



GESDAV

Oxidants and Antioxidants in Medical Science

available at www.scopemed.org

Original Article

Study of *Mentha piperita* against gamma radiation in mice

Pallavi Kaushik¹, Megha Mathur¹, Neha Rawat¹, Tanushree Saxena¹,
Sanjoli Mobar¹, Punar Dutt Meena²

¹Department of Life Science, The IIS University, Jaipur;

²Department of Zoology, Government College, Thanagazi, Alwar; Rajasthan, India

Received May 22, 2013

Accepted July 19, 2013

Published Online September 3, 2013

DOI 10.5455/oams.190713.or.050

Corresponding Author

Pallavi Kaushik

Department of Life Sciences,

The IIS University,

Gurukul Marg, SFS, Mansarovar,

Jaipur-302020, Rajasthan, India.

drpallavikaushik@rediffmail.com

Key Words

Hepatoprotective; Lipid Peroxidation;
Mentha piperita; Radiolesions; Reduced
Glutathione

Abstract

The aim of this study is to analyze the potential hepatoprotective efficacy of alcoholic extract of *Mentha piperita* (Linn) against radiation-induced liver injury in Swiss albino mice after whole body irradiation in terms of histopathological lesions and levels of lipid peroxidation and reduced glutathione in liver tissue. *M.piperita* leaves were extracted in 50% ethanol and administered at the dose of 100 mg/kg b.wt/day for three consecutive days before whole body irradiation (6, 8 and 10 Gy). The extract provided significant hepatoprotection against different doses of radiation in terms of higher number of normal hepatocyte count, lower abnormal hepatocyte count and lower binucleated cell population along with other radiolesions as compared to irradiation alone group. The optimum dose of 100 mg/kg b.wt/day for three consecutive days also arrested decline in the level of reduced glutathione and elevation in lipid peroxidation significantly at autopsy intervals of 1, 3, 7, 14 and 30 days post-irradiation (6 and 8 Gy). The results of this study confirmed the hepatoprotective potential of *M.piperita* leaves alcoholic extract in controlling the development of radiolesions and number of abnormal hepatocytes in liver. This protection can be attributed to the antioxidant and antiperoxidant properties of *Mentha piperita*.

© 2013 GESDAV

INTRODUCTION

The liver is a vital organ which performs wide range of functions, including detoxification, production of bile, metabolism of ingested nutrients, elimination of many waste products and plasma protein synthesis. In humans the radiation doses ranging between 2-8 Gray (Gy) are often used for treating cervical and bone cancers [1, 2]. Whole body or localized irradiation in humans often results in development of radiation-induced liver disease; thus, it seems reasonable to study liver for radiation injury as well as protection [3]. Radiotherapy has been widely used for treatment of abdominal neoplasms which often results in symptoms of radiation-induced liver disease (RILD) [4, 5]. Sub-acute RILD patients show development of veno-occlusive disease (VOD) characterized by marked centilobular congestion, yellow necrosis, collapse of the parenchyma, hyperemia, mild congestion of portal vein and increased size of Kupffer cells [6-8].

Whole body irradiation also results in biochemical changes in liver like decrease in glutathione (GSH)

levels while malondialdehyde (MDA) levels increased in tissues [9]. This can be induced because of free radicals which impair the antioxidant defense mechanism leading to damage of the membrane structure and inactivation of membrane bound enzymes [10].

Numerous drugs of synthetic or natural origin have been screened for their radioprotective efficacy. The molecular drug amifostine, the only drug which is approved by the Food and Drug Administration (FDA) in the United States, is the most thoroughly investigated drug but its radioprotective effects are short term and produce severe side effects at clinically effective doses [11, 12]. In fact, it can be said that there exist no single radioprotective agent which meets all the prerequisites of an ideal radioprotector [13, 14]. The role of the protective substances evidently accounts to a prevention of the secondary reactions which are the result of the primary radiochemical process induced by exposure. The primary mode of radiation induced injury is the production of reactive

oxygen species (ROS). Thus plant extracts which contain antioxidant properties seem promising in protection against ROS-mediated damage induced by radiation. Antioxidants delay the oxidation of biomolecules by inhibiting the initiation and propagation of oxidizing chain reactions and thus interfere with the process of apoptosis [15]. A number of plant extracts have exhibited considerable radioprotection due to their radical scavenging activity, which is examined as their effect on radiation-induced lipid peroxidation (LPO) and GSH levels [16, 17], but meager reports are available regarding the protection of histopathological lesions in liver. As large scale studies of various aspects of radiation exposure in human are limited and many times prohibited, animal models can provide useful information about radiation-induced injury and its protection using suitable drugs [18].

Mentha is one of the genera that are used in traditional Chinese, Thai and ayurvedic medicine for the treatment of fever, pain and dysentery [19, 20]. *Mentha piperita* (Linn) (peppermint), the 'medicinal plant of the year 2004' [21], is a perennial, glabrous and strongly scented medicinal herb belonging to the Labiatae family (also known as Lamiaceae) which has been shown to possess antioxidant, antimicrobial, antifungal, anticarcinogenic and antiallergic activity [22-33]. The aqueous extract of *Mentha piperita* has been studied for protection against radiation injury [34, 35] but its alcoholic extract has yet not been studied for hepatoprotection. Thus the present study deals with analysis of the hepatoprotective efficacy of the alcoholic extract of *M.piperita* (ALM) against radiation-induced liver injury in Swiss albino mice after whole body irradiation in terms of histopathological lesions and levels of LPO and GSH in liver.

MATERIALS AND METHODS

Animals

Six-eight week old female Swiss albino mice (*Mus musculus*) weighing 25 ± 2 g (procured from Hamdard University, Delhi, India) housed under controlled conditions of $25 \pm 2^\circ\text{C}$ temperature and 14:10 h light:dark cycle were used for the present study. The animals were given pelleted standard mice feed (obtained from Hindustan Lever Ltd, Delhi) and water *ad libitum*.

Irradiation

The Cobalt Teletherapy Unit (ATC-C9) in the Cancer Treatment Center, Radiotherapy Department S.M.S. Medical College and Hospital, Jaipur was used for irradiation exposure.

Unanesthetized animals were restrained in well-ventilated Perspex boxes and their whole body was

exposed to different doses (6, 8 and 10 Gy) of gamma radiation at a source-to-skin distance (SSD) of 77.5 cm to deliver the dose rate of 1.64 Gy/min.

Preparation of alcoholic extract of *Mentha*

Mentha piperita collected locally was identified and specimen was placed at Herbarium, Department of Botany, University of Rajasthan, Jaipur. The voucher number is RUBL-19443. Fresh leaves of *M.piperita* (Linn) were washed, air dried, powdered and extracted with 1500 ml of 50% ethanolic solution in double distilled water (DDW) and by refluxing for 36 h (3 x 12) at 60°C . The obtained extract was vacuum-evaporated and powdered. The optimum dose of ALM against gamma radiation-induced mortality was determined in the earlier study in our laboratory [36]. The extract was suspended in DDW and 0.1 ml of ALM suspension was given to each mouse by oral gavage dissolved at a dose of 100 mg/kg b.wt/day for 3 consecutive days before irradiation.

