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Hesperidin and Curdlan treatment ameliorates γ-radiation induced cellular damage and oxidative stress in the liver of Sprague-Dawley rats

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ABSTRACT

Rapid technological advancement and widespread application have resulted in increased human exposure ionizing radiation. As radiation exposures are known to cause deleterious effects to the biological systems, there is an urgent need to protect humans from the side effects of radiation exposure. In the present study the radioprotective effect of pre and post treatment of hesperidin + curdlan against γ-radiation (5Gy) induced hepatotoxicity was investigated in Sprague-Dawley rat model. Whole-body γ-radiation to rats resulted in an increase in serum AST, ALT, ALP, γ-GT, LDH, liver LPO as well as decrease in the levels of liver SOD, CAT, GPx, GSH, vit-C and vit-E post 2 days of irradiation. While pre and post treatment with hesperidin + curdlan for 2 days did not offer any significant protection, administration of hesperidin + curdlan orally for 7 days post irradiation was found to restore the altered levels of the above parameters in serum and liver tissue to near normalcy. The histopathological findings also supported the biochemical findings as hesperidin + curdlan treated rats showed minimal hepatocellular damage compared to γ-radiation exposed rats. The protective effect offered by hesperidin + curdlan is attributed to its antioxidant and free radicals scavenging properties.

Key words: γ-radiation, radiotoxicity, hesperidin, curdlan, oxidative stress.

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INTRODUCTION

Human beings are being exposed to radiation from natural as well as man-made sources including cosmic rays and medical diagnostics. Exposure to such radiations can induce alterations in the cellular macromolecules and can affect their functions. Radiation toxicity to human cells results in immediate and widespread oxidative stress and the most important target of radiation is the DNA. In addition, there is an interaction of the radiation with cellular water (radiolysis of water), forming ion radicals, which then attack the DNA [1]. The interaction of ionizing radiation with water results in the generation of primary water radical species like $^{\cdot}OH$, $^{\cdot}H$ and H_2O^+. The direct action of majority of primary radicals to bio-molecules are limited due to their short life time and hence its inability to diffuse up to target molecule These primary radicals generated during water radiolysis react with molecules like oxygen producing secondary radicals (H_2O_2 and O_2^-), which are not only relatively stable but could also diffuse to vital cellular targets like DNA, proteins and membrane [2]. It is now well established that radiation causes deleterious effects on the organisms and the current widespread use of radiation in diagnostic therapy, industry, power generation, unintentional exposure from air and space travel, nuclear accidents and nuclear attacks have necessitated an urgent need to safeguard humans against radiation exposure [3].

The potential application of radioprotective chemicals in the event of planned exposures or radiation accidents have been investigated from the beginning of the nuclear era [4]. It has also been considered possible that radiation therapy for cancer patients could be improved by the use of radioprotectors to protect normal tissues from unwanted radiation exposure. The most pragmatic approach in selecting a drug candidate to evaluate its radioprotective effect is to look into the available properties of the compound. Generally, a substance that either has anti-inflammatory, antioxidant, antimicrobial, immuno-modulatory, free radical scavenging or anti-stress properties may act as a potential radioprotector and could be the right candidate for the evaluation of radioprotective property [3]. Several compounds have been investigated over the years and some have also been approved for use either as a therapeutic agent or as dietary supplement to counteract the adverse effects of radiation exposure. As free radicals play a major role in the etiology of radiation induced tissue damage along with immunosuppression, the use of phytochemicals with antioxidant and immunomodulatory activity as *in vivo* radioprotectants has received much interest over recent years.

Hesperidin, a flavanone-type flavonoid, is found abundant in the peel and membraneous parts of citrus fruit [5]. Hesperidin is comprised of the flavanone hesperitin and the disaccharide rutinose and has been reported to have many biologically important properties, including anti-inflammatory, antimicrobial, anticarcinogenic, antioxidant and capillary strengthening effects [6]. Hesperidin has also been reported to possess antihypercholesterolemic activity [7], anti-inflammatory and analgesic activity [8], antifungal activity [9] and anticarcinogenic activity [10]. The antioxidant activity and radical scavenging

properties of hesperidin have been analyzed and reported by several investigators using a

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variety of assay systems [11, 12, 13, and 14]. Further, hesperidin was found to be effective in protecting liposomes from UV-irradiation induced peroxidation, probably by scavenging the oxygen free radicals generated by UV-irradiation [15]. Curdlan is an insoluble bacterial polysaccharide composed almost exclusively of ß-(1, 3)-glucosidic linkages. Apart from its immnuo-stimulating activity, the sulfated ß-(1, 3)-glucans have been shown to possess antiviral, antitumor and anticoagulant properties and are reported to interact with growth factors [16, 17, 18, 19 and 20].

