

# Antioxidant activity of capsaicin on radiation-induced oxidation of murine hepatic mitochondrial membrane preparation

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**Abstract:** Capsaicin is the major capsaicinoid in chili peppers and is widely used as a spice. It is also used for topical applications in cases of peripheral neuropathy. The present study deals with its role in modulation of gamma radiation-induced damages of the biochemical constituents of rat liver mitochondrial membrane (RLM) preparation. The extent of lipid hydroperoxide formation, depletion in protein thiols, and formation of protein carbonyls have been biochemically assessed in the presence of varying concentrations of capsaicin in RLM. Decrease in the activities of the important antioxidant enzyme superoxide dismutase, which is involved in the scavenging of free radicals, and the mitochondrial marker enzyme succinate dehydrogenase have been also looked into. Capsaicin has been found to efficiently inhibit radiation-induced biochemical alterations, namely lipid peroxidation and protein oxidation. It also significantly prevented radiation-induced loss in the activity of antioxidant enzyme and the important endogenous antioxidant glutathione. The study suggests that capsaicin can act as an antioxidant and radioprotector in physiological systems.

**Keywords:** capsaicin, gamma radiation, radioprotection, lipid peroxidation, protein oxidation, enzyme activity

## Introduction

Phenolic compounds are an important group of secondary metabolites, which are synthesized by plants in the process of the plant's adaptation to biotic and abiotic stress conditions.<sup>1,2</sup> Taking a cue from nature, the same phenolic compounds present in food and medicines are also considered as powerful antioxidants. These compounds can protect the different biochemical components of the human body from oxidative stress conditions, producing highly reactive free radicals by scavenging them and inhibiting oxidative chain reactions. The natural metabolism of aerobic cells is associated with controlled formation of free radicals that are removed quickly and systematically by antioxidant defense system (enzymes and antioxidants). However, uncontrolled formation by exposure to ionizing radiation, chemicals, and disease conditions (inflammation, cancer, etc) can cause oxidative stress leading to damage and, ultimately, death of the cells.<sup>3,4</sup> Many synthetic chemicals are known to inhibit oxidative damage to different extents, but they are also associated with unfavorable side effects. Therefore, investigation on free radical-scavenging aspects of antioxidant drugs made from natural food constituents has become not only very important but also essential due to their nontoxic nature, everyday intake through food, and favorable interaction with various free radicals in the living cells generated both from exogenous sources and in endogenous metabolic reactions.<sup>5-7</sup>

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In this context, capsaicin (*trans*-8-methyl-*N*-vanillyl-6-nonenamide) present in chili fruits, which gives pungent flavor, is an important phenolic food constituent. Capsaicin (Figure 1) is present in several varieties of chili powders, with concentrations ranging from ~0.3% to ~2% wt/wt.<sup>8</sup> In addition to being a food constituent, it is soluble in both aqueous (~40  $\mu$ M) and lipid phase ( $\log P = 3.04$ ), has low toxicity ( $LD_{50}$  [median lethal dose]  $\geq 40$  mg/kg), high stability ( $pK_a = 9.75$ ), and high bioavailability in different tissues which peaks at different times after oral administration (~3  $\mu$ M to 36  $\mu$ M).<sup>9-11</sup>

Capsaicin is reported to show a wide range of pharmacological properties, including antimutagenic, anticarcinogenic,<sup>12-15</sup> and antioxidant activities;<sup>16-22</sup> DNA protection against strand breaks and inhibition of chromosomal aberrations;<sup>23</sup> protection of tissues against free radical-mediated damage induced by exogenous chemicals;<sup>24</sup> inhibition of generation of reactive oxygen species;<sup>25</sup> and induction of apoptosis.<sup>26,27</sup>

There are many studies using chemical, *in vitro*, and animal models indicating its strong antiradical activities.<sup>12-38</sup> The peroxy radical-scavenging ability of capsaicin is reported to be greater than that of both melatonin and caffeine.<sup>16,21</sup> It was also shown to have antioxidant activity similar to that of flavonoids and  $\alpha$ -tocopherol.<sup>18,28</sup> Further, capsaicin is also suggested to interact with xenobiotic metabolizing enzymes, for instance, microsomal P450-dependent monooxygenases. Capsaicin is assumed to convert to a phenoxy radical intermediate through catalysis by hepatic cytochrome P450 2E1. This capsaicin phenoxy radical is a highly reactive species and can chemically bind not only to the active site of the enzyme in a covalent manner but also with other biologically active macromolecules in the cells. It is proposed that suicidal inhibition of microsomal cytochrome P450 may prevent subsequent activation of chemical carcinogens, mutagens, and other toxic xenobiotics. Some studies indicate such chemopreventive roles of capsaicin against some of the chemical carcinogens and mutagens.<sup>12,39,40</sup>