Experimental design

The animals selected for this study were divided into four groups:

-**Group I (normal)**; animals of this group were sham-irradiated

-**Group II (drug alone)**; animals of this group were given orally 100 mg/kg b.wt/day of ALM for 3 consecutive days

-**Group III (control; radiation alone)**; animals were given DDW for 3 consecutive days equal to the volume of ALM and then exposed to different doses of radiation:

-**Subgroup IIIa**; DDW + 6 Gy

-**Subgroup IIIb**; DDW + 8 Gy

-**Subgroup IIIc**; DDW + 10 Gy

-**Group IV (experiment; ALM + radiation treatment)**; animals of this group were given orally 100 mg/kg b.wt/day of ALM for 3 consecutive days and then exposed to different doses of gamma radiation as follows.

-**Subgroup IVa**; ALM + 6 Gy

-**Subgroup IVb**; ALM + 8 Gy

-**Subgroup IVc**; ALM + 10 Gy

Autopsy intervals

A minimum of five animals from all the above groups were autopsied by cervical dislocation at 1, 3, 7, 14 and 30 days post-treatment/irradiation and their liver tissues were removed and excised immediately after the autopsies, and fixed in Bouin's fixative. Following 16-24 h of fixation, tissues were washed, dehydrated, cleared and embedded in paraffin wax ($58-60^\circ\text{C}$) followed by their microtome sectioning at 5μ . The staining techniques employed for histological studies involved hematoxylin-eosin (HE).

Qualitative studies

The stained slides were observed at 100x and 400x magnifications for qualitative changes as hyperemia, degranulation in hepatoplasm, edema, lymphocytic infiltration, hydropic degeneration, giant cells and swollen Kupffer cells.

Quantitative studies

The stained slides were employed for the Histometric measurements on several 16 x 40 microscopic fields. At least 500 counts were taken in each case and then average values representing the percentage of normal, abnormal (pycnotic nuclei, necrotic cells) and binucleated cells were obtained.

Biochemical study

-Reduced glutathione assay: The level of GSH was determined by the method of Moron *et al* [37]. GSH content in liver was measured spectrophotometrically by using 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB or Ellman's reagent) as a coupling reagent. The absorbance was read at 412 nm with a UV-VIS spectrophotometer (Systronics).

-Lipid peroxidation assay: The LPO level in liver and serum was measured in terms of thiobarbituric acid reactive substances (TBARS) by the method of Ohkawa *et al* [38]. The absorbance was read at 532 nm.

Statistical analysis

The results obtained from the present study were expressed as mean \pm SEM. Student's t-test [39] was used to make a statistical comparison between the groups. Statistical comparisons were completed with the irradiation alone group vs normal, normal vs drug alone and irradiation alone vs radiation and ALM-treated combined group. The significance level was set at $P < 0.05$.

RESULTS

Qualitative study of liver

In the present study, no toxic effects on liver were observed in mice treated with ALM alone (Fig.1) and the histology of liver was found to be similar to normal group (Fig.2).

The liver of control (irradiation alone) mice showed radiolesions in the form of dilated sinusoidal spaces, cellular edema, hydropic degeneration, hyperemia, lymphocytic infiltration, degranulated and vacuolated cytoplasm, swollen Kupffer cells, giant cells, multinucleated cells, enucleated cells and necrotic cells along with many pycnotic nuclei in post-irradiation autopsy intervals. At 6 and 8 Gy irradiation dose maximum damage was seen on day 3 post-irradiation with a trend towards normalization from day 7, relapse of damage was observed on day 14 (Figs.3&4).

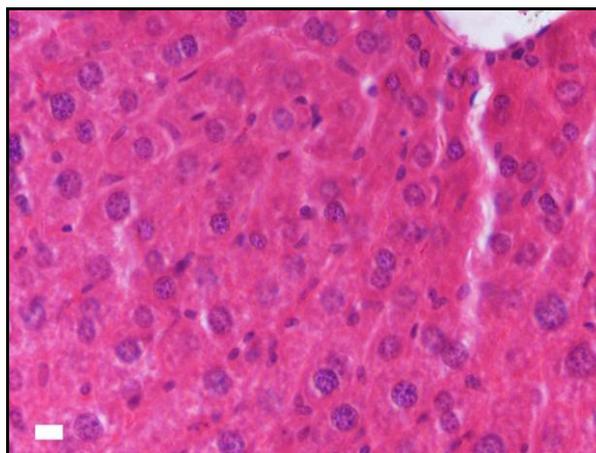


Figure 1. Drug alone group; showing normal liver structure (400x HE).

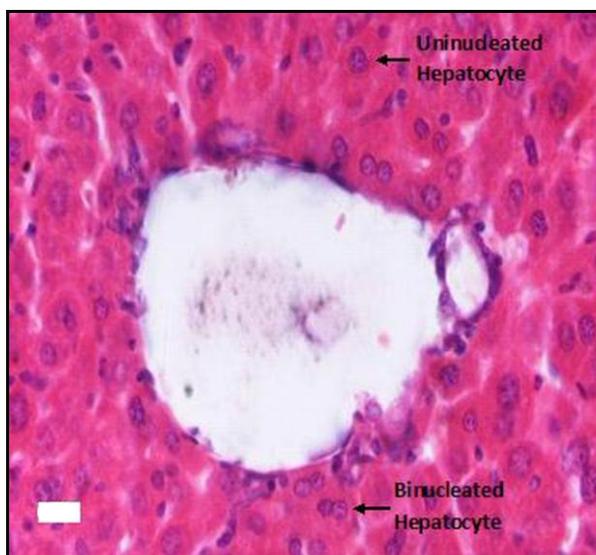


Figure 2. Normal group; showing normal liver structure with granulated cytoplasm, uninucleated and some binucleated hepatocytes (400x, HE).

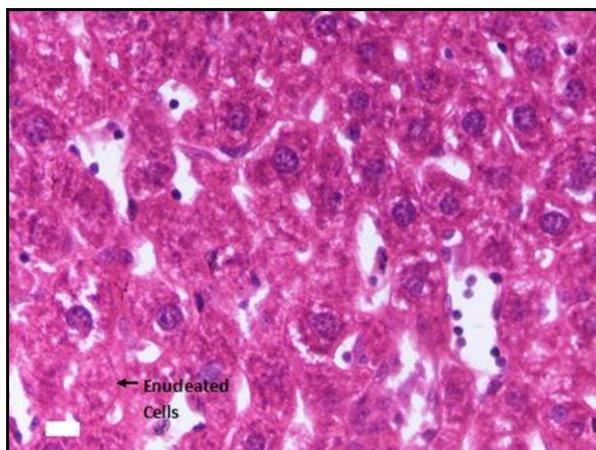


Figure 3. 6 Gy irradiation alone group; day 3 liver histology showing enucleated cells, increased sinusoidal spaces and lymphocytic infiltration (400x, HE).

However, by day 30 almost normal liver structure was attained, but the severity of damage was dose dependent.