The present investigation was performed to investigate the radioprotective effect of combined administration of hesperidin + curdlan in the liver of rats exposed to whole body γ-radiation.

MATERIALS AND METHODS

Chemicals and animals

All chemical including hesperidin were purchased from M/s. Sigma Chemical Co., St. Louis, USA. Curdlan was obtained from Takeda Chemical Industries Ltd. Healthy adult male Sprague-Dawley rats (160 ± 10 g) purchased from Central Lab Animal Inc., Seoul, Korea were used for the experiments. Rats were initially acclimatized to animal house conditions and were maintained under controlled conditions of temperature (25 ± 2 ^{o}C), humidity (55 ± 5%) and light (12 h of light and dark cycle) at the Central Animal Research laboratory, ARTI, KAERI. The animals had free access to sterile pellet diet and water. The study was approved by the Institutional Animal Ethical Committee and was performed in accordance with the Guide for the Care and Use of Laboratory Animals.

Exposure to γ-radiation and drug dosing

Unanaesthetized animals were placed in well ventilated acrylic restrainers and exposed to whole body γ-radiation (^{137}Cs at a dose rate of 1.10 Gy/min) from the Gammacell 40 Exactor (MDS Nordion, Canada). Immediately after irradiation the animals were sorted into individual cages and were monitored for the development of symptoms of radiation sickness and mortality throughout the study period, during which they had free access to food and water. The dose of γ-radiation (5Gy) was selected from the previously published reports of Meister [21]. Hesperidin and curdlan were freshly prepared on each day and administered as a combination at the same time throughout the study period using a ball tipped needle.

Experimental design

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After the animals were acclimatized to the animal house conditions, they were divided into 6 groups with 6 animals in each group. The experimental design and treatment schedule are as follows:

Group 1	Rats did not receive any treatment.
Group 2	Rats exposed to 5Gy of γ-radiation and sacrificed after 2 days.
Group 3	Rats exposed to 5Gy of γ-radiation and treated with H+C (100 mg/Kg) for 2 days and sacrificed.
Group 4	Rats exposed to 5Gy of γ-radiation and treated with H+C (100 mg/Kg) for 7 days and sacrificed.
Group 5	Rats administered H+C (100 mg/Kg) for 2 days before irradiation exposed to 5Gy of γ-radiation and sacrificed.
Group 6	Rats treated with Curdlan alone for 7 days.

Blood and tissue collection

At the end of the experimental period the animals were anaesthetized with diethyl ether following an overnight fasting and blood was collected from the orbital plexus using a sterile capillary tube. The serum was separated by centrifugation (1000 g for 20 min) according to the standard protocol and stored at 4 ^{o}C until further analysis. The animals were then decapitated by cervical dislocation and the liver was excised, washed in ice cold saline, weighed and stored at – 80 ^{o}C. The tissues were homogenized in ice-cold buffer (0.1M Tris-HCl, pH 7.4), centrifuged and used for biochemical analysis. A piece of the liver tissue from each group was fixed in 10% buffered formalin for histological analysis.

Biochemical analysis

Aspartate and alanine transaminases (AST and ALT) were assayed in the serum and liver tissue homogenate according to the method of Bergmeyer et al [22]. Alkaline phosphatase (ALP) was assayed in the serum and liver tissue homogenate according to the method of Williamsen [23] using disodium phenyl phosphate as substrate. Lactate dehydrogenase (LDH) was assayed by the method of King [24] using lithium lactate as substrate. γ-Glutamyl transpeptidase (γ-GT) in the serum and liver was estimated as described by Rosalki and Rau [25] using L-γ-glutamyl-p-nitroanilide as substrate . Protein was estimated by the method described by Bradford [26] using bovine serum albumin as standard. Lipid peroxidation was determined in the liver tissue as described by Ohkawa et al [27]. Superoxide dismutase (SOD) and Catalase (CAT) in the liver tissue were assayed using commercial assay kits (Calbiochem, Germany) as per the manufacturer's instruction. The activity of glutathione peroxidase in the liver was estimated by the methods of Rotruck et al [28]. Liver glutathione (GSH) content was estimated by the method of Beutler et al [29]. Vitamin C in the liver tissue was quantified as described by Omaye et al [30] and vitamin E was estimated by the method of Kayden et al [31]. Histopathology of

