Radioprotective effect of *Curcuma longa* extract on γ-irradiation-induced oxidative stress in rats

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Abstract: This study was conducted to evaluate the modulatory effect of aqueous extract of *Curcuma longa* (L.) against γ-irradiation (GR), which induces biochemical disorders in male rats. The sublethal dose of GR was determined in primary hepatocytes. Also, the effect of *C. longa* extract was examined for its activity against GR. In rats, *C. longa* extract was administered daily (200 mg/kg body mass) for 21 days before, and 7 days after GR exposure (6.5 Gy). The lipid profile and antioxidant status, as well as levels of transaminases, interleukin-6 (IL-6), and tumour necrosis factor α (TNFα) were assessed. The results showed that in hepatocytes, the aqueous extract exhibited radioprotective activity against exposure to GR. Exposure of untreated rats to GR resulted in transaminase disorders, lipid abnormalities, elevation of lipid peroxidation, trace element alterations, release of IL-6 and TNF, and decrease in glutathione and protein level of superoxide dismutase-1 (SOD-1) and peroxiredoxin-1 (PRDX-1). However, treatment of rats with this extract before and after GR exposure improved antioxidant status and minimized the radiation-induced increase in inflammatory cytokines. Changes occurred in the tissue levels of trace elements, and the protein levels of SOD-1 and PRDX-1 were also modulated by *C. longa* extract. Overall, *C. longa* exerted a beneficial radioprotective effect against radiation-induced oxidative stress in male rats by alleviating pathological disorders and modulating antioxidant enzymes.

Key words: γ-irradiation, *Curcuma longa*, hepatocytes, rats, oxidative stress, trace elements, cytokines.

Résumé : Cette étude a été réalisée afin d'évaluer les effets modulateurs d'un extrait aqueux de *Curcuma longa* sur l'irradiation γ (IG), qui induit des perturbations biochimiques chez les rats mâles. La dose sublétale de rayons γ a été déterminée sur des hépatocytes primaires. Les effets de l'extrait de *C. longa* sur l'IG ont aussi été examinés. L'extrait de *C. longa* a été administré quotidiennement (200 mg/kg de poids corporel) à des rats pendant 21 jours avant et 7 jours après l'exposition aux rayons γ (6,5 Gy). Les transaminases, le profil lipidique, l'interleukine-6 (IL-6), le nécrose des tumeurs α (TNF-α) et le statut antioxydant ont été évalués. L'extrait aqueux montrait une activité radioprotectrice sur les hépatocytes exposés à des radiations γ. L'exposition des rats aux rayons γ affectait les transaminases et les lipides, augmentait la peroxydation lipidique, altérait les oligoéléments, induisait la production d'IL-6 et de TNF, et diminuait les niveaux de glutathion, de superoxyde dismutase-1 (SOD-1) et de peroxiredoxin-1 (PRDX-1). Cependant, le traitement des rats à l'extrait aqueux avant et après l'exposition aux rayons γ améliorait le statut antioxydant et atténuait l'augmentation de la production de cytokines pro-inflammatoires induites par les radiations. Les modifications observées sur les oligoéléments et les niveaux de SOD-1 et de PRDX-1 étaient aussi modulées par l'extrait de *C. longa*. Globalement, *C. longa* a exercé un effet radioprotecteur bénéfique contre le stress oxydant induit par la radiation chez les rats mâles, en atténuant les troubles pathologiques et en modulant les enzymes anti-oxydantes.

Mots-clés : irradiation γ, *Curcuma longa*, hépatocytes, rats, stress oxydant, oligoéléments, cytokines.

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Introduction

It is evident that oxidative stress is associated with the development of many chronic and degenerative diseases. Apparently, reactive oxygen species (ROS) such as hydroxyl radical ($OH^{\bullet}$), superoxide anion ($O_2^{\bullet -}$), and hydrogen peroxide (H_2O_2) produced during normal metabolic activities or as a consequence response to abnormal stress, might be implicated in the pathogenesis of aging and several diseases. ROS exhibits deleterious effects on biological macromolecules such as proteins, lipids, and DNA. The human body develops a very delicate mechanism to eliminate free radicals from the body (Davies 2000). Oxidative stress occurs when the gener-

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ation of ROS exceeds the capacity of the defense system (Halliwell & Gutteridge 2007).

Extracts from a number of medicinal plants have been investigated for their capacity to scavenge specific reactive oxygen species (ROS), both in vitro and in vivo. Dietary antioxidants play an important role in mitigating cell damage from oxidative stress (Wu et al. 2004; Rao et al. 2006). Diets rich in curcumin are considered an excellent source of antioxidants (Kang et al. 2002). A high correlation was found between the amounts of curcumin extract from *C. longa* and the effectiveness of their antioxidant activity (Piper et al. 1998; Azab and Nada 2004). Curcumin is the main biologically photochemical compound of turmeric, a spice prepared from dried rhizomes of *C. longa* that is widely used as a seasoning in Asian countries. It is of particular interest to note that *C. longa* has a high content of essential trace elements, especially copper (Sorenson 2002). Turmeric is a powerful antioxidant, anti-inflammatory, and antihepatotoxic herb. It contains a mixture of powerful antioxidants: phytonutrients known as curcuminoids (Deshpande et al. 1998). The antioxidant activities in curcuminoids could be attributed to the presence of tetrahydrocurcumin, which is a major metabolite of curcumin in the body (Varadkar et al. 2001; Kang et al. 2002).

Many investigators have considered curcumin, the active constituent of *C. longa* extract, as hepatoprotective (Deshpande et al. 1998), renoprotective (Shoskes 1998), antiproliferative (Mehta et al. 1997), or as an anti-tumor agent (Piper et al. 1998). Also, it has hypolipidemic (Babu and Srinivasan 1997) and anti-inflammatory properties (Joe et al. 1997), and is involved in the attenuation of myocardial toxicity (Venkatesan 1998) and chromosomal damage (Abraham et al. 1993). Moreover, curcumin prevents irradiation-induced tumor, and minimizes and ameliorates the acute and chronic effects of radiation (Inano and Onoda 2002).