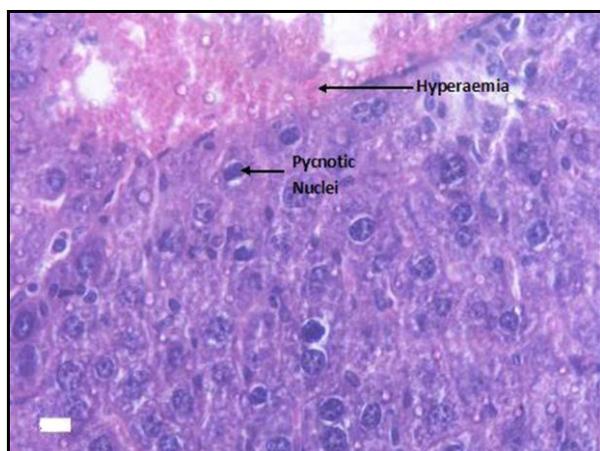


Figure 4. 8 Gy irradiation alone group; day 1 liver histology showing hyperemia, pycnotic nuclei, enucleated cells and degranulated cytoplasm (400x, HE).

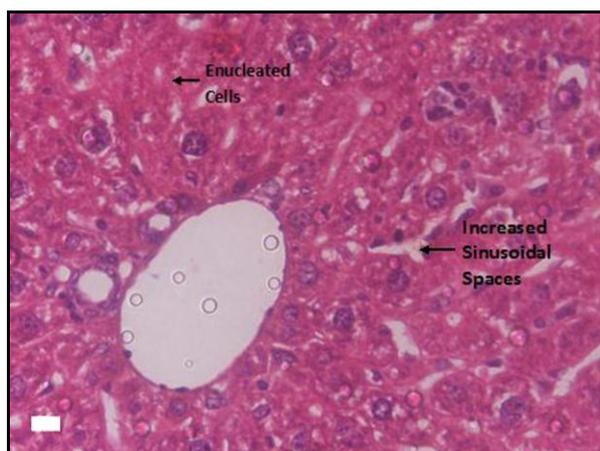


Figure 5. 6 Gy irradiation + ALM group; day 3 liver histology showing few enucleated cells and lymphocytic infiltration (400x).

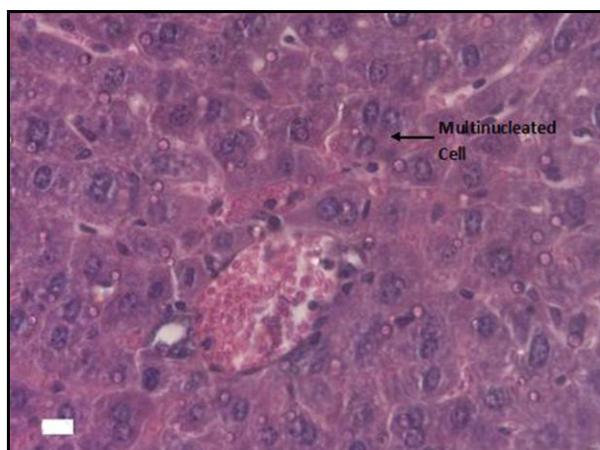


Figure 6. 8 Gy irradiation + ALM group; day 1 liver histology showing hyperemia lymphocytic infiltration, binucleated and multinucleated cells (400x, HE).

ALM pretreatment afforded significant protection against radiation-induced hepatic lesions at all the autopsy intervals and 6 and 8 Gy doses of radiation (Figs.5&6). The protection by ALM at irradiation dose of 10 Gy was exhibited in form of minor suppression in severity of radialesions as compared to control (Figs.7&8).

Quantitative study

-Normal hepatocytes: drug alone group did not showed any significant variation in number of normal hepatocytes as compared to normal. In the control group a dose dependent decline in normal cell population was observed as compared to normal with highest decline at 10 Gy followed by 8 Gy and 6 Gy. The decline was observed up to day 3 followed by a slight increase on day 7, further decline on day 14, but by day 30 post-irradiation considerable recovery in normal hepatocyte number was observed in the survivors of 6 and 8 Gy treated groups. The population of normal hepatocytes also decreased after irradiation in the ALM and radiation combined group. This decline was always significantly lower than the respective control groups (Table 1).



Figure 7. 10 Gy irradiation alone group; day 3 liver histology showing enucleated, necrotic cells and hydropic degeneration (400x).

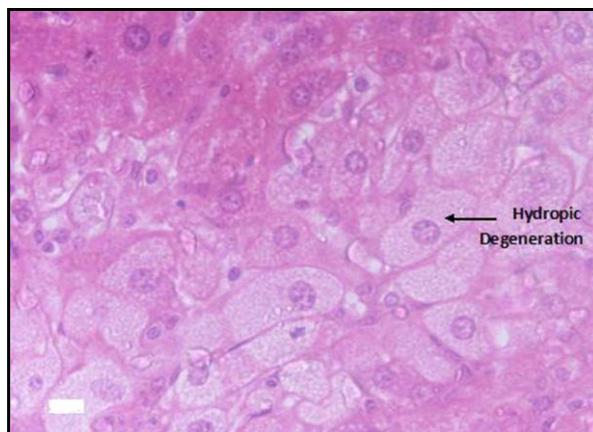


Figure 8. 10 Gy irradiation + ALM group; day 3 liver histology showing enucleated, necrotic cells and hydropic degeneration (400x).

Table 1. Percentage of normal, abnormal and binucleated hepatocytes after exposure to 6, 8 and 10 Gy gamma rays with (Exp) and without (Control) pretreatment of ALM

Dose	Hepatocyte	Group	Autopsy intervals (days)				
			1	3	7	14	30
6 Gy	Normal	Control	71.24 ± 1.52 ^c	70.58 ± 2.26 ^c	78.44 ± 2.16 ^b	71.55 ± 1.81 ^c	81.32 ± 2.38 ^a
		Exp	81.24 ± 2.51 ^b	80.24 ± 3.34 ^b	88.18 ± 1.32 ^b	84.18 ± 1.42 ^c	89.24 ± 1.48 ^b
	Abnormal	Control	10.4 ± 0.125 ^c	11.46 ± 0.86 ^c	9.22 ± 0.64 ^c	12.46 ± 0.26 ^c	7.84 ± 0.34 ^c
		Exp	8.16 ± 1.16 ^b	9.18 ± 0.36 ^a	8.46 ± 0.37	9.52 ± 1.18 ^b	4.67 ± 0.46 ^c
	Binucleated	Control	13.62 ± 1.42 ^b	14.22 ± 0.18 ^c	11.62 ± 0.32 ^c	12.16 ± 1.16 ^c	10.68 ± 0.15 ^c
		Exp	10.18 ± 1.55 ^b	10.08 ± 0.42 ^c	9.24 ± 1.46 ^b	9.08 ± 1.86 ^a	8.42 ± 1.182 ^c
8 Gy	Normal	Control	70.02 ± 1.52 ^c	68.08 ± 52.46 ^c	75 ± 1.82 ^c	70.46 ± 1.127 ^c	71.42 ± 1.192 ^c
		Exp	80.56 ± 3.32 ^c	79.86 ± 1.136 ^c	84.36 ± 2.148 ^a	82.48 ± 3.131 ^b	83.24 ± 2.22 ^b
	Abnormal	Control	11.2 ± 0.204 ^c	12.42 ± 0.35 ^c	12.98 ± 0.149 ^c	13.41 ± 0.148 ^c	9.76 ± 0.21 ^c
		Exp	9.12 ± 0.24 ^c	9.84 ± 0.184 ^c	10.16 ± 0.65 ^b	10.46 ± 1.4 ^c	7.16 ± 0.55 ^b
	Binucleated	Control	14.15 ± 0.33 ^c	14.96 ± 0.35 ^c	13.48 ± 0.38 ^c	14.26 ± 0.29 ^c	11.42 ± 0.5
		Exp	11.42 ± 0.55 ^b	12.96 ± 0.34 ^b	11.24 ± 2.24 ^b	10.25 ± 0.64 ^c	9.25 ± 1.152 ^b
10 Gy	Normal	Control	69.45 ± 1.131 ^c	68.83 ± 1.129 ^c	58.34 ± 3.96 ^c	---	---
		Exp	75.52 ± 3.118 ^a	74.67 ± 1.22 ^b	72.25 ± 2.81 ^c	56.44 ± 2.40	---
	Abnormal	Control	12.04 ± 1.101 ^c	18.46 ± 0.4 ^c	24.36 ± 0.55 ^c	---	---
		Exp	9.03 ± 0.55 ^a	12.18 ± 0.18 ^c	13.22 ± 0.31 ^c	20.21 ± 1.98	---
	Binucleated	Control	14.32 ± 1.136 ^c	15.22 ± 1.3 ^c	18.48 ± 1.22 ^c	---	---
		Exp	12.25 ± 0.56 ^b	9.64 ± 0.58 ^b	10.52 ± 1.16 ^b	16.56 ± 0.16	---