the liver tissue was performed by processing the tissue in ethanol followed by xylene washing

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and embedded in paraffin wax. Five micron thick sections were cut using a microtone (Leica Microsystems, Germany). The sections were de-waxed, stained with Hematoxylin and Eosin and observed under a microscope (Nikon Eclipse E 400) and photographed.

Statistical analysis

Statistical analysis was done using one way analysis of variance (ANOVA) and inter group comparisons were made using Tukey's multiple comparison test using SPSS statistics software. The values are expressed as mean ± S.D. for six samples in each group. *P* value < 0.05 was considered as significant.

RESULTS AND DISCUSSION

Serum AST, ALT, ALP, LDH and γ-GT induced by γ-radiation exposure and the effects of post and pre-treatment with hesperidin + curdlan are shown in Fig. 1 and 2. In the rats exposed to 5 Gy of γ-radiation, there was a highly significant increase in the activities of the above enzymes post 7 days of irradiation. The status of these marker enzymes are sensitive indices of hepatocellular necrosis as cell damage results in the leakage of these enzymes into the systemic circulation [32]. It is reported that ionizing radiation damage is caused by either a direct interaction with target molecules or indirect action by the formation of chemically and pharmacologically active elements produced mainly by water molecules [33]. The increase in the activities of these enzymes in the serum after exposure to 5 Gy of γ-radiation can be attributed to the possible release of these enzymes from different tissues associated with the obstruction of the blood flow to the liver. The increase in serum transaminase activities by γ-radiation may be due to the damage of cellular membranes of hepatocytes, which in turn leads to an increase in the permeability of cell membranes and facilitates the passage of cytoplasmic enzymes outside the cells leading to the increase in the transaminase activities in blood serum [34]. The results show that exposure of rats to whole-body γ-radiation at 5 Gy resulted in an increase in γ-GT activity that reached its maximum value after 2 days post-exposure. These results are in agreement with those of Reva et al [35], who observed an increase in liver and serum γ-GT activity during exposure to x-rays. Pre and Post treatment with hesperidin + curdlan for 2 days resulted in minor decrease, whereas post treatment for 7 days produced a highly significant decease in the activities of AST, ALT, ALP, LDH and γ-GT when compared with γ-radiation exposed rats. This is indicative of the protective effect of hesperidin + curdlan in ameliorating the hepatocellular damage caused by γ-radiation.

An important phenomenon, which is suggested to play a major role in radiation-induced toxicity, is the oxidative stress. As water is the major constituent of all living cells, exposure to γ-radiation causes radiolysis of water producing radical species like OH• and H• and some molecular products like H_2O_2 and H_2^-. These highly unstable free radicals attack the

cell membrane lipids in their vicinity leading to a cascade of reaction called lipid peroxidation

[36]. The lipid peroxidation levels in the liver tissue of control, radiation exposed and drug treated rats is shown in Fig. 3. At the dose of 5Gy, there was significant increase in the levels of TBARS post 2 days of irradiation. Several other investigators have reported a dose dependent elevation in the lipid peroxidation levels in the tissues of γ-radiation exposed rats and our results are in agreement with their findings. While pretreatment with hesperidin + curdlan at a dose of 100 mg/Kg, 2 days prior to 5Gy irradiation did not significantly bring back the levels of

TBARS to near normal, post treatment for 7 days resulted in significant decrease in the levels of TBARS in the liver tissue. This is a clear indication of the anti lipid peroxidative effect of hesperidin and curdlan.