It is known that free radicals produced during the natural metabolism of aerobic cells and stimulus (chemicals, ionizing radiation, etc)-induced oxidative stress can also be produced using gamma radiation. These free radicals react

nonselectively with proteins, lipids, and others molecules in the vicinity due to their high reactivity and oxidize them to nonfunctional/deleterious products. In this context, the reported antiradical and antioxidant activity of capsaicin prompted us to investigate its protective effects against gamma radiation-induced oxidative damages in different components of mitochondrial preparation, namely, lipids, proteins, and enzymes. Mitochondrial preparation has been used as a model because it is the powerhouse of the cell, is involved in important electron transport event, and in the generation and breakdown of highly reactive oxygen species.

## Materials and methods

### Chemicals

Capsaicin (*trans*-8-methyl-*N*-vanillyl-6-nonenamide) was procured from Sigma Chemicals (Perth, Australia). All other chemicals were of analytical grade. Solutions were prepared in Nanopure water from the Barnstead cartridge filtration system.

### Animals used

Female Wistar rats (about 3–4 months old) housed in temperature- and humidity-controlled room ( $24^{\circ}\text{C} \pm 2^{\circ}\text{C}$ ) with a 12-hour light/dark cycle and fed the standard laboratory diet and water *ad libitum* were used. The guidelines issued by the Institutional Animal Ethics Committee of the Bhabha Atomic Research Centre, (Mumbai, India), regarding the maintenance and dissections of small animals were strictly followed.

### Mitochondria isolation from rat liver and irradiation

Female Wistar rats (~14 weeks old) of about 250–300 g have been used for the liver mitochondrial membrane preparation. The rats were sacrificed by cervical dislocation (without anesthesia) with due and proper permission and recommendation from the animal ethics committee. The liver was excised and homogenized in 0.25 M cold sucrose containing 1 mM EDTA, then centrifuged at  $3,000 \times g$  for 10 min, and the supernatant was centrifuged at  $10,000 \times g$  for 10 min to sediment mitochondria. This pellet containing broken mitochondria was washed thrice with 5 mM potassium phosphate buffer, pH 7.4, to remove sucrose, and samples were maintained at  $4^{\circ}\text{C}$  throughout.<sup>41</sup> Lowry's<sup>42</sup> method was used for protein estimation in the samples, (10 mg/mL) which were then stored for subsequent use.

Rat liver mitochondrial membrane (RLM) samples in the presence and absence of capsaicin were exposed to gamma ( $\gamma$ ) rays from a  $^{60}\text{Co}$  source to cause radiation-induced

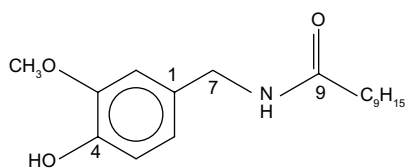


Figure 1 Structure of capsaicin.

oxidative damage. It is known that gamma radiation exposure causes damage to lipids and proteins, producing oxidized products such as lipid hydroperoxides, aldehydes, protein carbonyls, and oxidized thiol. The radiation-induced products of lipids and proteins were measured using different standard assays to understand the effect of capsaicin on the extent of damage and compared with the control set.

## TBARS assay

Thiobarbituric acid reactive substances (TBARS) assay was performed by the standard method to measure malondialdehyde and other aldehydes, which are products of lipid peroxidation (LP). One aldehyde molecule condenses with two molecules of thiobarbituric acid (TBA) to produce a pink-colored species that absorbs at 532 nm. The RLM were heated with TBA reagent (TBA–TCA–HCl–EDTA) for 20 min in a boiling water bath. The solution was cooled and then centrifuged, and the absorbance of supernatant was recorded at 532 nm.<sup>43,44</sup>