Normal values: normal hepatocyte 91.27 ± 1.84%; abnormal hepatocytes 2.56 ± 0.15%; binucleated hepatocytes 8.46 ± 0.22%. Statistical comparisons were performed Control vs normal values and Exp vs Control; ^aP < 0.05, ^bP < 0.01, ^cP < 0.001.

-Abnormal hepatocytes: drug alone group did not showed any significant variation in number of abnormal hepatocytes as compared to normal. In the irradiation alone group a dose-dependent increase in abnormal cell population was observed at all irradiation doses with maximum increase at 10 Gy followed by 8 Gy and 6 Gy. The increase in number of abnormal hepatocytes was observed up to day 3 followed by a slight decline on day 7, further increase on day 14, followed by considerable decline on day 30 post-irradiation in the control groups. The population of abnormal hepatocytes also increased after irradiation in the experimental groups but this increase was always significantly lower than the respective control groups (Table 1).

-Binucleated cells: the number of binucleated cells increased up to day 3 in 6 Gy and 8 Gy and up to day 7 in 10 Gy irradiation-treated groups. Their number showed a slight decrease on day 7 followed by increase on day 14 and considerable recovery by reaching close normal on day 30 post-irradiation in 6 Gy and 8 Gy treated groups. The experimental groups showed similar trend of increase and decrease of binucleated cells but were always significantly lower than the respective control groups (Table 1).

Biochemical estimation of lipid peroxidation and glutathione

-Lipid peroxidation: a dose dependent increase in

TBARS level of liver was evident in control animals with maximum on day 14. However, no significant difference was noticed in levels of TBARS in normal and drug alone groups. ALM pretreatment exhibited considerable radioprotection by significant arrest of LPO elevation at all autopsy intervals as compared to control reaching near normal values on day 30 (Fig.9).

-Reduced glutathione: the normal group did not exhibit any significant variation in GSH levels but the drug alone group showed higher values than normal at all autopsy intervals. Animals of irradiation alone showed dose dependent decline in GSH levels at all autopsy intervals as compared to normal. ALM pretreatment before irradiation exhibited significantly elevated values of GSH level in liver as compared to control at all autopsy intervals (Fig.10).

DISCUSSION

The efficacy of any hepatoprotective drug is essentially dependent on its capacity of either reducing the harmful effects or maintaining the normal physiologic function which has been distributed by hepatotoxic agents [40]. Radiation exposure results in severe damage to liver. This causes necrosis of liver cells at varying level, depending on the radiation dose. Extensive necrosis of parenchymatous cells of liver after irradiation has long been known [41].

Figure 9.
Lipid peroxidation (LPO) level
in liver tissue of mice
with or without pretreatment of ALM
and/or exposed
to different doses of gamma radiation.
^aP < 0.05, ^cP < 0.001.

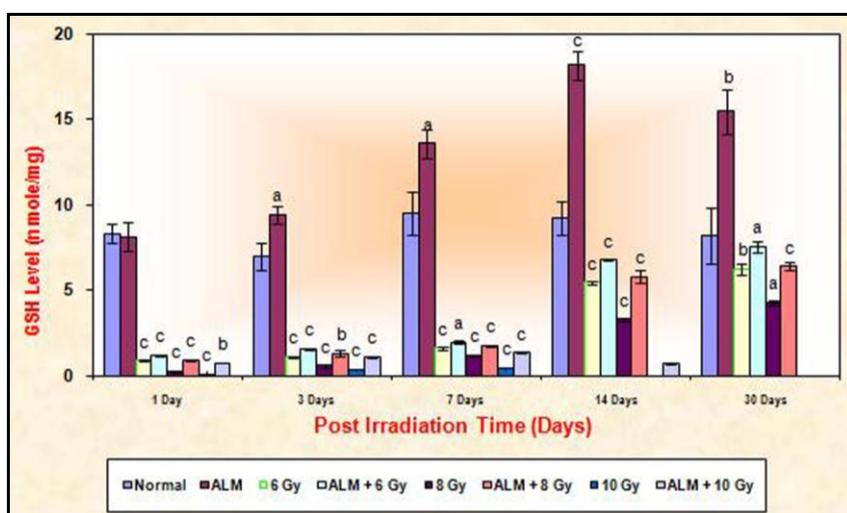
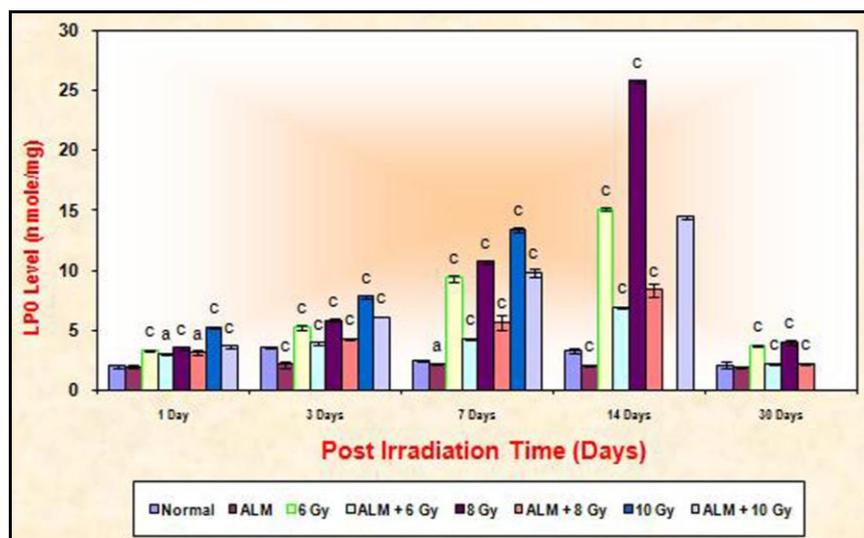


Figure 10.
Reduced glutathione (GSH) level
in liver tissue of mice
with or without pretreatment of ALM
and/or exposed
to different doses of gamma radiation.
^aP < 0.05, ^bP < 0.01, ^cP < 0.001.