In the present study, we observed a decrease in enzymatic (SOD, CAT and GPx) and non-enzymatic antioxidant (GSH, vit-C and vit-E) status, along with an increase in the lipid peroxidative index (TBARS) in the liver tissue during radiation exposure (Fig. 4). The results suggest that cellular endogenous antioxidants are not able to counteract the increased amount of free radicals generated during radiolysis and subsequently, these increased free radicals attack membrane lipids and progresses lipid peroxidation. Post treatment with hesperidin + curdlan for 7 days resulted in highly significant almost complete restoration in the status of enzymic and non-enzymic antioxidants in the liver tissue thereby maintaining a balance in the oxidant/antioxidant status in the liver. This is suggestive of the possible antioxidant effect of hesperidin + curdlan. Saada et al [37] observed a significant decrease in GPx and GR post 72 h of radiation exposure. They suggested that the GPx activity increased to destroy the excess H_2O_2 formed after exposure to radiation. The reaction is catalyzed in the presence of reduced glutathione and the increase in glucose-6-phosphate dehydrogenase that generates NADPH which is essential to reduce oxidized glutathione. This necessitates an increase in the activity of GR that uses NADPH to reduce glutathione with the generation of NADP. The net result is the destruction of H_2O_2. On the other hand, the decrease in GSH content, SOD, CAT and GPx activities as recorded in the present study after 7 days post-irradiation are in agreement with those recorded by Othman [38] and Saada et al. [37]. They recorded a significant depletion in the antioxidant system accompanied by enhancement of lipid peroxides in rats after whole-body γ-radiation and which is in accordance with our observations.

Non-enzymic antioxidants such as reduced glutathione, vitamin C and vitamin E play an excellent role in protecting the cells from oxidative damage [39, 40]. It is well established that GSH in blood keeps up the cellular levels of the active forms of vitamin C and vitamin E by neutralizing the free radicals. When there is a reduction in the GSH content the cellular levels of vitamin C is also lowered, indicating that GSH, vitamin C, and vitamin E are closely interlinked to each other [41]. In agreement with this report, the decreased levels of GSH, vitamin C and vitamin E were observed in our study following γ-radiation exposure (Fig. 5 and 6). Administration of hesperidin + curdlan to γ-radiation exposed rats for 7 days, maintained the level of non-enzymic antioxidants to near normal, by the possible role of hesperidin + curdlan in

improving the GSH status. Thus hesperidin + curdlan treatment mitigates lipid peroxidation in

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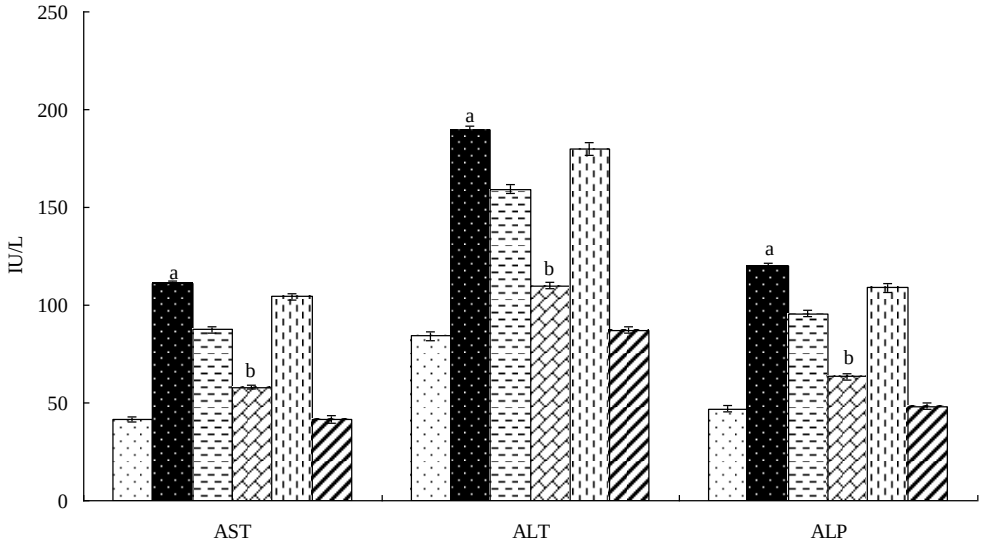
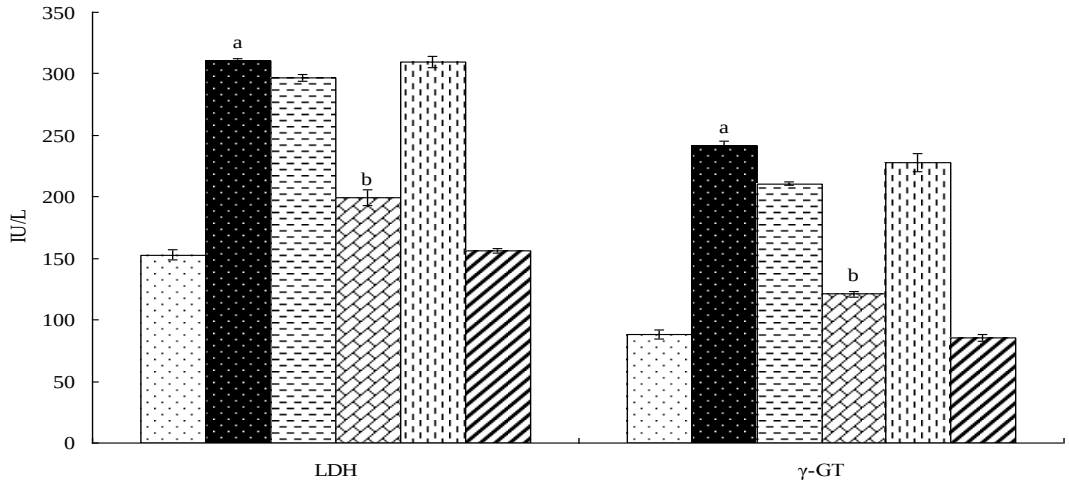
the liver by maintaining the status of cellular non-enzymic antioxidants.