## Lipid hydroperoxide

Lipid hydroperoxides were estimated by the modified FOX (ferrous oxidation in xylenol orange) method.<sup>45</sup> Hydroperoxides oxidize  $\text{Fe}^{2+}$  to  $\text{Fe}^{3+}$  under acidic conditions, and xylenol orange (dye) complexes with an equal molar concentration of  $\text{Fe}^{3+}$  to produce a blue-purple colored complex with an apparent extinction coefficient of  $1.5 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$  at 560 nm. FOX reagent consists of two solutions, solution A comprises of 98 mg ammonium ferrous sulfate, 100 mL 250 mM  $\text{H}_2\text{SO}_4$ , and 79 mg xylenol orange, and solution B comprises of 969 mg butylated hydroxytoluene in 900 mL methanol, mixed in 1:9 ratio (stored in dark). About 875  $\mu\text{L}$  of FOX reagent was added to 125  $\mu\text{L}$  of the reaction mixture and incubated at 37°C for 30 min. It was centrifuged at  $10,000 \times g$  for 15 min at 20°C, and the absorbance was measured at 560 nm.

## Protein thiol

In this assay, RLM was suspended in 14% perchloric acid, centrifuged at  $4,500 \times g$  for 5 min, and the pellet was suspended in 7% perchloric acid and again centrifuged at  $4,500 \times g$  for 5 min. To the pellet were added, 10% Triton X-100, 0.2 M potassium phosphate buffer (pH 7.4). Subsequently, addition of 2 mM 5,5'-dithiobis-(2-nitrobenzoic acid) or DTNB in the buffer was followed by measurement of absorbance at 412 nm after incubation for 5 min in the dark.<sup>46,47</sup>

## Protein carbonyl assay

This assay involves the reaction of carbonyl groups with 2,4-dinitrophenylhydrazine (DNPH) to form a 2,4-dinitrophenylhydrazone, which was estimated spectrophotometrically

at 366 nm. The experimental tubes and the corresponding blank set of tubes were treated with 20% TCA, vortexed, chilled on ice, and followed by centrifugation at 2,000 rpm for 20 min. The pellets in the blank set of tubes were treated with 2 mL of 2 N HCl, whereas the pellets in experimental tubes were treated with 2 mL of 10 mM DNPH in 2 N HCl. All the tubes were incubated at room temperature for 1 h followed by addition of 20% TCA to the tubes. The contents of the tubes were precipitated as pellets, washed thrice with a mixture of ethanol and ethyl acetate (1:1) to remove excess DNPH, and then the pellets were dried and dissolved in 6 M guanidine hydrochloride at 37°C for 20 min. The absorbance was read at 366 nm. The amount of carbonyls formed was calculated from the difference in absorbance between the blank and the corresponding experiment tube.<sup>48</sup>

## Superoxide dismutase

The epinephrine method was used for superoxide dismutase (SOD) measurement. One mL of reaction mixture contained 50 mM sodium carbonate buffer (pH 10.0), 25  $\mu\text{L}$  of 20 mM epinephrine in 0.1 N HCl, and about 20  $\mu\text{g}$  of protein in the enzyme sample. In the blank cuvette, the same amount of enzyme and buffer were taken, except epinephrine. Absorbance was recorded at 320 nm for 6 min. The activity was calculated using the difference between absorbance of standard and absorbance of enzyme and is expressed as units/mg protein.<sup>49</sup>

## Succinate dehydrogenase

Succinate dehydrogenase (SDH) possesses dye reductase properties and is the only membrane bound enzyme in citric acid cycle. Phenazine methosulphate and 2,6-dichlorophenolindophenol are electron acceptor dyes in the assay.<sup>50</sup> In this assay, the reaction mixture containing RLM is mixed with 0.1 M potassium phosphate buffer (pH 7.4) and 0.1 M sodium cyanide and incubated at 37°C for 10 min. Sodium cyanide completely inhibits oxygen consumption in respiring cells and thus fairly prevents the loss of succinate by oxidation. The contents were split into two cuvettes followed by the addition of 2 mM phenazine methosulphate and 50  $\mu\text{M}$  2,6-dichlorophenolindophenol. Addition of 50  $\mu\text{L}$  of 0.4 M succinate to the experimental cuvette marks the start of the reaction, whereas 50  $\mu\text{L}$  of deionized water was added in the blank cuvette. Absorbance was recorded at 600 nm for 6 min. SOD concentration is given in terms of “units”, where one unit SOD causes 50% inhibition of auto-oxidation of epinephrine.

