Modulation of glutathione and antioxidant enzymes by *Ocimum sanctum* and its role in protection against radiation injury

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Aqueous extract (OE) of the leaves of *Ocimum sanctum*, the Indian holy basil, has been found to protect mouse against radiation lethality and chromosome damage and to possess significant antioxidant activity in vitro. Therefore a study was conducted to see if OE protects against radiation induced lipid peroxidation in liver and to determine the role, if any, of the inherent antioxidant system in radioprotection by OE. Adult Swiss mice were injected intraperitoneally (ip) with 10 mg/kg of OE for 5 consecutive days and exposed to 4.5 Gy of gamma radiation 30 min after the last injection. Glutathione (GSH) and the antioxidant enzymes glutathione transferase (GST), reductase (GSRx), peroxidase (GSPx) and superoxide dismutase (SOD), as well as lipid peroxide (LPx) activity were estimated in the liver at 15 min, 30 min, 1, 2, 4 and 8 hr post-treatment. LPx was also studied after treatment with a single dose of 50 mg/kg of OE with/without irradiation. OE itself increased the GSH and enzymes significantly above normal levels whereas radiation significantly reduced all the values. The maximum decline was at 30-60 min for GSH and related enzymes and at 2 hr for SOD. Pretreatment with the extract checked the radiation induced depletion of GSH and all the enzymes and maintained their levels within or above the control range. Radiation significantly increased the lipid peroxidation rate, reaching a maximum value at 2 hr after exposure (~3.5 times that of control). OE pretreatment significantly (P<0.0001) reduced the lipid peroxidation and accelerated recovery to normal levels. The results indicate that *Ocimum* extract protects against radiation induced lipid peroxidation and that GSH and the antioxidant enzymes appear to have an important role in the protection.

Radiation induced free radicals produce peroxidation of lipids, leading to structural and functional damage to cellular membranes. The natural antioxidant system of the body, consisting of GSH and the related enzymes as well as superoxide dismutase, are believed to be the major cellular constituents involved in the defence against lipid peroxidation. Glutathione (GSH), which amounts to about 90% of the non-protein thiols in the cell, is involved in a number of reductive reactions in the cell and acts as a substrate or cofactor for the antioxidant enzymes, GSH peroxidase, GSH transferase and reductase, which are involved in the termination of peroxidation. GSH has been shown to protect cells against oxidative stress by reacting with peroxides and hydroperoxides. Increase in GSH was found to be correlated with thiol induced radioprotection and chemoprotection in mouse. Superoxide dismutase (SOD) reacts with superoxide radicals and converts them to H2O2, which is catalyzed by catalase or GSH peroxidase.

Our earlier studies have shown that the leaf extract of *Ocimum sanctum* protects against radiation lethality and bone marrow damage in mouse and has strong radical scavenging activity in vitro and recently**. Therefore, the present investigation was undertaken to study the effect of Ocimum leaf extract (OE) on radiation induced lipid peroxidation in mouse liver and to examine the role, if any, of intracellular glutathione and antioxidant enzymes in the radioprotection by OE.

Materials and Methods

**Chemicals—**Nitroblue tetrazolium, xanthine oxidase, reduced nicotinamide adenine dinucleotide phosphate (NADPH), glutathione reductase, reduced glutathione (GSH), 1-chloro-2,4-dinitrobenzene (CDNB), malonaldehyde (MDA), 5,5'-dithio-bis-2-nitrobenzoic acid (DTNB) and bovine serum albumin (BSA) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). The remaining chemicals were purchased from local firms (India) and were of highest purity grade.

The source of irradiation, animals and preparation of Ocimum extract are described in an earlier paper.**

**Animals**—Random bred adult Swiss mice of 6 to 8 weeks, of either sex, weighing 25±2 g, were selected from our mouse colony, maintained in an air-conditioned animal house on standard mouse feed and water ad libitum.