In the present study histopathological alterations showed a correlation with the number of abnormal cells (pycnotic nuclei, necrotic cells). Pycnotic nuclei of cells depict interphase cell deaths which were more numerous on day 1 and day 14. The peroxidation of lipid portion of the membranes which was found to be elevated on day 14 appears to be responsible for pycnosis and other structural and functional changes in liver. Other nuclear changes can be attributed to DNA damage, as DNA is the critical target and is irreversibly damaged by ionizing radiation [8].

Liver with its metabolic, detoxicating, secretory and excretory functions is extremely vulnerable to harmful substances. This is particularly true for the highly differentiated parenchyma which allows the organ to perform its specific functions. Liver function is disturbed if there is interference with the hepatocellular metabolic processes or bile secretion. Such interference may take place after radiation exposure resulting in disturbed metabolic processes and cytological

abnormalities.

In the present study two types of cytological abnormalities were distinguished:

1. Cell injury, directly showing cellular edema, balloon cells (10 Gy), cytoplasmic vacuolization, hydropic degeneration, swelling, enucleation, degranulation, pyknosis and karyolysis (Fig.7)
2. Mitosis related abnormalities, resulting in giant cells, binucleated cells and multinucleated cells.

Similar distinction in cellular abnormality has also been reported earlier [42]. The increase in cellular water content causes swelling of the cell, or cellular edema. In cellular edema, there is an increase in cell size; the cytoplasm stains less intensely and often has a somewhat foamy structure [43]. This may occur due to membrane damage or disturbances in the energy metabolism of cells, resulting in increased uptake of water. The improper functioning of Na⁺/K⁺-pump may also be responsible for cellular edema since ATP

production is said to be reduced in certain cells following moderate doses of radiation [44]. Among the cellular organelles mitochondria are one of the key components of cells killed by radiation-induced oxidative stress [45]. Since mitochondria are the source of ATP, their damage would deplete ATP levels in cells.

If the disturbance in the water balance is not counteracted in time, the reversible phase of cellular edema changes into an irreversible phase, known as hydropic degeneration or ballooning [46]. Thus, it can be suggested that whole body exposure to radiation at 6, 8 and 10 Gy results in cellular edema because of reduced ATP production and disturbance in Na⁺/K⁺-pump; secondly animals exposed to 6 and 8 Gy show considerable recovery as they are less prone to enter in irreversible phase of cellular injury (hydropic degeneration) whereas at 10 Gy considerable hydropic degeneration is seen which is irreversible and may be one of the reason for mortality at high dose (10 Gy).

Degranulation of cytoplasm can be directly correlated with reduction in the amount of glycogen. A decrease in the glycogen content of tissues is frequently observed if there is a shortage of oxygen in the tissues (hypoxia) or if the oxygen supplied cannot be used because of mitochondrial damage [45]. By way of adaptation, the cells then change to anaerobic respiration, *i.e.* increased glycogen degradation via glycolysis [46].

At higher irradiation dose the cells exhibit ballooning. In these balloon cells the cavities of endoplasmic reticulum swells [47]. As we know that smooth endoplasmic reticulum (RER) plays a role in glycogenesis, the ballooning of cells may cause disturbance in the proper functioning of endoplasmic reticulum and thus causing depletion in glycogen synthesis.

Formation of giant cells has been reported by several workers in different pathological conditions like tumor [48, 49], infection [50] and administration of cytolethal toxin [51]. Giant cells in liver after internal irradiation were also reported [52]. The giant cells result in part presumably from continued DNA synthesis causing polyploidy in the cells that were unable to enter mitosis whereas the formation of bi- or multinucleate cells is considered as a result of undisturbed karyokinesis with an inhibited cytokinesis which may be partial or complete [53-56]. Mononucleate giant cells might be formed due to a failure to enter mitosis after completion of interphase. Binucleate and multinucleate giant cells might also be formed as a result of the failure of cell separation after completion of mitosis, or due to fusion of cells [57]. Giant cell formation is an irreversible phenomenon and it seems to be a step before degeneration and cell death [58].

An increase in binucleated cells may be due to failure of cell separation after mitosis, or fusion of two mononuclear cells after irradiation [59, 60]. The recovery phase of liver also marked an elevation in binucleated cells. Two types of binucleated cells could be distinguished. The binucleated cell can be formed by incomplete cell division, which can be observed as the two nuclei are joined to each other while cells formed by fusion of mononucleate cells showed two separate nuclei [57, 59]. In the present study the former type of binucleated cells were found more frequently on day 14 and even day 30 while the latter type were more frequent on day 1 and day 3.

Hyperemia was frequently observed at all radiation doses in the initial autopsy intervals. This might be because of VOD [6-8], which is the anatomic substratum of RILD. Animals pretreated with ALM and irradiated to different doses of radiation exhibited lesser degree of damage to liver. The initial injury by radiation to liver was mild and recovery was early as compared to control (irradiation alone), however, the injury was dose dependent.

Damage incurred to a tissue can be divided into three parts [61]: a period of reversible alterations (in this period the damage can be repaired to certain extent); a point of no return (beyond which the cells will be irreversibly changed); and a period of irreversible changes (culminating in the total destruction of the cell). From the present study, the period of reversible alterations can be considered as early post-irradiation period when considerable damage occurred, followed by gradual recovery. The recovery of the tissue is not complete as many cells show pycnosis and necrosis, these cells can be considered to cross the point of no return and enter into period of irreversible changes as observed in the 10 Gy treated group.

The extent of injury and repair can be evaluated on the basis of quantitative study of cells which have not crossed the point of no return. As seen in the histological slides the liver tissues of ALM treated group showed mild damage and faster recovery at 6 and 8 Gy as compared to irradiation alone group. At a lethal radiation dose (10 Gy) the treatment of ALM resulted in delayed cellular damage, but was possibly not able to prevent considerable number of cells from crossing the point of no return followed by period of irreversible change and cellular death.

Radioprotection by ALM can be supported by the biochemical estimations of GSH and LPO. The levels of LPO and GSH reached close to normal levels in the ALM treated group at irradiation dose of 6 Gy and 8Gy and these levels significantly varied with those of irradiation alone group. The study suggests considerable hepatoprotection afforded by alcoholic extract of *Mentha piperita* when administered for 3

consecutive days prior to irradiation at sub-lethal dose of 6 Gy and 8 Gy in Swiss albino mice. The percentage of normal, abnormal and binucleated cells showed significant difference in the ALM treated group as compared to irradiation alone. The GSH levels dropped in both the groups as compared to normal in the initial autopsy intervals (1 and 3 days) thereafter showed gradual recovery (7, 14 and 30 days).

But at the lethal dose (10 Gy) irradiation, ALM treatment does not provide much benefit [36]. Although the levels of LPO were significantly lower than irradiation alone group they remained still 2-3 folds higher than its normal values and at 14 day autopsy interval it reached extremely high levels resulting in no survivors after 15 days. Similarly, the GSH levels in the ALM treated group were significantly higher as compared to the control group (10 Gy irradiation alone) but were many folds lower than the normal values; moreover, its levels even did not recovered by day 14.