Fig. 1. Status of AST, ALT and ALP in the serum of control and experimental animals following γ-radiation exposure



Values expressed as mean ± S.D. for 6 animals in each group. Comparisons were made between: a – compared with control group; b – compared with radiation exposed group. P<0.001 was considered significant.

Fig. 2. Status of LDH and γ-GT in the serum of control and experimental animals following γ-radiation exposure



Values expressed as mean ± S.D. for 6 animals in each group. Comparisons were made between: a – compared with control group; b – compared with radiation exposed group. P<0.001 was considered significant.

Fig. 3. Status of lipid peroxidation in the liver tissue of control and experimental animals following γ-radiation exposure

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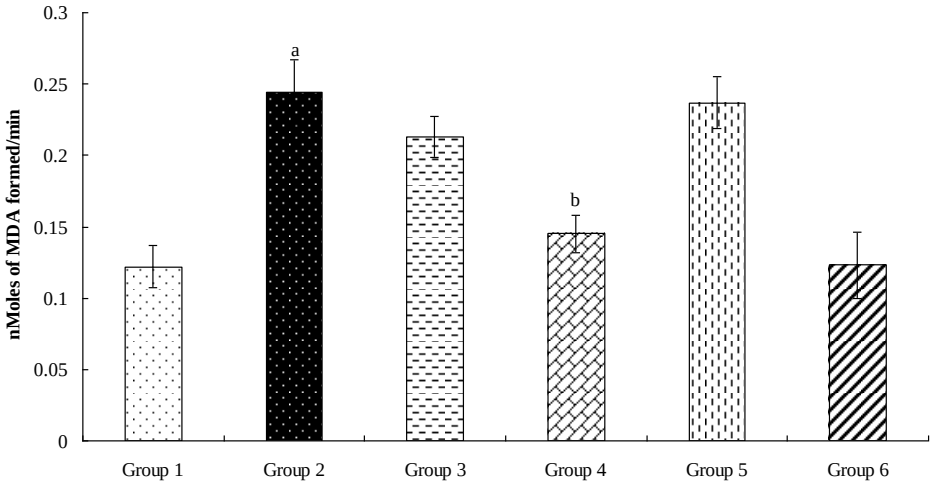
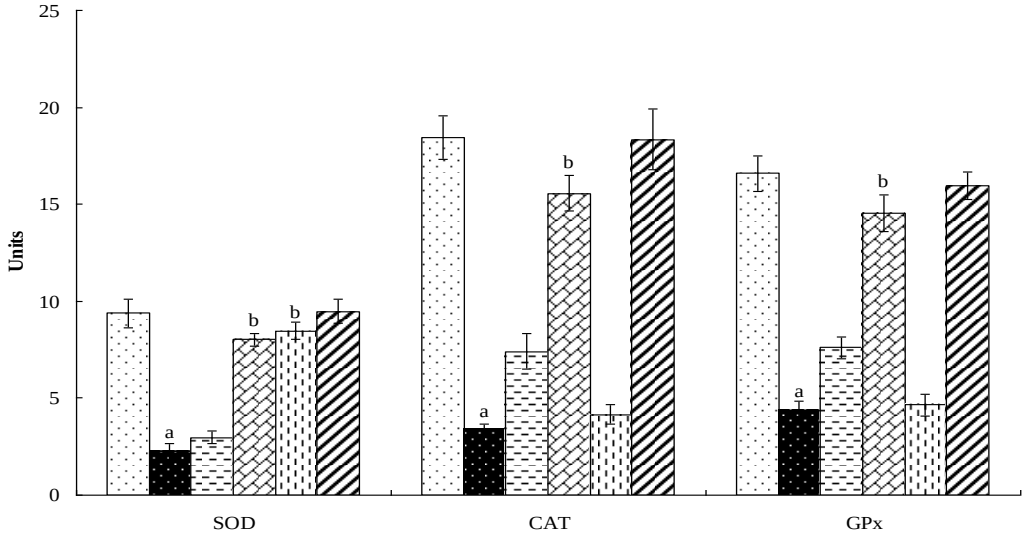