**Preparation of extract**—Fresh leaves of *Ocimum sanctum* were collected locally, shade-dried and powdered. Aqueous extract was prepared by refluxing with double distilled water (DDW) at 80°C and

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concentrated under vacuum. The weight/volume of the extract to solvent after complete dissolution was fixed at 1 mg/ml for all injections.

Irradiation—Animals were restrained in well ventilated polypropylene boxes and whole body exposed to Cobalt-60 gamma rays from a Gammatron teletherapy unit (Siemens, Germany) at a dose rate of 1 Gy/min.

Experimental design—Animals were divided into four groups and treated as follows:

- **Group 1 (Control):** DDW (0.3 ml/mouse) injected (ip) for 5 consecutive days and sham irradiated.
- **Group 2 (OE):** 10 mg/kg of OE injected ip for 5 consecutive days and sham-irradiated.
- **Group 3 (RT):** DDW (0.3 ml/mouse) injected for 5 consecutive days as in group 1 and whole-body irradiated with 4.5 Gy of gamma rays, 30 min after the last injection.
- **Group 4 (OE + RT):** 10 mg/kg OE injected for 5

![Fig. 1A](image)

**Fig. 1A**

*Glutathione, μmol/g tissue*  

![Fig. 1B](image)

**Fig. 1B**

*GSH reductase, μmol/mg protein*  

Fig. 1—Changes in (A) glutathione, (B) glutathione reductase, in mouse liver after exposure to 4.5 Gy of gamma radiation (RT) with or without OE pre-treatment (Mean±SE). □—□ Control; ○—○ 10 mg/kg OE; ×—× V RT; *—* 10 mg/kg + RT. Error bars are not shown as they fall within the symbols. *P<0.05, †P<0.001, ‡P<0.0001 compared to control; †P<0.05, ‡P<0.001, ‡P<0.0001 compared to RT.*
consecutive days as in group 2, followed by whole-
body exposure to 4.5 Gy of gamma radiation, 30 min
after the last injection.

Lipid peroxidation, GSH and the antioxidant
enzymes were measured in the liver at 15 min, 30 min,
1, 2, 4 and 8 hr post-treatment.

In addition, two groups of mice were treated with
single dose of OE as follows and lipid peroxidation in
liver was studied at 15 min to 8 h post-treatment.

Group 5: A single dose of 50 mg/kg of OE injected
ip and sham irradiated.

Group 6: A single dose of 50 mg/kg of OE injected
ip and whole body irradiated with 4.5 Gy of gamma
rays, 30 min later.

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**Fig. 2A**

![Graph showing changes in GSH peroxidase activity](image1)

**Fig. 2B**

![Graph showing changes in GSH-S-transferase activity](image2)

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*Fig. 2—Changes in (A) glutathione peroxidase, (B) glutathione S transferase, in mouse liver after exposure to 4.5 Gy of gamma radiation (RT) with or without OE pretreatment (Means±SE) Control; OE 10 mg/kg ×5; RT; OE (10 mg/kg) + RT. Error bars are not shown as they fall within
the symbols. *P<0.05, †P<0.001, ‡P<0.0001 compared to control; †P<0.05, ‡P<0.001, ‡P<0.0001 compared to RT.*
Preparation of liver homogenate—Animals were killed by cervical dislocation and the liver was perfused immediately with 0.84% NaCl and excised in toto carefully. The gall bladder was removed and liver was blotted dry, weighed quickly and homogenised in ice-cold saline-EDTA to yield a 10% homogenate. An aliquot of this was used to assay GSH\textsuperscript{12}. The remaining sample was centrifuged at 10,000 rpm for 10 min. The supernatant was collected and again centrifuged at 19,000 rpm for 1 hr at 4°C. The supernatant, after removing any floating lipid layer and appropriate dilution, was used for the assay of protein\textsuperscript{13}, GSH-S-
transferase (GST)\textsuperscript{14}, GSH peroxidase (GSPx)\textsuperscript{15}, GSH reductase (GSR)\textsuperscript{16}, superoxide dismutase (SOD)\textsuperscript{17} and lipid peroxidation (LPx)\textsuperscript{18}.