The LPO level can be related to changes in permeability of membranes [62] which may result in hydropic degeneration as seen in present study. The lower LPO in ALM treated group may be responsible for milder changes in membrane permeability and hydropic degeneration. The decline of LPO on day 7 and elevation on day 14 paralleled with the abnormal hepatocyte population. Similar radiation response of LPO indices was also reported in an earlier study [63]. Most likely hepatocytes are not main target, but they do suffer secondary damage (atrophy, necrosis) probably due to anoxia [8]. Moreover, the lysosomal membrane stabilization by flavonoids and polyphenolic compounds present in medicinal plants like *M.piperita* can also be implicated control in the decline of LPO levels in the ALM treated group [64]. Endogenous antioxidants constitute important defense systems in cells and elicit their action by suppressing the formation of ROS, by scavenging or by repairing the damage caused [45]. This is one of the most important systems capable of protecting cells against free radicals formed during ionizing radiation [65].

GSH effectively scavenges free radicals and other ROS [67]. Thus it can be suggested that ALM raises the levels of GSH in liver and thus increases the radical scavenging activity of liver cells. Raised level of GSH would protect mitochondrial damage and thus increase ATP production to maintain Na⁺/K⁺-pump and protect against hydropic degeneration of liver. GSH is essential for the activation of T-lymphocytes and polymorphonuclear leukocytes [67]. The higher degree of lymphocytic infiltration and thus immunological protection in ALM treated and radiation combined group can be explained by this study. Some swollen Kupffer cells were frequently observed in ALM treated and radiation combined group. The sinusoidal wall is

made up of endothelial cells and Kupffer cells. Kupffer cells are elongated, irregular cells displaying marked phagocytic activity [46]. The Kupffer cells have been shown to phagocytize denatured serum proteins [68, 69]. It is suggested that the Kupffer cells may physiologically remove any denatured or modified serum proteins from the circulation, which may help in faster recovery from radiation insult to liver. Immunomodulatory effect has been reported in peppermint oil which seems to be exerted through the components of *M.piperita* like 1-menthol, menthol and 8-cineole [70].

The degeneration of cellular membrane may be responsible for increase in binucleated cells which may be formed due to inhibition of cytokinesis or fusion of mononucleated cells. The LPO levels showed higher values on day 3 and 14 autopsy intervals which can be correlated with the higher number of binucleated cells. Lower LPO levels can also explain the milder degranulation in cytoplasm (glycogen depletion). Lower peroxidation of lipids can reduce radiation induced oxidative stress and thus protect mitochondria [45]. Thus the ATP generation may not be hampered and all metabolic processes including glycogenesis. The free radical-scavenging activity of *M.piperita* extract has been earlier demonstrated [71].

The radioprotective property of *M.piperita* can be attributed to the presence of antioxidants, namely α -tocopherol, rosmarinic acid, menthone, isomenthol, eugenol, caffeic acid, rutin, β -carotene, geranial [15-17, 20, 21]. α -Tocopherol has also been found to attenuate oxidative stress and to exhibit liver morphological protection [72]. Limonene, another component of *M.piperita*, is shown to exhibit anti-inflammatory property [73] which may help prevent inflammatory symptoms in liver. Radical scavenging activities of three *Mentha* species' (*M.aquatica* L, *M.longifolia* L, and *M.piperita* L) essential oils on 2,2-diphenyl-1-picrylhydrazyl (DPPH) and hydroxyl (\bullet OH) radicals have been studied by earlier workers. The highest \bullet OH radical scavenging activity by reducing \bullet OH radical generation in the Fenton reaction was exhibited by *M.piperita* essential oil and the most powerful scavenging compounds were monoterpene ketones (menthone and isomenthone) [74].

The aqueous extract of *M.piperita* has earlier been shown to provide significant radioprotection at a dose of 1 g/kg b.wt/day for 3 consecutive days before irradiation at 8 Gy [75]. In the present study the 50% alcoholic extract of *M.piperita* leaves was tested which provides significant protection at 0.1 g/kg b.wt/day for 3 consecutive days before irradiation at 8 Gy which is $\frac{1}{10}$ th of the aqueous extract dose. The antioxidant potential of *M.piperita* oil has been found comparable to synthetic antioxidants such as butylated

hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) in terms of lipid peroxidation inhibition [32].

The conclusion drawn from the present study is that the alcoholic extract of *Mentha piperita* provides significant protection from gamma ray irradiation if given 3 days prior to exposure. The protection provided by ALM can be attributed to the antioxidant properties of biochemical constituents in the *Mentha piperita* leaves.

ACKNOWLEDGEMENT

We sincerely thank Cobalt Teletherapy Unit, Department of Radiology, S.M.S. Medical College and Hospital, Jaipur for providing us with irradiation facility.

CONFLICTS OF INTEREST

The authors have no conflict of interest or relationship, financial or otherwise to declare.

REFERENCES

1. Kleinerman RA, Smith SA, Holowaty E, Hall P, Pukkala E, Vaalavirta L, Stovall M, Weathers R, Gilbert E, Aleman BM, Kaijser M, Andersson M, Storm H, Joensuu H, Lynch CF, Dores GM, Travis LB, Morton LM, Curtis RE. Radiation dose and subsequent risk for stomach cancer in long-term survivors of cervical cancer. *Int J Radiat Oncol Biol Phys* 2013; 86:922-9.
2. Maranzano E, Trippa F, Casale M, Costantini S, Lupattelli M, Bellavita R, Marafioti L, Pergolizzi S, Santacaterina A, Mignogna M, Silvano G, Fusco V. 8Gy single-dose radiotherapy is effective in metastatic spinal cord compression: results of a phase III randomized multicentre Italian trial. *Radiother Oncol* 2009; 93:174-9.
3. Khozouz RF, Huq SZ, Perry MC. Radiation-induced liver disease. *J Clin Oncol* 2008; 26:4844-5.
4. Reed GB, Cox AJ. The human liver after radiation injury. *Am J Pathol* 1966; 48:597-612.
5. Cheng JC, Wu JK, Huang CM, Huang DY, Cheng SH, Lin YM, Jian JJ, Yang PS, Chuang VP, Huang AT. Radiation-induced liver disease after radiotherapy for hepatocellular carcinoma: clinical manifestation and dosimetric description. *Radiother Oncol* 2002; 63:41-5.
6. Fajardo LF. *Pathology of Radiation Injury*, Masson Publishing New York, NY, USA, pp 57-70, 1982.
7. Lawrence TS, Robertson JM, Anscher MS, Jirtle RL, Ensminger WD, Fajardo LF. Hepatic toxicity resulting from cancer treatment. *Int J Radiat Oncol Biol Phys* 1995; 31:1237-48.
8. Fajardo LF, Berthrong M, Anderson RE. Small intestine. In: *Radiation Pathology*, Oxford University Press, New York, NY, USA, p 228, 2001.
9. Sener G, Kabasakal L, Atasoy BM, Erzik C, Velioglu-Ogunc A, Cetinel S, Gedik N, Yegen BC. *Ginkgo biloba* extract protects against ionizing radiation induced oxidative organ damage in rats. *Pharmacol Res* 2006; 53:241-52.
10. Halliwell B, John H, Gutteridge MC. Production of hydroxyl radicals in living systems. In: *Free Radicals Biology and Medicine*, Clarendon Press, Oxford, UK, p 31, 1989.
11. Cairnie AB. Adverse effects of radioprotector WR2721. *Radiat Res* 1983; 94:221-6.
12. Kligerman MM, Glover DJ, Turrisi AT, Norfleet AL, Yuhans JM, Coia LR, Simone C, Glick JH, Goodman RL. Toxicity of WR-2721 administered in single and multiple doses. *Int J Radiat Oncol Biol Phys* 1984; 10:1773-6.
13. Maisin JR. Chemical radioprotection: past, present and future prospects. *Int J Radiat Biol* 1998; 73:443-50.
14. Coleman NE, Blakely WF, Fike JR, MacVittie TJ, Metting NF, Mitchell, JB, Moulder JE, Preston RJ, Seed TM, Stone HB, Tofilon PJ, Wong RS. Molecular and cellular biology of moderate-dose (1-10 Gy) radiation and potential mechanisms of radiation protection: report of a workshop at Bethesda, Maryland, December 17-18, 2001. *Radiat Res* 2003; 159:812-34.
15. Halliwell B, Gutteridge JMC. Role of free radicals and catalytic metal ions in human disease: an overview. *Methods Enzymol* 1990; 186:1-85.
16. Arumugam P, Ramesh A. Antigenotoxic and antioxidant potential of aqueous fraction of ethanol extract of *Mentha spicata* (L.) against 4-nitroquinoline-1-oxide-induced chromosome damage in mice. *Drug Chem Toxicol* 2009; 32:411-6.
17. Sinha M, Das DK, Datta S, Ghosh S, Dey S. Amelioration of ionizing radiation induced lipid peroxidation in mouse liver by *Moringa oleifera* Lam leaf extract. *Indian J Exp Biol* 2012; 50:209-15.