Turmeric is considered as a potential candidate against radiation because it has antioxidant properties (Salimath et al. 1986), and it is not toxic at higher levels of intake (Ravindranath and Chandrasekhara 1982). For this reason, aqueous extract of turmeric was chosen to be used, as this spice is largely consumed in recipes containing substantial amounts of water.

An interesting study reported by Shalini and Srinivas (1987) indicated that the inhibition of lipid peroxidation using the aqueous extract of turmeric incorporated into the liposome itself, was very efficient. This indicated the presence of yet another antioxidant in turmeric besides the lipophilic curcumin. Moreover, lipid peroxidation of the abundant lipid components in the cell membranes is reported to be particularly susceptible to radiation damage (Chevion et al. 1999). In view of the above, our study was designed to evaluate the possible modulatory effects of the prolonged administration of an aqueous extract of *C. longa* on γ-radiation-induced biochemical and trace element changes in male rats.

Materials and methods

Cell culture and treatment

Rat hepatocyte primary cultures were prepared after teasing the liver by flushing the tissue with phosphate-buffered saline (PBS) with the aid of a syringe, as previously described (Syng-Ai et al. 2004). Primary cultures were maintained in Dulbecco's modified Eagle medium/F12 (1:1 *v*/*v*) supplemented with 1% penicillin–streptomycin and 10% fetal bovine serum (Gibco, Invitrogen). Hepatocytes were incubated at 37 °C with humidified air containing 5% CO_2. Different concentrations of the aqueous extract of *C. longa* (0–200 μg/mL) and curcumin dissolved in dimethyl sulfoxide (DMSO, 0–100 μmol/L; Sigma–Aldrich, St. Louis, Missouri, USA) were prepared. Cells were cultured in 96-well plates for 24 h and then treated with either *C. longa* or curcumin in addition to the controls (water or DMSO). After 72 h, a cell proliferation assay was performed to determine the IC_{50} using Cell Counting Kit-8 according to the manufacturer's instructions (Dojindo Molecular Technologies, Inc., Maryland, USA).

Experimental animals and preparation of *Curcuma longa* extract

Male albino Wistar rats weighing 150 ± 20 g were obtained from the Egyptian Organization for Biological Products and Vaccines. Animals were housed in polypropylene cages with stainless steel mesh, at a temperature of 26–30 °C and under a light period of 16–18 h daily. The animals were maintained in commercially available standard laboratory diet (Vacsera, Egypt) and water, ad libitum. All animals received the necessary professional care in compliance with the guidelines of the Ethical Committee of the Centre of Radiation Research and Technology, Atomic Energy Authority, Cairo, Egypt. The fresh rhizomes of *C. longa* were purchased from the Faculty of Agriculture, Cairo University (Egypt), and authenticated by the Department of Botany at NCRRT. The rhizome was ground, sieved through BP 100 mesh, and suspended in warm double-distilled water that was boiled gently to reach the final volume of the appropriate concentration dose level (5% aqueous extract). The suspension was vortexed thoroughly in a magnetic stirrer for 30 min and allowed to stand overnight at 4 °C. The resulting suspension was filtered and saved as aliquots at –20 °C for oral administration to the rats (Hussain 2002; Chethankumar 2010).

High performance liquid chromatography

The high performance liquid chromatography (HPLC) system consisted of a Waters Controller 600 pump, a Model Waters 1500 Rheodyne manual injector, a Waters 486 UV–visible detector, and a data workstation with Millennium software. A phenomenex C_{18} column was used (250 mm × 4.6 mm internal diameter; 5 μm), from Waters Corporation (USA). The mobile phase consists of acetonitrile/water – 2% acetic acid (70:30 *v*/*v*). The separation was performed at 40 °C at a flow rate of 1.0 mL/min. The injection volume was 20 μL for both the standard curcumin solution (100 μg/mL) and the sample extract. The UV detector was operated at 420 nm.

Radiation processing

Cultured hepatocytes were exposed to different doses of γ-irradiation (0, 2, 4, 6, and 8 Gy) to determine the sublethal dose (ID_{50}). To determine the efficacy of the aqueous curcumin extract against γ-radiation, cells were pre-incubated with either aqueous extract of *C. longa* (45 μg/mL) or curcumin

dissolved in DMSO (36.5 μmol/L) for 24 h. Pretreated cells were γ-irradiated at the dose of 3.2 Gy (0.61 Gy/min) using ^{137}Cs, a biological irradiator source (γ-Cell-40) located at the NCRRT. After the irradiation procedure, cells were washed twice with PBS, and completly fresh culture medium was added. Cells were cultured in 96-well plates for 72 h and then the cell viability for irradiated and nonirradiated cells was assessed as before. For the animal experiments, the whole body was exposed to a single dose of 6.5 Gy γ-irradiation using ^{37}Cs, using the same procedure as described above.

Experimental design

After one week of adaptation, animals were divided among 4 groups, with 6 rats in each. The first group was not irradiated and was used as a control group. Each animal in the second group received, by gavage, a daily dose of 200 mg of aqueous curcumin extract per kilogram body mass in 0.6 mL water, for 28 successive days. The physiological dose of 200 mg/kg aqueous extract used in this study, is even lower than the doses previously reported (Hussain 2002; Ashok and Meenakshi 2004). The third group of animals was exposed to a single dose of whole body γ-irradiation (6.5 Gy). The fourth group received the aqueous extract (200 mg/kg body mass) daily for 21 successive days, then was exposed to a single dose of γ-irradiation 6.5 Gy, followed by daily administration of the extract for an extra 7 days. At the end of experiment, the rats were sacrificed, and serum and liver tissue were collected for biochemical analyses.