Values expressed as mean ± S.D. for 6 animals in each group. Comparisons were made between: a – compared with control group; b – compared with radiation exposed group. P<0.001 was considered significant.

Fig. 4. Status of SOD, CAT and GPx in the liver tissue of control and experimental animals following γ-radiation exposure



Values expressed as mean ± S.D. for 6 animals in each group. Comparisons were made between: a – compared with control group; b – compared with radiation exposed group. P<0.001 was considered significant.

Fig. 5. Status of vitamin-C and vitamin-E in the liver tissue of control and experimental animals following γ-radiation exposure

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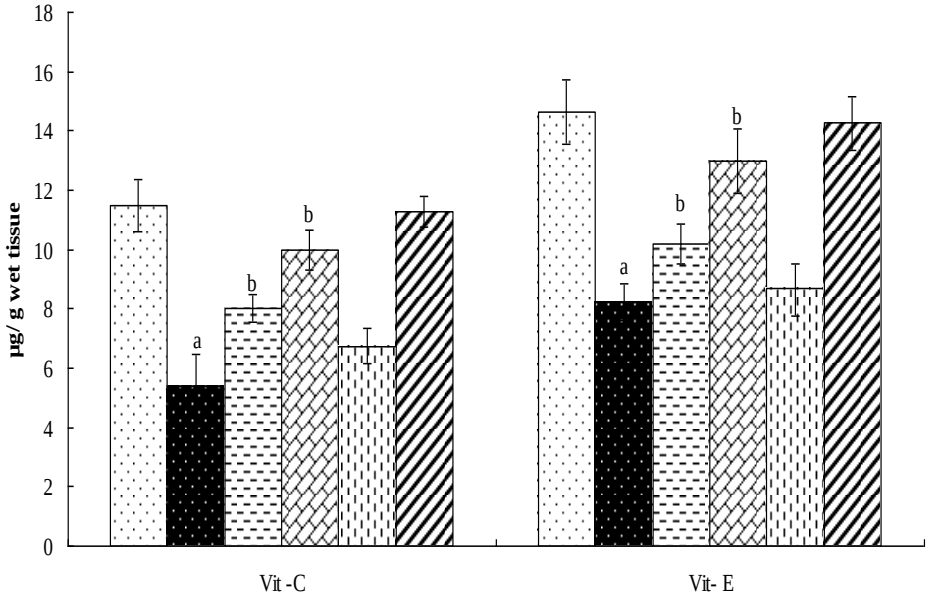
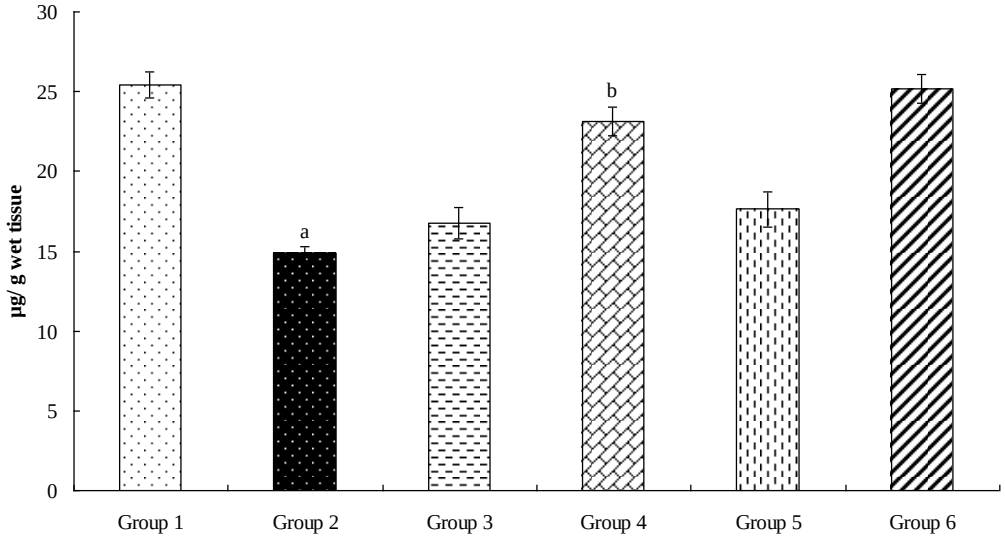