## Total reduced glutathione (GSH) assay

About 60  $\mu\text{L}$  of *O*-phosphoric acid was added to RLM and centrifuged. The supernatant was then mixed with 0.1 M sodium phosphate buffer, 0.05 M EDTA (pH 8.0), and 100  $\mu\text{L}$  *O*-phthaldehyde (1 mg/mL) and incubated at room temperature for 15 min to measure GSH levels. Fluorescence was then recorded using an excitation wavelength of 350 nm and an emission wavelength of 420 nm.<sup>51,52</sup>

## Statistical analysis

The statistical significance of the difference in the parameters between the capsaicin-containing samples and the corresponding controls was tested. The measured values in different assays from six sets of experiments are reported as mean  $\pm$  standard error of mean. Analysis of variance was performed using "Origin6.1-Scientific Graphing and Data Analysis Software". The level of statistical significance  $P < 0.05$  is shown as \* in Figures 2–5.

## Pulse radiolysis

Electron beam irradiation with 7 MeV pulses of 100 ns duration was used in the present work for in situ generation and study of transient species that was described earlier.<sup>53</sup> Kinetics of the reactions were studied by observing formation or decay of the concerned transient's absorption with time at different concentrations of sample.<sup>54</sup> Mechanism of reaction and kinetics of transients were studied by observing absorption at different wavelengths at different time points after irradiation.

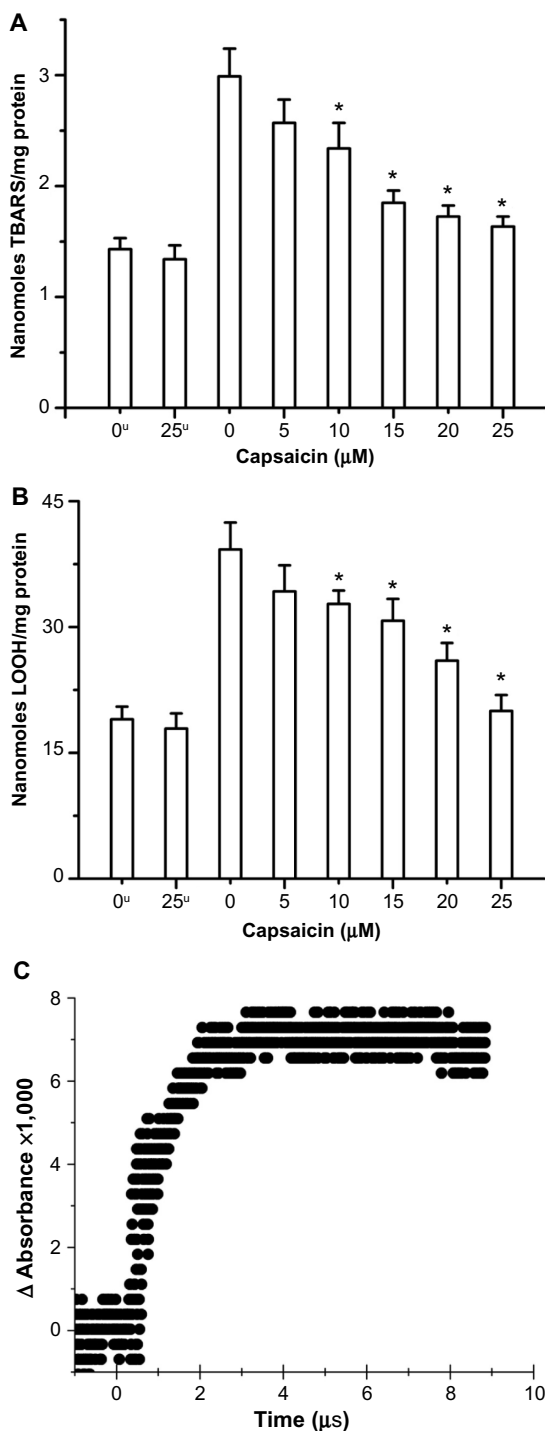
## Results and discussion

High-energy gamma radiation nonselectively deposits energy in the medium to produce oxidizing and reducing free radicals. Water-derived free radicals are produced in the physiological system on exposure to ionizing radiation since water is the major component. As the dose of radiation is increased, the extent of damage produced also increases and then reaches a plateau. Therefore, the radiation dose before the attainment of plateau has been chosen to study the influence of capsaicin on the radiation-induced oxidative damage. The effect of capsaicin has been investigated in terms of its ability to impart protection to isolated RLM preparation from gamma radiation-induced damages to lipids, proteins, antioxidant enzyme (SOD), and SDH.