Biochemical analysis

Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), cholesterol, triglycerides (TG), and low-density lipoprotein (LDL) were measured according to the standard laboratory protocols. Glutathione content was estimated according to the method of Tietz (1986). The lipid peroxidation products were estimated as thiobarbituric acid reactive substances (TBARS) in accordance with the protocol provided by Yoshioka et al. (1979). Interleukin-6 (IL-6) and tumour necrosis factor α (TNFα) were measured in the serum using enzyme-linked immunosorbent assay, according to the manufactures protocol (Thermo Scientific, USA).

Trace elements were determined both in the liver tissue of rats and in the rhizome of *C. longa*. After digestion in pure concentrated nitric acid and hydrogen peroxide at 5:1 ratio (*v*/*v*) (IAEA 1980), sample digestion was carried out using a Milestone MLS-1200 Mega High Performance Microwave Digestor Unit. The selected elements were estimated using a UNICAM 939 atomic absorption spectrometer, equipped with deuterium background correction. All solutions were prepared with ultra-pure water having a specific resistance 18 Ω/cm, obtained from ELGA. The biochemical analysis was achieved using a Heλios γ UV–VIS spectrophotometer.

Western blot

Liver tissues were homogenized in radio-immunoprecipitation assay lysis buffer. The lysate was centrifuged, and the supernatant was collected for measuring the protein concentration using a BCA protein kit (Pierce, USA). Thirty micrograms were resolved on 12% polyacrylamide gels, and electroblotted onto nitrocellulose membranes. Membranes were probed with anti-SOD-1 (1:500), anti-PRDX-1 (1:1000) and anti-actin (1:2000) purchased from Abcam (Abcam, Massachussetts, USA). The membrane was incubated with secondary antibodies labeled with horseradish peroxidase conjugate and then detected using an enhanced chemiluminescence substrate (ECL; Pierce, USA).

Histopathology of liver

After the animals were dissected, small pieces of liver were washed and fixed in 10% (*v*/*v*) buffered formalin. Fixed samples were embedded in paraffin blocks, processed in an alcohol series, and stained with haematoxylin and eosin according to standard histological techniques.

Statistical analysis

Data were expressed as the mean ± SEM. Statistical analysis was performed using GraphPad software version 5 (GraphPad, California, USA). One way analysis of variance (ANOVA) was used to compare differences among each group by Tukey multiple comparison test. A value for $p < 0.05$ was considered to be statistically significant.

Results

Establishment of optimal γ-irradiation dose and radioprotective effect of the aqueous extract of *C. longa* on rat hepatocytes

Before studying the effect of γ-irradiation on primary rat hepatocytes, we determined the cell viability at different γ-radiation doses (0–8 Gy). The dose–response curve for rat hepatocytes indicated that 3.2 Gy was the 50% inhibitory dose (ID_{50}). This dose was selected for exposing hepatocytes to γ-irradiation (Fig. 1*a*). To determine the IC_{50} for *C. longa* and curcumin (used as a standard), cells were treated with different concentrations of either extract for 72 h and then cell viability was assessed. The IC_{50} for aqueous extract of *C. longa* was 45 μg/mL, compared with 36.5 μmol/L for curcumin (data not shown). Using curcumin as a standard, hepatocytes were pretreated with *C. longa* extract (45 μg/mL) for 24 h and then exposed to a single dose of γ-radiation (3.2 Gy), which demonstrated the radioprotective properties of the extract ($p < 0.05$) (Fig. 1*b*).

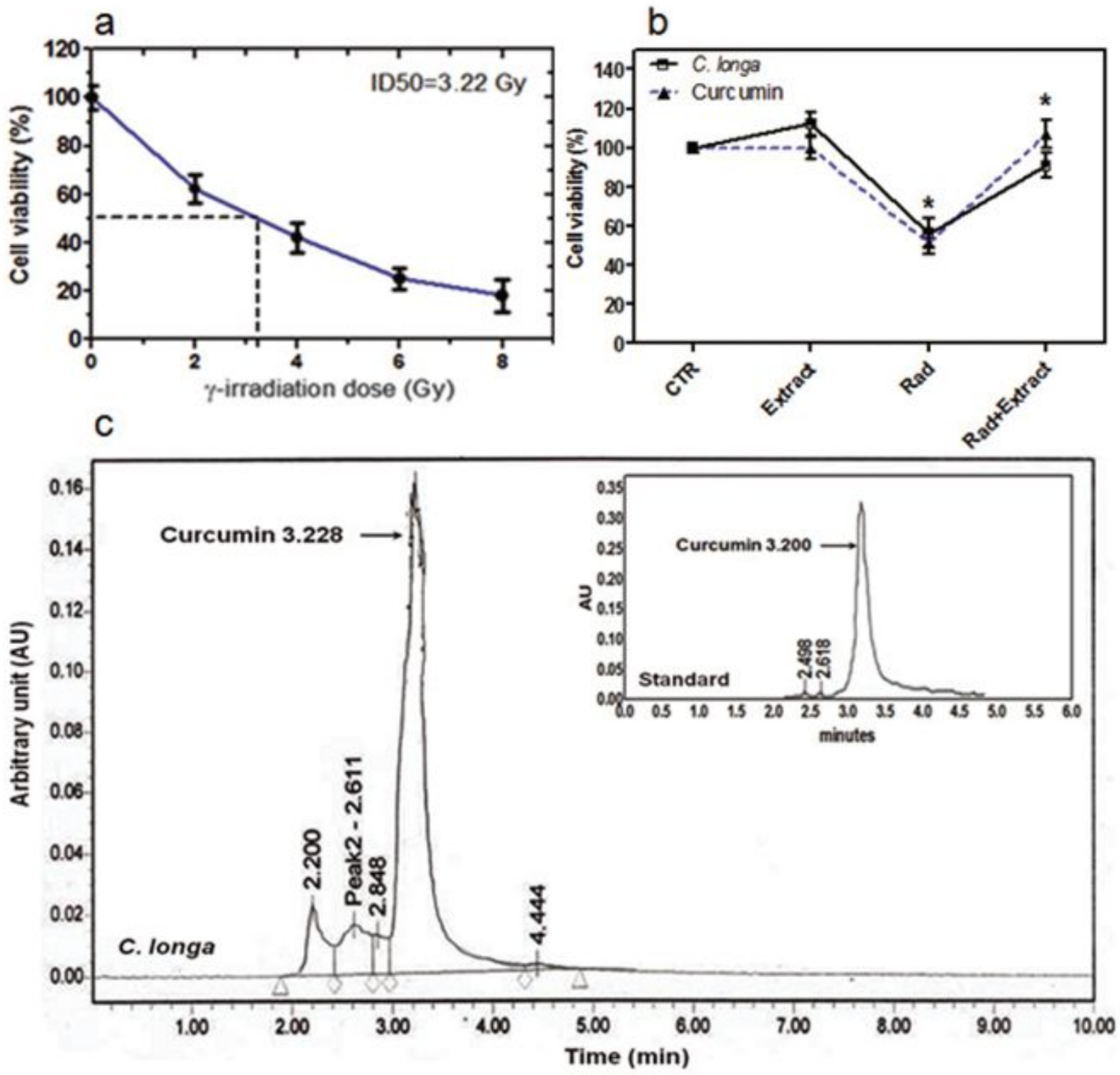
High performance liquid chromatography data analysis