18. Gerweck LE, Dorr W. Normal Tissue Radiobiology: Animal Models of Radiation Injury, Wolters Kluwer/Lippincott Williams & Wilkins, Philadelphia, PA, USA, pp 21-25, 2011.
19. Kavitha C, Rajamani K, Vadivel E. *Coleus forskohlii*: a comprehensive review on morphology, phytochemistry and pharmacological aspects. *J Med Plant Res* 2010; 4:278-85.
20. Vertuani S, Angusti A, Manfredini S. The antioxidant and Proantioxidants network: an overview. *Curr Pharm Des* 2004; 10:1677-94.
21. Saller R. Peppermint (*Mentha piperita*), medicinal plant of the year 2004. *Forsch Komplementarmed Klass Naturheilkd* 2004; 11:6-7.
22. Samarth RM, Panwar M, Kumar M, Kumar A. Radioprotective influence of *Mentha piperita* (Linn) against gamma irradiation in mice: antioxidant and radical scavenging activity. *Int J Radiat Biol* 2006; 82:331-7.
23. Rastogi RP, Mehrotra BN. Compendium of Indian Medicinal Plants, Volume 3. CDRI & PID, New Delhi, India, pp 420-422, 1991.
24. Krishnaswamy K, Raghuramulu N. Bioactive phytochemicals with emphasis on dietary practices. *Indian J Med Res* 1988; 108:167-81.
25. Al-Sereiti MR, Abu-Amer KM, Sen P. Pharmacology of rosemary (*Rosmarinus officinalis* Linn.) and its therapeutic potential. *Indian J Exp Biol* 1999; 37:124-30.
26. Inoue T, Sugimoto Y, Masuda H, Kamei C. Anti-allergic effect of flavonoid glycosides obtained from *Mentha piperita* L. *Biol Pharm Bull* 2002; 25:256-9.
27. Iscan G, Kirimer N, Kurkcuoglu M, Baser KH, Demirci F. Antimicrobial screening of *Mentha piperita* essential oils. *J Agric Food Chem* 2002; 50(14): 3943-6.
28. Mimica-Dukic N, Bozin B, Sokovic M, Simin N. Antimicrobial and antioxidant activities of *Melissa officinalis* L. (Lamiaceae) essential oil. *J Agric Food Chem* 2004; 52:2485-9.
29. Ortmann EK, Mayerhofer T, Getoff N, Kodym R. Effect of Antioxidant vitamins on radiation induced apoptosis in cells of a human lymphoblastic cell line. *Radiat Res* 2004; 161:48-55.
30. Kumar A, Samarth RM, Yasmeen S, Sharma A, Sugghara T, Terado T, Kimura H. Anticancer and radioprotective potentials of *Mentha piperita*. *Biofactors* 2004; 22:87-91.
31. Schmidt E, Bail S, Buchbauer G, Stoilova I, Atanasova T, Stoyanova A, Krastanov A, Jirovetz L. Chemical composition, olfactory evaluation and antioxidant effects of essential oil from *Mentha piperita*. *Nat Prod Commun* 2009; 4:1107-12.
32. Sharafi SM, Rasooli I, Owlia P, Taghizadeh M, Astaneh SD. Protective effects of bioactive phytochemicals from menths piperita with multiple health potentials. *Pharmacogn Mag* 2010; 6:147-53.
33. Jain D, Pathak N, Khan S, Raghuram GV, Bhargava A, Samarth R, Mishra PK. Evaluation of cytotoxicity and anticarcinogenic potential of *Mentha* leaf extracts. *Int J Toxicol* 2011; 30:225-36.
34. Samarth RM, Kumar A. *Mentha piperita* (Linn.) leaf extract provides protection against radiation induced chromosomal damage in bone marrow of mice. *Indian J Exp Biol* 2003; 41:229-37.
35. Baliga MS, Rao S. Radioprotective potential of mint: a brief review. *J Cancer Res Ther* 2010; 6:255-62.
36. Kaushik P, Mathur M, Rawat N, Dutt P. Radioprotective effect of alcoholic extract of *Mentha piperita* (Linn) on Swiss albino mice exposed to whole body gamma radiation: e preliminary study. *Int J Pharm Bio Sci* 2012; 3:598-610.
37. Moron MS, Depierre JW, Mannervik B. Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. *Biochem Biophys Acta* 1979; 582:67-78.
38. Ohkawa H, Ohishi N, Yogi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem* 1979; 95:351-8.
39. Bourke GJ, Daly LE, McGilvary J. Interpretation and Uses of Medical Statistics, 3rd edition, Blackwell, Oxford, UK, 1985.
40. Heibatollah S, Reza NM, Izabpanah G, Sohailla S. Hepatoprotective effect of *Cichorium intybus* on CCl₄-induced liver damage in rats. *Afr J Biochem Res* 2008; 2:141-4.
41. Kolodny A. Tissue changes after experimental deep roentgen irradiation. *Am J Path* 1925; 1:285-93.
42. Kumar A, Paul P. Cytologic and quantitative studies on the effects of radiocalcium (C-45) on the bone marrow of Indian desert Gerbil (*Meriones hurricinae*, Jerdon). *J Radiat Res* 1979; 20:225-35.
43. Penninks A. Cytopathology: General response patterns and morphologic aspects. In: Toxicology- Principle and Applications. CRC Press Boca Raton. New York. pp 445-470, 1995.
44. Casarett AP. Radiation Biology, Prentice Hall Englewood Cliffs, New Jersey, NJ, USA, 1968.
45. Lakshmi B, Tilak JC, Adhikari S, Devasagayam TP, Janardhanan KK. Inhibition of lipid peroxidation induced by γ -radiation and AAPH in rat liver and brain mitochondria by mushroom. *Curr Sci* 2005; 88:484-8.
46. Niesink RJM, Hollinger MA, de Vries J. Toxicology: Principles and Applications, CRC Press, Boca Raton, FL, USA, 1995.
47. Oberling CH, Rouiller CH. Les effets de l'intoxication aigue are tetrachorure de carbone sur le foie de rat. Etude au microscope electronique. *Ann Anat Pathol* 1956; 1:401-27.
48. Kawaguchi H, Miyoshi N, Souda M, Maeda H, Kawashima H, Gejima K, Uchida K, Umekita Y, Yoshida H. Renal adenocarcinoma in a ferret. *Vet Pathol* 2006; 43:353-6.
49. Willenbrock K, Kriener S, Oeschger S, Hansmann ML. Nodular lymphoid lesion of the liver with simultaneous focal nodular hyperplasia and hemangioma: discrimination from primary hepatic MALT-type non-Hodgkin's lymphoma. *Virchows Arch* 2006; 448:223-7.
50. Szeredi L, Molnar T, Glavits R, Takai S, Makrai L, Denes B, Del Piero F. Two cases of equine abortion caused by *Rhodococcus equi*. *Vet Pathol* 2006; 43:208-11.
51. Ceelen LM, Harsebrouck F, Favourel H, Ducatelle R, Deceostere A. The cytolethal descending toxin among helicobacter pullorum strains from herman and poultry origin. *Vet Microbial* 2006; 113:45-53.
52. Sharma S. Modification of radiocalcium (⁴⁵Ca) induced histopathological changes in mice by 2-mercaptoproprionylglycine (MPG). Ph.D. Thesis, Himachal Pradesh University, Shimla, India, 1986.
53. Bond VP, Fliedner TM, Archambeau JO. Mammalian Radiation Lethality. A Disturbance in Cellular Kinetics, Academic Press, London, UK, 1965.
54. Bond VP. Radiobiological bases for the understanding of the hematological consequences of radiation exposures. In: Manual of Radiation Hematology. IAEA, Vienna, Austria, pp 71-78, 1971.
55. Fukui Y. Formation of multinuclear cells induced by dimethyl sulfoxide: inhibition of cytokinesis and occurrence of novel nuclear division in *Dictyostelium* cells. *J Cell Biol* 1980; 86:181-9.