In the present work, RLM has been used as an in vitro model of the biological system and not as a model of the living organism. The dose selected for the present study (450 Gy) produces  $\sim 270 \mu\text{M}$  (calculated) primary free

radicals which can cause sufficient damage in the RLM to be measured conveniently and explicitly. The objective of the present study is to get radiation damage in the model system and not to mimic the conditions of cancer treatment. Further, capsaicin concentrations of up to 50  $\mu\text{M}$  have been used to study the protective effect of capsaicin against oxidative damage induced by  $\sim 270 \mu\text{M}$  primary free radicals. The effect of capsaicin on the studied parameters (without gamma irradiation) has been also shown in the figures.

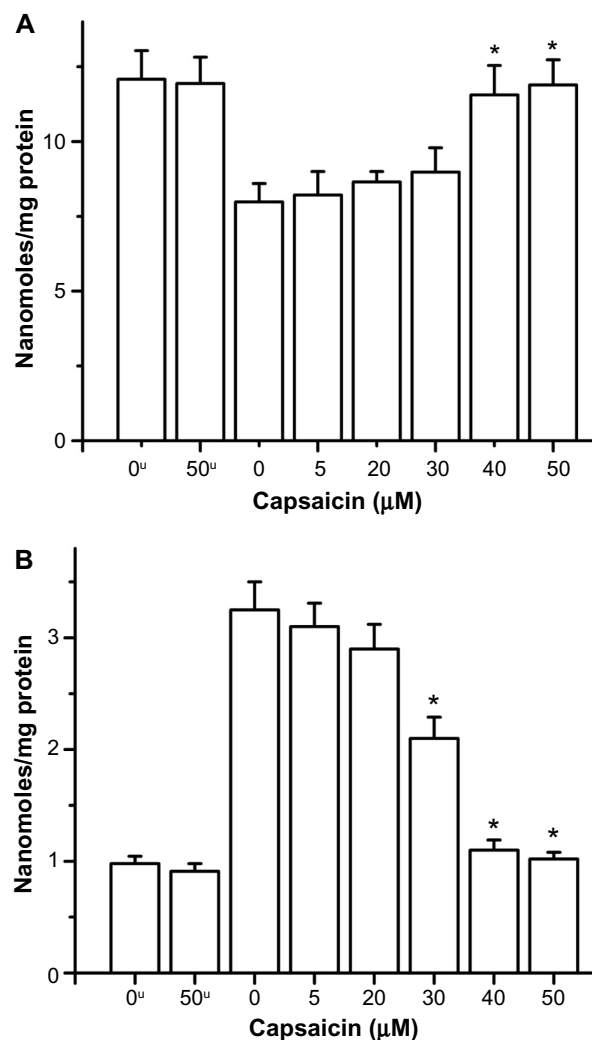
Gamma radiation-induced damage to RLM components has been measured using standard assays. LP is defined as an oxidative deterioration of polyunsaturated fatty acids involving free radical-mediated chain reaction. Conjugated dienes, lipid hydroperoxides, aldehydes, and ketones are oxidation products of LP. These are reasonably stable molecules at physiological temperatures and can be estimated using different standardized assays. Capsaicin in the range of 5–25  $\mu\text{M}$  imparted protection to RLM in terms of decrease in formation of radiation-induced TBARS and lipid hydroperoxides. Capsaicin has shown significant protection for lipids even at 5  $\mu\text{M}$  concentration, and this protection further increased linearly with concentration. Lipids (LH) are known to react with oxidizing hydroxyl radical ( $\cdot\text{OH}$ ) to produce carbon-centered radical by: 1) hydrogen atom abstraction, and 2) addition to unsaturation. These lipid radicals ( $\text{L}\cdot$ ) react with oxygen to produce lipid peroxy radical ( $\text{LO}_2\cdot$ ), which initiates chain reaction causing lipid damage. The presence of capsaicin during irradiation significantly inhibited the formation of these lipid oxidation products in RLM (Figure 2A and B) in a concentration-dependent manner. The  $\text{IC}_{50}$  value has been found to be around 11.3 and 16.7  $\mu\text{M}$  for TBARS and LOOH (linoleic acid hydroperoxide) assays, respectively. We have also studied direct scavenging of  $\text{LO}_2\cdot$  with capsaicin, using pulse radiolysis technique. Capsaicin is known to react with oxidizing radicals to produce transient with absorption band at 380 nm.<sup>55</sup> High concentration of lipid (linoleic acid, 11.5 mM) has been reacted with hydroxyl radical (8.4  $\mu\text{M}$ ) formed in situ in the presence of air (0.25 mM oxygen) and low concentrations of capsaicin at pH 11. Capsaicin has been found to scavenge lipid peroxy radical with a high bimolecular rate constant of  $3.3 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$  (Figure 2C). This is a limiting value obtained with 0.11 mM capsaicin due to 1) low absorption signal of capsaicin radical at 380 nm at lower concentrations and 2) saturation at higher capsaicin concentrations. The measured lipid peroxy radical scavenging rate constant ( $3.3 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ ) at pH 11 is higher than that reported with  $\text{CCl}_3\text{O}_2\cdot$  ( $2.0 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ ) and  $\text{CHCl}_2\text{O}_2\cdot$