The mobile phase comprised acetonitrile/water – 2% acetic acid (70:30 *v*/*v*). This is the result of several trials that were performed to find the conditions that provided the extraction. The curcumin standard revealed a clear peak at 3.2 min, and the area under peak was considered as 100% curcumin, equivalent to 100 μg/mL. The sample of aqueous extract of *C. longa* showed few peaks. The largest area under a curve showed curcumin to be the major component of turmeric, with a concentration of 42 μg/mL. However, a peak tailing was identified in the *C. longa* chromatogram, which could be due to an overlap of 2 peaks (Fig. 1*c*).

Effect of γ-irradiation and *C. longa* extract on serum AST, ALT, and lipid metabolite content

Daily treatment of rats with *C. longa* extract (200 mg/kg body mass) for 28 days showed no detectable changes in AST and (or) ALT activity, or lipid metabolites in the sera

Fig. 1. Determination of a sublethal dose of γ-radiation and the protective role of *Curcuma longa* (CL) extract on rat hepatocytes. Primary hepatocytes were cultured for 24 h and exposed to different doses of γ-irradiation (0–8 Gy). (*a*) The ID_{50} for γ-irradiation was assessed by a cell viability assay, as mentioned in the Materials and methods. (*b*) Hepatocytes were pretreated with aqueous extract of *C. longa* (45 µg/mL) for 24 h, then the cells were exposed to 3.22 Gy. (*c*) Chromatogram of HPLC analysis for the aqueous extract of *C. longa* using curcumin as the standard. Each value represents the mean ± SEM of triplicates for 3 repeated experiments. *, $p < 0.05$ compared with the control or irradiated cells.



of rats. Thus, and as expected, the plant extract showed no toxic effects on the biochemical parameters measured in the serum. However, deleterious effects were observed after a single dose of γ-radiation exposure to the whole body of rats. These effects noted by the increase of AST and ALT enzymatic activity and lipid profile (cholesterol, triglycerides, and LDL) (Figs. 2*a* and 2*b*). Surprisingly, animals treated with the aqueous extract for 21 days before taking a single dose of γ-irradiation, followed by treatment for additional 7 days, exhibited a significant decrease in AST and ALT activity and lipid profile. This effect was manifested when treated rats were compared with those exposed to γ-irradiation alone ($p \leq 0.05$) (Figs. 2*a* and 2*b*).

Hepatoprotective effect of *C. longa* on liver antioxidant status of rats exposed to γ-irradiation

The effect of the aqueous extract of *C. longa* on levels of GSH and TBARS was investigated. The results indicated that exposure of rats to γ-irradiation caused a significant decrease in levels of GSH and a significant increase in levels of TBARS when compared with the control rats (Fig. 3*a*). Also, superoxide dismutase (SOD-1) and peroxiredoxin (PRDX-1) were down regulated after γ-irradiation (Fig. 4). Treating rats with the *Curcuma* extract brought the levels of GSH, TBARS, and proteins (SOD-1 and PRDX-1) back up to normal (Figs. 3*a* and 4). These observations indicate the role of *C. longa* extract in modulating the antioxidant enzymes and (or) inhibiting the signaling pathways involved in oxidative stress induced by γ-irradiation.

Modulation of hepatic changes caused by *Curcuma* extract occurring in heavy metal content, and levels of inflammatory cytokines during γ-irradiation

Gamma irradiation induced an increase in the level of Fe and Zn in fresh liver tissue, while the levels of Cu decreased (Fig. 3*b*). Simultaneously, inflammatory cytokines (IL-6 and TNFα) were elevated in the serum of rats (Fig. 3*c*). In contrast, there was insignificant effect on heavy metal levels in rats treated with the *Curcuma* extract, compared with the untreated rats. The modulating effect of *C. longa* on hepatic levels of heavy metals as well as IL-6 and TNFα was observed by exposing the rats to γ-irradiation and treating them with the extract (Figs. 3*b* and 3*c*). These observations indicate a modulating effect by the extract on the transport of intrahepatic Fe and Zn, either directly by regulating hepatic enzymes, or indirectly by stabilizing the plasma membranes and endoplasmic reticulum.