56. Yang X, Yu K, Hao Y, Li DM, Stewart R, Insogna KL, Xu T. LATS1 tumour suppressor affects cytokinesis by inhibiting LIMK1. *Nat Cell Biol* 2004; 6:609-17.
57. Sharma R, Sharma J. Modification of Gamma Ray induced changes in the mouse Hepatocytes by *Centella asiatica* extract: *in vivo* studies. *Phytother Res* 2005; 19:605-11.
58. Gupta ML, Umadevi P. Formation of giant hepatocytes in response to radiation. *Curr Sci* 1981; 50:637-8.
59. Kura S, Sasaki H, Aramaki R, Yoshinaga H. Binucleate cell formation induced by X-irradiation. *Radiat Res* 1978; 76:83-94.
60. Rodilla V, Pellicer JA, Serrano A, Pertusa J. Possible relationship between micronucleated and binucleated cells induced by cisplatin in cultured CHO cells. *Mutat Res* 1993; 291:35-41.
61. Majno, G. Death of liver tissue. A review of cell death, necrosis, and autolysis. In: Rouiller C (ed) *The Liver, Morphology, Biochemistry, Physiology*, volume 2, Academic Press, New York, NY, USA, pp 267-313, 1964.
62. Halliwell B, Aruoma OI. *Diet and Free Radicals*, Ellis Horwood, London, UK, p 95, 1993.
63. Baraboi VA, Dziatkovskaia NN, Klimenko TV, Orel VE, Redkousova SF. Dynamics of the indices of lipid peroxidation. *Radiobiologiya* 1990; 30:735-9.
64. Kamal ME, Nermien, ZA. Comparison of the affects of some essential oils as anti-inflammatory agents on the activities of lysosomal acid-hydrolases in rat Liver *in-vitro*. *Nature Science* 2011; 9:90-98.
65. Tamba M, Simone G, Quintiliani M. Radiation induced reaction of glutathione with oxygen and their possible role in biological systems. In: Cerutti PA, Nygaard OF, Simic MG (eds) *Anticarcinogenesis and Radiation Protection*, Plenum Press, New York, NY, USA, pp 25-32, 1987.
66. Fang YZ, Yang S, Wu G. Free radicals, antioxidants and nutrition. *Nutrition* 2002; 18:872-9.
67. Townsend DM, Tew KD, Tapiero H. The importance of glutathione in human disease. *Biomed Pharmacother* 2003; 57:145-55.
68. Benacerraf B, Halpern BN, Stiffel C, Cruchaud S, Biozzi G. Phagocytose d'une fraction du serum chauff e et iode por de systeme reticulo-endothelial at comportement consecutif de ses cellules a legard d' autres colloides. *Ann Inst Pasteur* 1955; 89:601-20.
69. Biozzi G, Benacerraf B, Stiffel C, Halpern BN, Mouton D. Influence de la quantite d'iode fixe sur les proteines seriques normales et modifiees par la chaleur sur la phagocytose de ces colloides par les cellules du. *SRE. Ann Inst Pasteur* 1957; 92:89-111.
70. Arakawa T, Shibata M, Hosomi K, Watanabe T, Honma Y, Kawasumi K. Antiallergic effects of peppermint oil, chicle and jelutong. *J. Food Hyg Soc Japan* 1992; 33:569-75.
71. Samarth RM, Panwar M, Kumar M, Kumar A. Protective effect of *Mentha piperita* Linn on benzo[a]pyrene-induced lung carcinogenicity and mutagenecity in Swiss albino mice. *Mutagenesis* 2006; 21:61-6.
72. Bharrhan S, Chopra K, Rishi P. Vitamin E supplementation modulates endotoxin-induced liver damage in a rat model. *Am J Biomed Sci* 2010; 2:51-62.
73. Mahmoud SS, Williams M, Croteau R. Cosuppression of limonene-3-hydrolyases in peppermint promotes accumulation of limonene in the essential oil. *Phytochemistry* 2004; 65:547-54.
74. Mimica-Dukic N, Bozin B, Sokovic M, Mihajlovic B, Matavulj M. Antimicrobial and antioxidant activities of three *Mentha* species essential oils. *Planta Med* 2003; 69:413-9.
75. Samarth RM, Kumar A. Radioprotection of Swiss albino mice by plant extract *Mentha piperita* (Linn.). *J Radiat Res.* 2003; 44:101-9.

This is an open access article licensed under the terms of the Creative Commons Attribution Non-Commercial License which permits unrestricted, non-commercial use, distribution and reproduction in any medium, provided that the work is properly cited.