**Figure 2** Radiation-induced increase in (A) TBARS and (B) lipid hydroperoxide and its restoration by capsaicin in RLM (C) lipid peroxy radical scavenging by capsaicin. **Notes:** \* $P < 0.05$ . <sup>u</sup>Represents unirradiated. **Abbreviations:** TBARS, thiobarbituric acid reactive substances; LOOH, linoleic acid hydroperoxide; RLM, rat liver mitochondrial membrane.

( $1.2 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ ) at pH 9.4.<sup>55</sup> This agrees well with the fact that oxidation rate of any chemical increases significantly above its  $pK_a$  value (capsaicin  $pK_a = 9.75$ ).

Gamma radiation-induced depletion of protein thiols in RLM has also been restored toward basal level (Figure 3A)

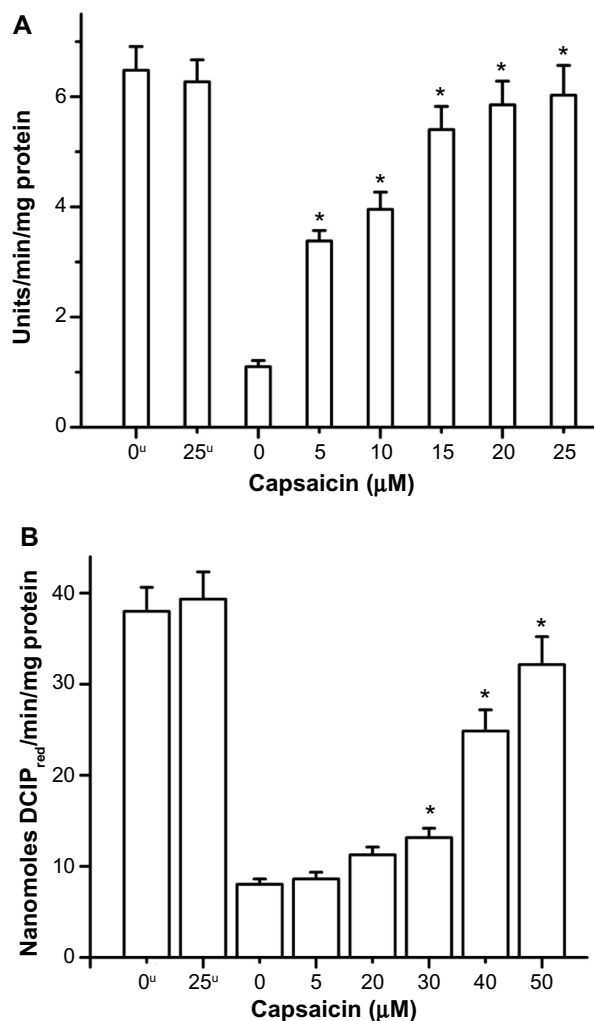


**Figure 3** Radiation-induced (A) depletion of protein thiols and (B) increase in protein carbonyls and their restoration by capsaicin in RLM. **Notes:** \* $P < 0.05$ . <sup>u</sup>Represents unirradiated. **Abbreviation:** RLM, rat liver mitochondrial membrane.

by the presence of 40  $\mu\text{M}$  capsaicin during exposure. Capsaicin protected protein thiol in the concentration range of 5–30  $\mu\text{M}$ , but the effect is more significant in the concentration range of 30–40  $\mu\text{M}$ . Similarly, the presence of capsaicin decreased radiation-induced formation of protein carbonyl in RLM, and the effect is significant in the concentration range of 20–40  $\mu\text{M}$  (Figure 3B).