Assessment of metal contents (Fe, Cu, Zn, Mn, Ca, Mg, and Se) in *C. longa* plant tissue

To identify the contents of trace metals in the tissue of *C. longa*, which may involve the radioprotection process

Fig. 2. Radioprotective effect of aqueous extract of *C. longa* (200 mg/kg body mass) after exposure of rats to γ-irradiation (6.5 Gy) (*a*) on serum aspartate aminotransferase, alanine aminotransferase, and lipid metabolites, and (*b*) liver antioxidant status in rats. Each value represents the mean ± SEM of 6 rats. Level of significant difference is considered at $p < 0.05$ when comparing data with the corresponding value for (a) the control or (b) irradiated rats.

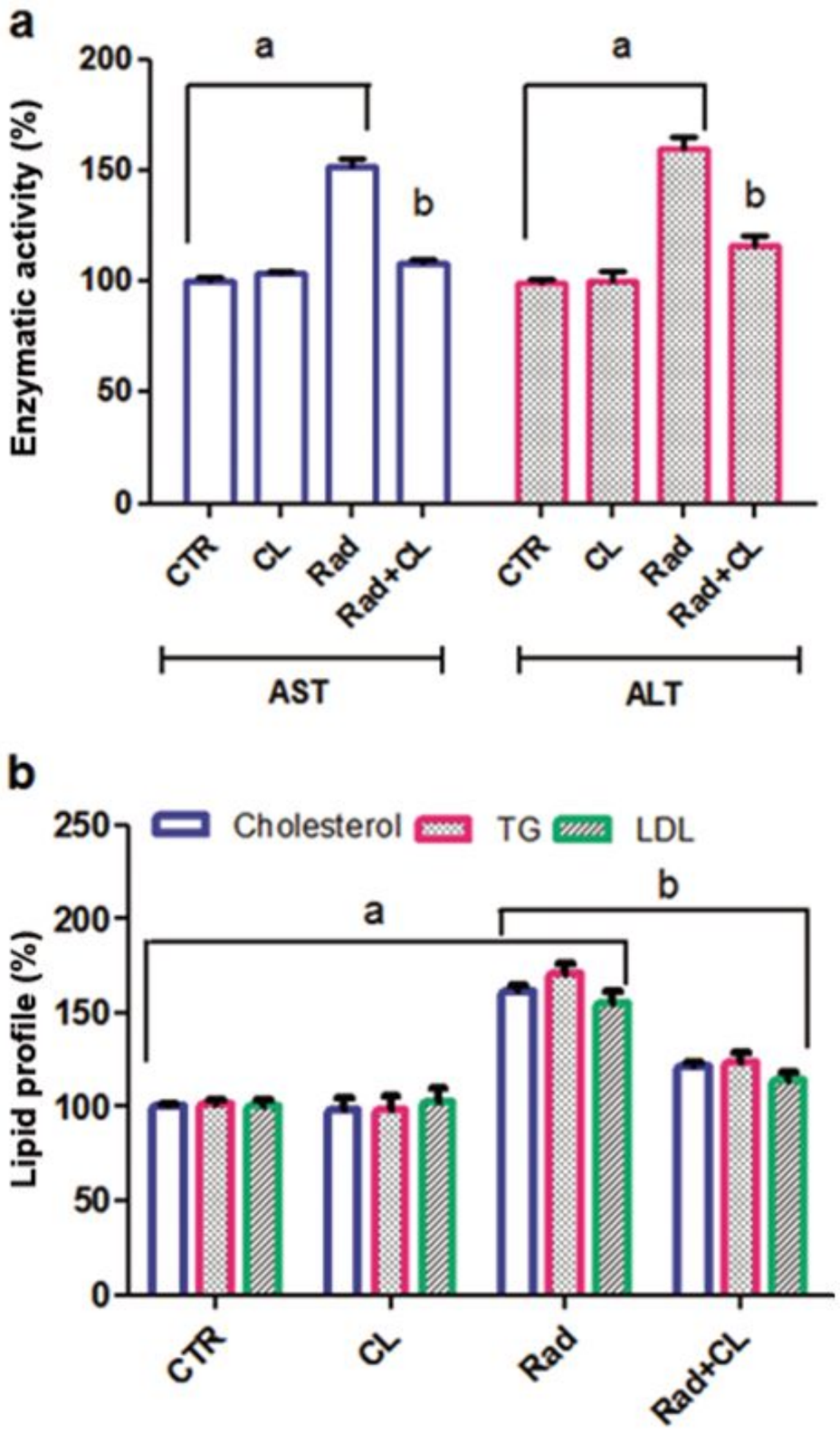
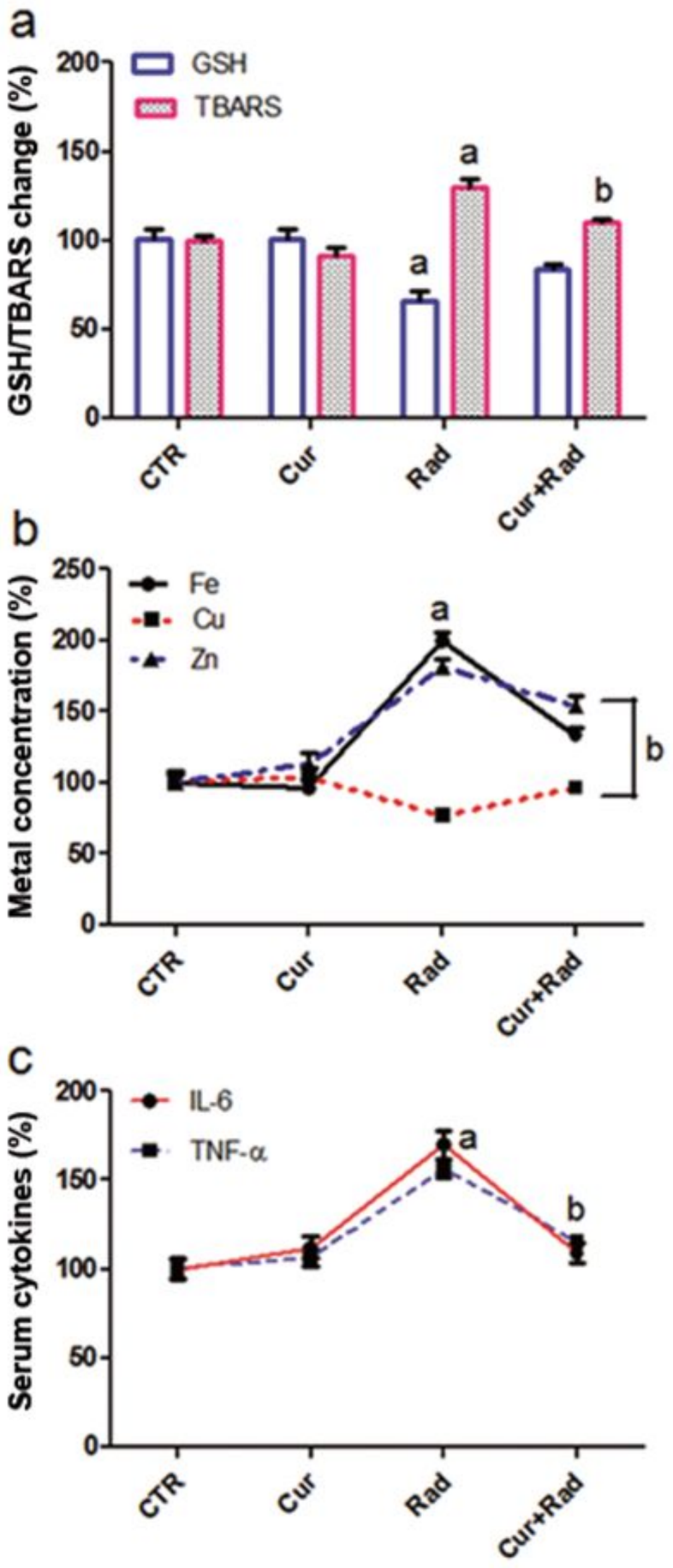