Values expressed as mean ± S.D. for 6 animals in each group. Comparisons were made between: a – compared with control group; b – compared with radiation exposed group. P<0.001 was considered significant.

Fig. 6. Status of reduced glutathione in the liver tissue of control and experimental animals following γ-radiation exposure



Values expressed as mean ± S.D. for 6 animals in each group. Comparisons were made between: a – compared with control group; b – compared with radiation exposed group. P<0.001 was considered significant.

Fig. 7. H & E Stained sections of the liver tissue of control and experimental rats (100X)

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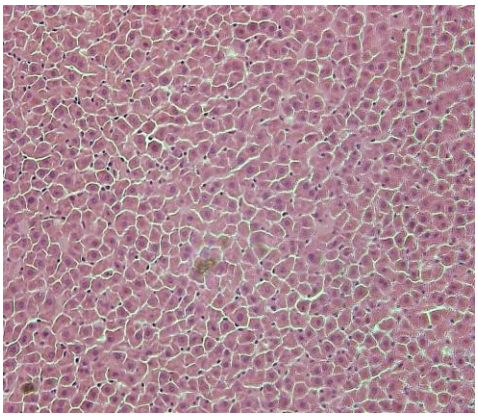
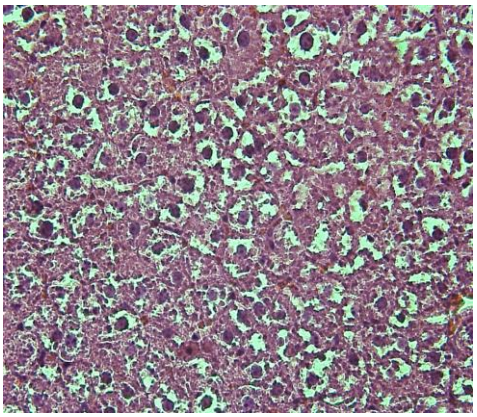


Group 1



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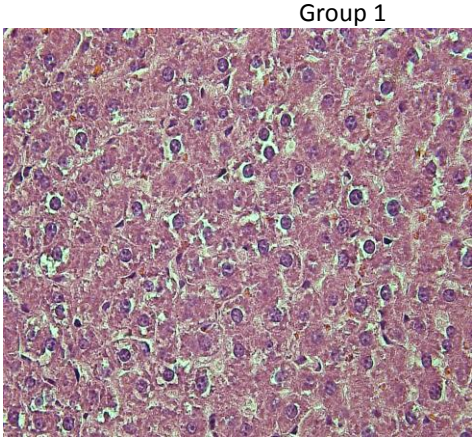