Five animals were used per treatment per point of observation. Data presented is the mean of five animals ± standard error. GSH is expressed as micromole/g (μmole/g) of tissue, GSPx, GST and GSR as μmole/mg of protein, SOD as units/mg protein and LPx as μmole of MDA/mg of protein. Statistical analysis: The data were statistically analyzed by Student’s t test, using GraphPAD Software, on an IBM computer.

Results

Glutathione and enzymes—OE injection resulted in a significant increase in GSH level of liver at 15 min after injection, with a further increase to a maximum at 4 hr, which was maintained at 8 hr. RT produced a significant decrease in GSH from 30 min to 2 hr, followed by recovery by 4 hr. OE treatment before RT prevented the radiation induced depletion of liver GSH and maintained the values at normal or above normal levels at all observation times (Fig. 1A).

GSR also showed a similar trend of change. OE produced a significant increase at 15 min, which further increased to a maximum value at 4 hr; the higher than normal level was maintained throughout the observation period. RT resulted in a significant decrease in GSR at 30 min, followed by recovery towards normal; a higher than normal value was observed at 8 hr. OE pretreatment checked the depletion and maintained the reductase above normal levels (Fig. 1B).

GSPx increased to a peak value at 30 min after OE treatment, followed by a decrease by 2 hr, though higher than normal values were maintained during the remaining period of observation. After RT, there was a significant decrease in the value at 15 min and the minimum level was reached at 1 hr, after which it rose to normal range by 4 hr. When OE was injected before RT, though there was an increase at 15 min, it was not statistically significant from RT alone group. The GSPx value increased to a maximum at 30 min which was significantly higher than control as well as RT alone values, followed by a drop to normal at 1 hr, but a higher than normal level was maintained from 2 to 8 hr (Fig. 2A).

GST also showed a significant increase at 30 min after OE treatment and this level was retained more or less unchanged at the later intervals. RT produced a significant decrease in GST at 15 min, but the values recovered to normal range by 2 hr. As in the case of the other enzymes, OE treatment before RT prevented the radiation induced decrease in GST and the values remained higher than normal throughout the observation period (Fig. 2B).

SOD showed a significant increase at 2 hr after OE injection, and remained elevated from 2 to 8 hr. RT produced a significant decrease which was evident at 15 min and dropped to the lowest value at 2 hr, with recovery at later intervals. Higher than normal level was reached at 8 hr. OE pretreatment checked the initial fall in the SOD and maintained it at normal level thereafter. The pattern of changes was similar in OE as well as OE+RT groups (Fig. 3).

Lipid peroxidation—OE injection as a single dose of 50 mg/kg or as 5 fractions of 10 mg/kg each did not produce any significant change in liver LPx values. Irradiation resulted in a highly significant increase in LPx at 15 min after exposure, which further increased steeply after 1 hr to reach a peak value of ~ 3.5 times normal at 2 hr, after which the level dropped significantly by 4 hr, and remained at a higher than normal level even at 8 hr. Liver of animals treated with OE before irradiation also showed a higher than normal LPx value from 15 min to 2 hr; the pattern of changes was similar to that of the RT alone group, but the values were significantly lower than in the latter. Administration as a single dose of 50 mg/kg produced a higher reduction in LPx compared to fractionated dose (10 mg/kg × 5) at all study points from 15 min to 2 hr. The peak value at 2 hr was about 2.4 times that of normal value in OE (10 mg/kg × 5) + RT group, while it was further reduced to 2 times the normal in the OE (50 mg/kg × 1) + RT group; the values dropped by 4 hr and normalcy was restored by 8 hr (Fig. 4).

Discussion

The GSH/GST detoxification system is an important part of cellular defense against a large array of injurious agents. GSH offers protection against oxygen-derived free radicals and cellular lethality following exposure to ionizing radiation\textsuperscript{19}. GST enzyme also possesses peroxidase activity and can directly attack the peroxides that may be generated via oxidative reduction recycling, resulting in decreased cytotoxicity\textsuperscript{20}.