Fig. 3. Effect of γ-irradiation (6.5 Gy) and *Curcuma longa* (CL) extract (200 mg/kg body mass) on (*a*) liver antioxidant status (glutathione and thiobarbituric acid reactive substances), (*b*) metal contents, and (*c*) inflammatory cytokines in rats. Each value represents the mean ± SEM of 6 rats. Values for $p < 0.05$ are considered significant when comparing data with the corresponding value for the control (a) or irradiated rats (b).



against γ-irradiation, atomic absorption was used to assess these metals. Seven metals, including Fe, Cu, Zn, Mn, Ca, and Se, were detected in the plant tissue. Magnesium was the most abundant metal found in the plant tissue, followed by calcium, iron, copper, zinc, manganese, and selenium (Table 1).

Histopathological study

Treatment of normal rats (nonirradiated) with *C. longa* extract caused no histological changes, compared with (irradiated) untreated rats, and the liver architecture was normal, with many binucleated cells surrounding a normal central vein (Figs. 5*a* and 5*b*). However, γ-irradiation induced harmful effects on the liver morphology, including widening and dilated hepatic portal area, hemorrhage, and infiltration of inflammatory cells and fibroblasts around the portal vein. In addition, vacuolated cytoplasm, congested vessels, diluted sinusoids, necrotic cells, pyknotic nuclei, and ruptured hepatic cells were also observed (Fig. 5*c*).

A hepatoprotective effect of *C. longa* towards morphological changes in the liver of rats exposed to γ-irradiation was observed, as evidenced by the occurrence of regular hepatic lobules with normal circular nuclei and a normal central vein. Also, hepatic cells were undergoing mitotic division, with intact cell membranes and improved regenerating hepatic cells (Fig. 5*d*).

Discussion

Ionized radiation causes dramatic changes in the living body, including an increase in levels of reactive oxygen species (ROS), which damage proteins, lipids, and nucleic acids. Because of the abundance of lipid components in the cell

Fig. 4. Effect of γ-irradiation (6.5 Gy) and aqueous extract of *Curcuma longa* on antioxidant proteins. Thirty micrograms of protein was loaded on 12% polyacrylamide gels and probed with anti-superoxide dismutase-1 and peroxiredoxin-1 antibodies. Equal loading of the protein samples was assessed by reprobing the membrane with anti-actin antibody.