Capsaicin also imparted protection to the antioxidant enzyme SOD from radiation-induced oxidative damage, which has been measured in terms of enzyme activity. The radioprotective action of capsaicin toward SOD has been found to be significant, even at 5  $\mu\text{M}$ , and increased linearly from 5 to 25  $\mu\text{M}$  (Figure 4A). The presence of capsaicin also inhibited the gamma radiation-induced loss of activity of the mitochondrial marker enzyme SDH, but it is significant only above 30  $\mu\text{M}$  concentration (Figure 4B). The protection





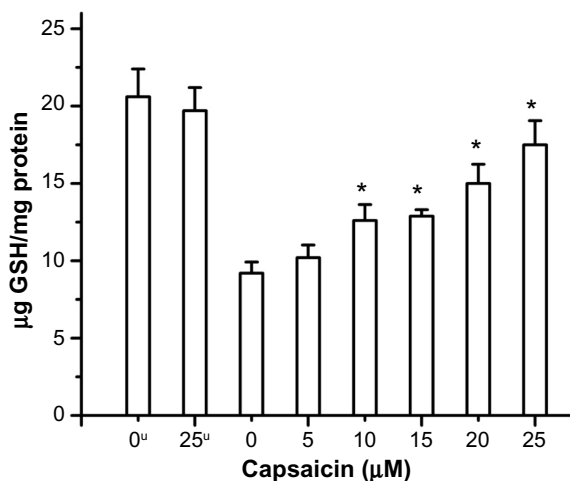
**Figure 4** Radiation-induced decrease in activity of (A) SOD (superoxide dismutase) enzyme and (B) SDH (succinate dehydrogenase) enzyme and their restoration by capsaicin.

**Notes:** \* $P < 0.05$ . <sup>u</sup>Represents unirradiated.

**Abbreviation:** DCIP, 2,6-dichlorophenolindophenol.

exerted by capsaicin against gamma radiation-induced loss of endogenous antioxidant GSH has been found even at 5 μM concentration and increased almost linearly in the 5–25 μM concentration range (Figure 5).

In the present study, capsaicin has shown protection against oxidative damages in the concentration range of 5–50 μM. However, a particular concentration has not exerted the same level of protection against oxidative damages to different subcellular components. Therefore, a difference in capsaicin level has been observed to reverse the effect of gamma radiation for different components in RLM. This can be attributed to the octanol–water partition coefficient of capsaicin ( $\log P = 3.04$ ), suggesting its high partitioning in lipid phase, which is reflected in terms of concentration dependent protection of lipids from 5 to 25 μM. Further, capsaicin completely inhibited LP process at ~40 μM concentration. It is



**Figure 5** Radiation-induced depletion of GSH and its restoration by capsaicin.

**Notes:** \* $P < 0.05$ . <sup>u</sup>Represents unirradiated.

**Abbreviation:** GSH, glutathione.

to be noted that capsaicin inhibited protein thiol damage and protein carbonyl formation above 20 μM and almost completely at ~40 μM concentration, which can be attributed to its solvation in some hydrophobic pockets of proteins/enzyme at lower concentrations. Moreover, radioprotection of GSH and SOD by capsaicin has been observed even at 5 μM and is linear at higher concentrations.

Radiation-induced damage to proteins (including enzymes) can be studied directly by observing transients of amino acids produced in this process. Tyrosyl radical (TyrO<sup>•</sup>, absorption maximum: 410 nm) is one of the marker transient produced in the oxidative damage of proteins in addition to other transients. Therefore, scavenging of TyrO<sup>•</sup> with increasing concentration of capsaicin has been used as a model to study protection of proteins as exerted by capsaicin. An approximate value of TyrO<sup>•</sup> radical scavenging by capsaicin could be measured due to overlap of transient absorption bands of capsaicin and tyrosine. Capsaicin (28–115 μM) has been found to scavenge TyrO<sup>•</sup> radical with a bimolecular rate constant of  $1.2 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$ .

