II)-induced lipid peroxidation. Under similar conditions, the positive control, α-tocopherol, prevented lipid peroxidation with an IC_{50} value of 4.27 ± 0.44 μg/ml.

3.6. Singlet oxygen scavenging activity of PE

The luminescence of singlet oxygen at 1270 nm decreased progressively in the presence of increasing concentrations of PE, as shown in Table 3. The effect of PE was reduced below a concentration of ~47 μg/ml, but, increased at higher concentrations. Since PE is a mixture of several organic compounds, we did not determine the rate constant for its reaction with the singlet oxygen. However, a plot of the inverse of the lifetime of singlet oxygen against the concentration of PE was linear. Under similar

Table 3
Concentration-dependent singlet oxygen quenching activities of PE

Concentration of PE (μg/ml)	Lifetime of singlet oxygen (s)[a]
0 (control)	6.2×10^{-5}
46.9	5.9×10^{-5}
273	5.1×10^{-5}
546	4.4×10^{-5}

[a] The values are mean of two experiments.

conditions, the rate constant for the positive control, sodium azide, was 2.41×10^8 mol^{-1} s^{-1}.

4. Discussion

Photosensitization is a widely-occurring phenomenon in biological systems, due to the ubiquitous nature of visible light and a number of pigments and related compounds, which can act as photosensitizers (Black, 1987). Even endogenous compounds have been reported to act as photosensitizers. Photosensitization might involve type I (superoxide radicals) and type II (singlet oxygen, $^1O_2$) processes, leading to damaging consequences (Foote, 1991; Sies, 1986). Singlet oxygen is an electronically excited species of oxygen produced in mammalian cells under various normal and pathophysiological conditions (Kanofsky, 1989). Due to its relatively long half-life in the range of 10–50 μs, it is capable of travelling appreciable distance in the cellular milieu causing damage to membrane lipids, DNA, etc. (Epe, 1991). Consequently, in the present study, the capacity of PE in preventing photosensitization-induced lipid peroxidation and protein damage were assessed.

For this, the rat liver mitochondria was used as a model source of lipids and proteins. Using methylene blue as the sensitizer, the extent of oxidation of lipids and proteins by photosensitization was investigated in the absence and presence of PE. The combination of methylene blue, light and oxygen mainly generates $^1O_2$ and can induce oxidative damage in membrane (Valenzeno & Tarr, 1991).

Membrane damage has been considered as a crucial event contributing to cytotoxicity of a photo-sensitizer. Polyunsaturated fatty acids, present in cellular membranes, are especially prone to ROS-mediated damage, resulting in drastic alteration of membranes, as well as generating toxic metabolites, capable of acting at sites far away from the sites of their generation. The induced alterations in membrane permeability, transport systems, loss of membrane-bound enzymes, etc., can eventually lead to cell lysis and death under specific conditions (Valenzeno & Tarr, 1991). Adverse effects of photosensitization to specific carriers for succinate, citrate and oxalacetate have been reported (Salet & Moreno, 1990).

In view of this, the capacity of PE to prevent the photosensitization-induced lipid peroxidation in rat liver mitochondria was studied by measuring the amounts of TBARS formed in its absence and presence. It was found that PE could effectively inhibit the TBARS formation in a concentration-dependent manner. Given that the TBA assay is fairly non-specific (Knight, Pieper, & McClellan, 1988), the protection offered by a fixed concentration of PE (50 μg/ml) was also assayed by measuring LOOH and CD, two relatively unstable products of lipid peroxidation. Addition of PE to the mitochondria prior to photo-irradiation could inhibit the formation of CD and LOOH very effectively. The results obtained from these experiments were better than that from the TBA assay.

Lipid peroxidation is a complex multistep process, wherein the initially formed lipid radicals are converted to TBARS, via intermediate CD and LOOH. PE could efficiently prevent photosensitization-induced lipid peroxidation at its various stages as is revealed from our results. However, the protective effect of PE against ionizing radiation is significantly higher (Bhattacharya et al., 2005) than that against photosensitization. This may be attributed to its lower reactivity with $^1O_2$, that is revealed from our data.

Protein oxidation is inherent to aerobic life. Oxidation of membrane proteins by ROS, a process independent of lipid peroxidation, is also a highly damaging event, the significance of which has been realized recently (Dean, Fu, Stacker, & Davies, 1997; Stadtman, 1992). Activated oxygen species and other free radicals, generated as by-products of cellular metabolism or by photochemical reaction, modify amino acids of proteins. Subsequently loss of protein structure and function can occur through denaturation, fragmentation and aggregation. It has been established that most amino acids are susceptible to oxidation by $^{\bullet}OH$ or $O_2^{\bullet-}$ radicals (Davies, 1987). Once oxidized, proteins are degraded by the proteosome complex and by lysosomal hydrolases. Alternatively, they can be repaired by antioxidants.

In the present studies, the protective activities of PE against photosensitization-induced protein oxidation of rat liver mitochondria was investigated in terms of protein carbonyl formation and SOD inactivation. Conversion of proteins into oxidized species (e.g., oxyacids) is one of the earliest observable events during the radical-mediated oxidation of proteins (Mukhopadhyay & Chatterjee, 1994). Likewise, oxidative inactivation of SOD is one of the most damaging events in oxidative stress, as the enzyme plays a crucial role in the cellular antioxidant defense mechanism (Hassan & Fridovich, 1981).

The increase in the formation of protein carbonyls, the products of protein oxidation, as well as inactivation of SOD, as observed in our studies, confirmed the potential deleterious effects of photsensitization. PE effectively prevented these damages even at a low concentration (50 μg/ml). The restoration of the SOD level can partly explain the observed protection against lipid peroxidation and protein oxidation. However, the protective effect of PE against oxidative damage of proteins was less pronounced when photo-irradiation was carried out for a longer period (1.0 h).

As discussed earlier, $^1O_2$ is the major oxidative species generated during photosensitization, especially in a system containing methylene blue and oxygen. Given that sodium azide, a specific $^1O_2$ scavenger, could prevent the photosensitization-induced damages to lipids and proteins, the involvement of $^1O_2$ in the process was apparent. Our studies on the $^1O_2$ scavenging activity of PE also confirmed this.

Besides $^1O_2$, the photsensitization-induced damages can also be mediated through various free radicals, including the superoxide radicals (produced in the type I process) and/or due to iron release (Haliwell & Gutterridge, 1989).

Earlier we have found (Bhattacharya et al., 2005) that PE can effectively scavenge superoxide radicals. The present studies established its efficacy in preventing iron-mediated lipid peroxidation. All of these factors might account for its protective activity against photosensitization.

The phenolic compounds such as chavibetol and 4-allylpyrocatechol found as the principal constituents of PE which might contribute to its protective activity. Earlier, we have found that PE does not show any toxic effect in rats, up to a dose of 3 g/kg body weight (Majumdar et al., 2002, Majumdar, Roy Chaudhury, Roy, & Bandyopadhyay, 2003). In these studies, it was also found that the antioxidant activity of the phenolics present in PE is retained even in the animal model. The present studies revealed their high potency as an in vitro antioxidant against photosensitization-induced cellular damage in rat liver mitochondria. It can protect against oxidative damage to both lipids and proteins. The results, taken together, suggest a possible use of PE against photosensitization-induced oxidative biological damage. Given that a large population of the Eastern World extensively consume the leaves of the *P. betel* plant without any toxic effect (Press note), PE appears to be a promising formulation for further investigation as a new natural photo-protector.

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