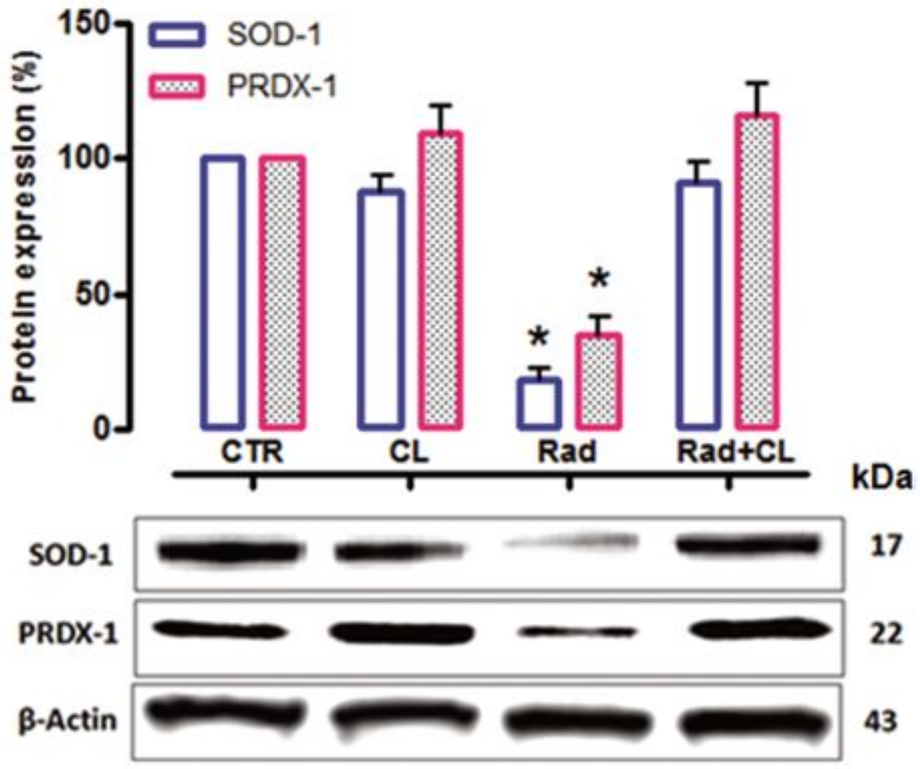


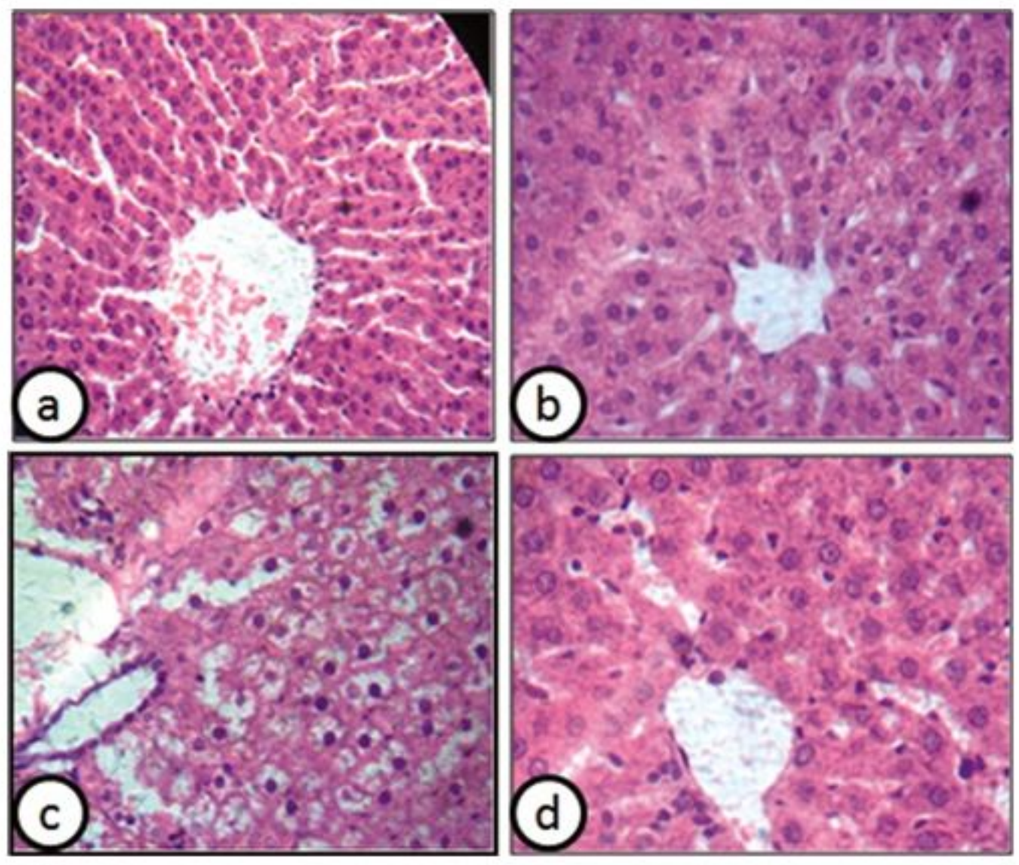
Table 1. Concentration of metals (Se, Mn, Zn, Cu, Fe, Mg, and Ca) found in *Curcuma longa* plant tissue, expressed as micrograms per gram of tissue.

Element	Concentration
Se	0.234±0.015
Mn	30.42±0.630
Zn	36.96±6.110
Cu	57.21±8.587
Fe	378.8±13.53
Mg	6957.0±503.4
Ca	2882.0±43.00

Note: Each value represents the mean ± SD of 6 samples.

membranes, lipid peroxidation is reported to be particularly susceptible to radiation damage (Chevion et al. 1999). Recent studies reported the importance of small peptide of IL-β or the whole form of IL-6 in protection of mice against potentially lethal doses (6.5–9.0) of ionizing radiation (Patchen et al. 1991; Singh et al. 2005). Antiproliferative, antiradical, and protective effects against induced oxidative stress were demonstrated in hepatocellular carcinoma after treatment of HepG2 cells with *C. longa* extract (Menghini et al. 2010). In the same line, our in-vitro study demonstrates a radioprotective effect of an aquaous extract from *C. longa* on rat hepatocytes, using curcumin as the standard. The results from HPLC revealed that *C. longa* contains 45 μg/mL of active materials with a major peak for curcumin at a concentration 42 μg/mL. However, the peak is highly distorted, which could be due to an overlap of 2 peaks. This could be a peak from the standard, curcumin, overlapped with another peak of a similar compound, or most probably, a curcumin isomer, as described previously by Tonnesen and Karlsen (1983) who reported that curcuminoids from turmeric could include bidesmethoxycurcum, desmethoxycurcumin, and (or) curcumin. Our results also indicated that the exposure of rats to whole body γ-radiation as a single dose (6.5 Gy) induced biochem-