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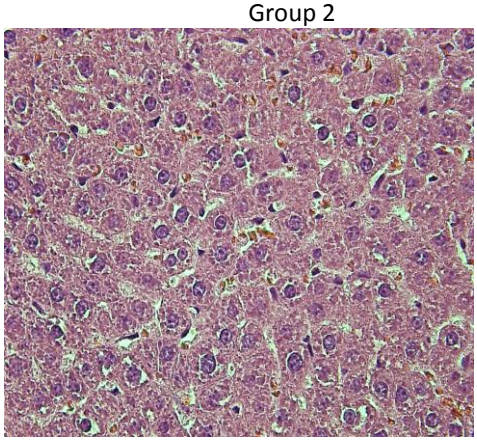

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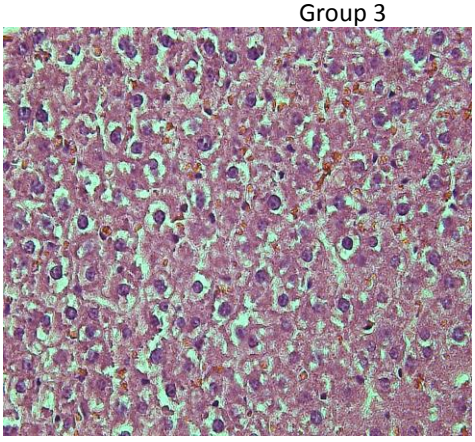
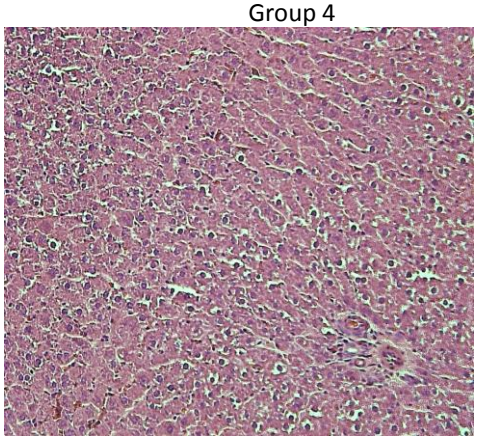


Group 5



Group 6

Figure Legends

- Control rats administered with the vehicle alone
- Rats exposed to 5 Gy of γ-irradiation.
- Rats exposed to 5Gy of γ-radiation and treated with H+C (100 mg/Kg) for 2 days.
- Rats exposed to 5Gy of γ-radiation and treated with H+C (100 mg/Kg) for 7 days.
- Rats administered H+C (100 mg/Kg) for 2 days before 5 Gy irradiation.

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- Rats administered H+C (100 mg/Kg) for 7 days

The histopathological observation of the control and experimental liver sections stained with H & E also substantiated the radioprotective effect of hesperidin + curdlan treatment. Severe enlargement of the heptocytes with cytoplasmic vacuolation, loss of hepatic cell plates, single cell necrosis or apoptosis with considerable dilation in the sinusoids with few red blood cells was observed in the liver sections of the rats exposed to 5 Gy of γ-radiation (Fig. 7). Hesperidin + curdlan treatment for 7 days post irradiation showed a recovery pattern of the

damaged hepatocytes characterized by a mild enlargement of the heptocytes, fairly good intact heptocytes in the centrilobular regions with a normal hepatic cell plate and a minor dilation of the sinusoids. Rats treated with hesperidin + curdlan did not show any signs of necrosis or apoptosis, with intact heptocytes in the centrilobular regions and a normal hepatic cell plate and normal endothelial cells and were comparable with the control rats. Administration of curdlan alone did not cause any alteration to most of the parameters investigated indicating its safe and non-toxic nature to the biological system.

In conclusion, the present investigation indicates that combined administration of hesperidin and curdlan offers significant protection against γ-radiation induced hepatocellular damage by its ability to ameliorate the lipid peroxidation through the free radicals scavenging activity, which enhanced the levels of antioxidant defense system. Further studies are underway to investigate the effect of hesperidin + curdlan treatment on the immune system during radiation exposure.

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