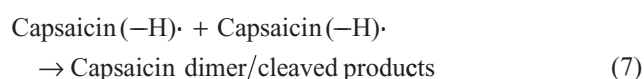
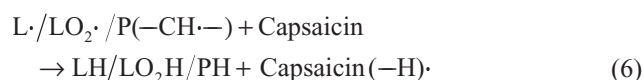
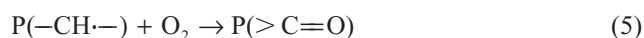
The protection provided to protein thiol and GSH agrees well with our earlier report that capsaicin scavenges glutathyl radical with a bimolecular rate constant of  $3.4 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ .<sup>55</sup> Our reported reduction potential value of capsaicin radical (0.467 V vs NHE at pH 10.3) suggests that it can scavenge radicals of biomolecules, which agrees well with the radioprotection activity of capsaicin.<sup>55</sup> The redox properties of capsaicin transients produced with hydroxyl radical can be studied by pulse radiolysis technique and redox standards, methyl viologen dication (MV<sup>2+</sup>) and ABTS dianion (ABTS<sup>2-</sup>).<sup>56,57</sup> Methyl viologen is a well-known scavenger of electrons, with known redox potential ( $E^0(\text{MV}^{2+}/\text{MV}^{\cdot+})$

$=-0.45$  V) and absorption maximum (605 nm). On the other hand,  $\text{ABTS}^{2-}$  anion is oxidized at  $+0.68$  V ( $\text{ABTS}^-/\text{ABTS}^{2-}$ ) to produce  $\text{ABTS}^{\cdot-}$  radical anion (absorption maximum at 650 nm). Electron transfer from transient/s obtained in the reaction of capsaicin with  $\cdot\text{OH}$  radical to methyl viologen suggests that only  $\sim 9\%$  of them can reduce methyl viologen. Further, the capsaicin transients with  $\cdot\text{OH}$  radical have not been found to oxidize ABTS dianion. Moreover, capsaicin transients with  $\cdot\text{OH}$  radical are found to be rather unreactive toward reaction with oxygen.

As mentioned earlier, the formation of capsaicin phenoxy radical in scavenging of oxidizing free radicals as well as by hepatic cytochrome P450 2E1 suggests an important role of both capsaicin and its phenoxy radical in physiological systems. The mechanism of radioprotection by capsaicin in the present study seems to be primarily by way of scavenging of free radicals generated during exposure to gamma radiation in vitro. In an earlier study, incubation of DNA with a mixture of  $\text{FeSO}_4$  and  $\text{H}_2\text{O}_2$  caused DNA strand scission, while addition of capsaicin to the incubation mixture decreased the strand scission in a concentration-dependent manner.<sup>58</sup> Kinetic competition studies using 5,5-dimethyl-1-pyrroline N-oxide indicated that the decrease in oxidative DNA damage was primarily because of scavenging of hydroxyl radical by capsaicin.<sup>58</sup> Capsaicin has been shown to scavenge peroxy radicals derived from 2,2'-azobis(2,4-dimethylvaleronitrile), as revealed by electron spin resonance spectroscopy.<sup>58</sup> Earlier studies on molecular mechanism of antioxidant activity of capsaicin are conflicting in nature. Those studies attribute either phenolic or C7-benzylic hydrogen in capsaicin to its antioxidant activities based on oxidation products of capsaicin.<sup>16,17</sup> However, using pulse radiolysis technique, we have explicitly shown that capsaicin scavenges oxidizing free radicals, namely hydroxyl, azidyl, glutathionyl, and trichloromethylperoxy radicals, to produce both phenoxy and carbon-centered radical.<sup>55</sup> The phenoxy radical is known to dimerize through *ortho*-carbon atoms producing biphenolic dimers, as also reported for tyrosine. Moreover, the carbon-centered benzylic radical can dimerize to produce biphenolic compound as reported earlier, using atmospheric pressure chemical ionization coupled mass spectroscopy study.<sup>16</sup>

The present study and literature suggest that radical scavenging activity of capsaicin plays a major role in protection of lipids (LH), proteins (PH), and enzymes from gamma radiation-induced oxidative damage. Since hydroxyl radical is the most oxidizing radical produced in normal metabolism and ionizing radiation exposure, the reactions taking place in oxidative damage of the studied biomolecules and protection

provided by capsaicin can be explained using it as a reactive radical [Equations (1)–(7)].



## Conclusion

The present study indicates that capsaicin is an efficient antioxidant in the RLM model even at  $5 \mu\text{M}$ . It is able to protect major biochemical components of the cells (lipids and proteins) from stress (radiation)-induced oxidative damage, and almost complete protection of lipids and proteins has been observed at  $40 \mu\text{M}$ . Its other features like, natural occurrence and dietary component makes it attractive and suitable candidate as an antioxidant both in vitro and in vivo. Further, the ongoing studies with cell system may reveal the extent of protection exerted by capsaicin in in vivo systems.

## Disclosure

The authors report no conflicts of interest in this work.

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