Fig. 5. Photomicrograph of normal rat liver showing (*a*) regular hepatic architecture of normal central vein, portal area, normal hepatic strands; (*b*) liver treated with *Curcuma longa* extract only (200 mg/kg body mass for 28 days) showed no morphological changes; (*c*) γ-irradiated rats (6.5 Gy) showing widening and dilated hepatic portal area, hemorrhage, inflammatory cells and fibroblasts surrounding the portal vein, vacuolated cytoplasm, congested vessels, diluted sinusoids, necrotic cells, pyknotic nuclei, ruptured hepatic cells, extremely diluted branch of portal vein with inflammatory cells, widening and dilated central vein with ruptured endothelial lining cells; (*d*) treatment of rats with *C. longa* alleviated the morphological deformations induced by exposure of rats to γ-irradiation. Magnification ×400.



ical disorders. These were manifested by elevation of transaminases, lipid peroxidation, and alteration of the level of trace elements in liver tissues, while levels of glutathione, and PRDX-1 and SOD-1 proteins were reduced. Treatment of rats with an aqueous extract of *C. longa* for 28 successive days did not cause any significant changes, indicating no detectable harmful effects produced from using this plant extract. The aqueous extract may contain several ingredients responsible for its radioprotective properties, such as proteins, polysaccharides, or other compounds. Turmerin, a water-soluble protein extracted from *C. longa*, is considered to be an efficient antioxidant/DNA-protectant/antimutagen. Also, turmerin is heat stable, insensitive to UV radiation, and contains 3 residues of methionine, which give the protein its antioxidant properties (Srinivas et al. 1992). Another study (Yue et al. 2010) has reported on the immunomodulatory activity exerted by new polysaccharide compounds isolated from whole aqueous extracts of *C. longa*.

Hyperlipidemia developed after exposure to ionizing radiation, which results in the accumulation of cholesterol, triacylglycerols (TGs), and phospholipids (Stepanov 1989). The present results revealed significant elevations in lipid profile in irradiated rats. The increase in serum TGs after irradiation resulted either from the inhibition of lipoprotein lipase activity (Sedláková et al. 1986) or the increase in 3-hydroxy-3-methylglutaryl coenzyme A reductase enzyme activity (Bok et al. 1999) Also, this effect could be attributed to the damage that occurs in biomembranes, which leads to the release

of structural phospholipids, in addition to mitochondrial dysfunction (Madamanchi and Runge 2007).

Glutathione is known to play an important role in the regulation of the cellular redox balance. However, the effect of γ-irradiation resulted in depletion of GSH. The depletion of GSH could be attributed to the enhanced utilization of the antioxidant system and the diminished activity of glutathione reductase (Půlpanova et al. 1982), or to the inhibition of GSH efflux across hepatocytes (Dahm et al. 1991). Radiation also induced significant changes in the levels of metalloelements in liver tissues. In this study, iron and zinc were increased, while copper levels decreased. Several studies have revealed marked alterations in trace element metabolism associated with radiation (Nada 2008). Gamma-irradiation caused hematological changes such as reduction of red blood cell count, hemoglobin concentration, and hematocrit value. These discrepancies may be observed during changes in iron level (Noaman and Gharib 2005).

The redistribution and acceleration of zinc metabolism might work as a defense mechanism against radiation damage (Nada 2008). In contrast with iron and zinc, radiation-induced depression in hepatic copper level, which is caused by excess utilization of cuproenzymes after irradiation or de novo syntheses of Cu-SODs and catalase (Fee and Valentine 1977). Also, radiolytic loss of essential metalloelement cofactors accounts for the loss of Cu- and Zn-SODs following irradiation (Summers et al. 1989). El-Nimr and Abdel-Rahim (1998) have attributed the alterations in trace element level induced by irradiation to the long term disturbances of enzymatic functions and possible retardation of cellular activities. The botanical antioxidants such as *C. longa* extract induce their activity through a free radical quenching mechanism and electron hydrogen donation (Chaudhary et al. 1999). The bioavailability of curcumin is due to the presence of tetrahydrocurcumin antioxidant action (Okada et al. 2001). These authors also reported that the phenolic groups in curcumin have hydrogen bond acceptor properties, while those in bisdemethoxycurcumin acted as hydrogen bond donors. Curcumin is involved in the prevention of mitochondrial membrane lipid peroxidation (Azab and Nada 2004), and can inhibit the peroxidation of liposomes (Ramsewak et al. 2000). Radiolytic loss of essential metalloelement cofactors accounts for 20% of the loss of both Cu- and Zn-dependent SODs in rats, after acute exposure to radiation (15 Gy), and then increased as a result of de-novo synthesis of the former enzymes (Summers et al. 1989).

The essential trace elements (Fe, Cu, Zn, Mn, Ca, Mg, and Se) in turmeric plants, as shown in Table 1, could be involved in multiple biological processes, such as constituents of the enzyme system including superoxide dismutase (Cu-, Zn-, Mn-SODs), oxide reductase, glutathione (GSP, GSH, GST), and metallothionein (MT) (Sorenson 2002). These metals increased the antioxidant capacities through the induction of metalloelement-dependent enzymes, which prevent the accumulation of pathological concentrations of oxygen radicals, or by repairing damage caused by irradiation injury (Wan et al. 2007). Gamma-irradiated rats treated with turmeric extract exhibited improved hepatic architecture with a normal central vein and intact cell membrane. Curcumin also restored hepatic architecture and reduced hepatic fibrosis through antiperoxidation and regulation of collagen metabolism in liver tissues (Subudhi et al. 2009).

It is obvious that treating rats with an aqueous extract of *C. longa*, which contains a mixture of bioactive compounds as well as essential trace elements, has a protective effect against γ-irradiation. Radioprotective properties are indicated by attenuation of hepatic transaminases, lipids, and lipid peroxidation. Also, the enhancement of antioxidant defense mechanisms by improved antioxidant status and reduction of inflammatory cytokines are beneficial.

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