Radiation interacts with biological molecules and produces toxic free radicals leading to DNA and membrane damage. While DNA damage is the crucial event in radiation induced reproductive cell death, membrane lipids are thought to be the critical targets in interphase cell death by radiation\textsuperscript{21}. Under normal conditions the inherent defense system, including glutathione and the antioxidant enzymes, protects against the oxidative damage. The present study demonstrated a significant reduction in GSH and the
activities of all the enzymes (between 30% and 60% of control) in the RT treated group. This could be due to the enhanced utilization of the antioxidant system in an attempt to detoxify the free radicals generated by radiation. In the intact and healthy cells the enzymes are restored immediately after each interaction and GSH is also restored by synthesis. But in the irradiated animals the normal synthesis/repair will be disrupted due to damage to DNA and membranes. As a result, restoration will be delayed till the cells are recovered. This could explain the slow recovery in the levels of GSH and enzymes after radiation treatment. OE treatment enhanced the levels of GSH and all the enzymes significantly above that of normal. The lower depletion of GSH and the antioxidant enzymes in the OE pretreated irradiated animals could be due to the initial higher availability, which increases the ability to cope with the free radicals produced by radiation. Radiation was given 30 min after OE, by which time the levels of GSH and antioxidant enzymes are already elevated significantly and thus the cells are made combat-ready against the toxic free radicals, making them more radioresistant. In addition, protection of DNA and membranes will preserve a larger number of healthy cells for synthesis and restoration of GSH and enzymes. Radiation protection of DNA by OE has already been demonstrated by a significant decrease in the radiation induced chromosomal aberrations. Therefore, after the initial depletion, the recovery is faster in the OE pretreated liver.

The basic effect of radiation on cellular membranes is believed to be the peroxidation of membrane lipids. LPx can be initiated by hydrogen abstraction from lipid molecules by lipid radiolytic products, including hydroxyl and hydroperoxyl radicals. This leads to permeability changes, secondary alterations in membrane proteins and other sequelae. There is considerable evidence that lipid peroxidation may have detrimental consequences for the cell and DNA, and may lead to structural and functional damage to membrane proteins.

There appears to be a close correlation between depletion of GSH and antioxidant enzymes and increase in LPx. Under normal conditions the antioxidant defense system of the body protects against the metabolic free radicals and oxidative stress. However, the oxidative stress due to the radiation induced free radicals can cause a dramatic fall in the hepatic GSH and enzymes and overwhelm the cellular defense and lead to membrane lipid peroxidation and loss of protective thiols. Such an effect is indicated in the present study, where maximum LPx was observed at 2 hr and maximum depletion of GSH and most of the enzymes by 30 min to 1 hr after RT, i.e. the LPx activity becomes dominant only after the defense mechanism becomes incapacitated. A similar correlation between maximum GSH depletion and increase in LPx in mouse liver induced by cyclophosphamide has been observed earlier. This supports the earlier observations that LPx develops when GSH depletion reaches critical values.

The significant reduction in the yield of lipid peroxides by OE pretreatment clearly demonstrates that OE protects the membranes against radiation induced oxidative damage. The finding that OE given 30 min before irradiation, at a time when GSH and antioxidant enzymes levels are elevated, brings about a significant decrease in LPx activity suggests that OE protection may be mediated through modulation of cellular antioxidant levels. The increased GSH levels by OE pretreatment will facilitate reduction of oxidative free radicals by H donation and the GSH will be restored by GSH reductase activity. In addition, GSPx also may help in the redox cycle. The significant increase of SOD in the OE treated group will facilitate removal of superoxide anions produced by radiation. The H2O2 formed in the process, will be removed by the GSPx and GST, which are also increased by OE treatment. A direct scavenging of OH radicals, as was shown in vitro, may also contribute to the OE protection against radiation injury.

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References
2 Kosower E M & Kosower N S, in Glutathione: Metabolism and function, edited by IM Arias & WB Jakoby (Raven Press, New York) 1976, 139.