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AMELIORATION OF MERCURIC CHLORIDE AND RADIATION INDUCED HISTOPATHOLOGY IN MICE BY *MORINGA OLEIFERA*

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ABSTRACT

The aim of the present study was to investigate the impact of the *Moringa oleifera* against combined administration of mercuric chloride and radiation on histopathological changes in the liver of mice. The mice were exposed to mercuric chloride (0.5ppm) and gamma radiation (5.0Gy) simultaneously and individually. The experimental groups were given *Moringa oleifera* seven days prior to radiation or mercuric chloride treatment. The changes included cytoplasmic degranulation, vacuolation, nuclear pycnosis, necrosis, hyperaemia and leucocytic infiltration etc. In the combined treatment groups the changes were more severe showing synergistic effect. An early and fast recovery in *Moringa* pre treated groups may be due to the protection provided by the drug.

KEYWORDS: *Moringa oleifera*, nuclear pycnosis, necrosis, hyperaemia and leucocytic.

INTRODUCTION

Biological effects begin with the ionization of the atoms the mechanism by which radiation causes damage to human tissue or any other material is by ionization of atoms in the material. Ionization absorbed by a tissue has enough energy to remove electrons from the atoms that make up molecules of the tissue. When the electron that was shared by the two atoms to form a molecular bond is dislodged by ionizing radiations. The bond is broken and thus the molecules fall apart. This is the basic model for understanding radiation damage. Potential biological effects depend on how much and how fast radiation dose is received.

Heavy metals are metallic elements which have a high atomic weight and a density much greater than water. There are more than twenty heavy metals, but four are of particular concern to human health; Lead (Pb), Cadmium (Cd), Mercury (Hg) and inorganic Arsenic (As) According to the U.S Agency for Toxic Substances and Disease Registry, these four heavy metals are four of the top six hazards present in toxic waste sides They are highly toxic and can cause damaging effects even at very low concentrations.

Mercury poisoning (also known as hydrargyria or mercurialism) is a type of metal poisoning and a medical condition caused by exposure to mercury or its compounds. Mercury (chemical symbol Hg) is a heavy metal occurring in several forms. All of these, except elemental liquid mercury (for which intravenous injection of a certain volume) produce toxicity or death with less than a gram. The damage done by elemental mercury is caused by blocking blood vessels. Mercury's zero oxidation state Hg^0 exists as vapor or as liquid metal, its mercurous state Hg^+ exists as inorganic salts, and its mercuric state Hg^{+2} may form either inorganic salts or organo-mercury compounds; the three groups vary in effects. Toxic effects include damage to the brain, kidneys, lungs and liver.[1]

In the present investigation, toxicological research efforts would do well to first establish the safety of consumption of the plant products and to the various extents; as this will be vital to the establishment of the use of the plant's products as standard nutritional supplements and natural or bio-medicinal products. To this end, we employed histological methods to evaluate the effects of *Moringa* leaf extracts on vital body tissue organs. The rationale is that histological methods of observations would provide a more reliable and consistent picture of the effects produced by the interactions of the photochemical with the body cells and tissue better than *in vitro* tests and analysis of the highly dynamic biochemical activities as contained in extracted tissue fluids. Also, the use of histological methods of assessment of *Moringa* leaf extract effects on body tissues is important because literatures are comparatively scarce on such methods of investigation of the plant's extracts' effects.

Moringa oleifera ethanolic leaf extract reportedly have hepatoprotective abilities in various induced conditions such as using diclofenac[2]; acetaminophen[3] antitubercular drug[4] and carbon tetrachloride.[5] The LD (50) for aqueous extract of *Moringa* leaf was estimated and tested various dosages of extract on the sperm,

haematological and biochemical parameters as well as histopathological preparations; and they concluded that orally administered *Moringa* leaf extract at their estimated sub-lethal dosages were relatively safe for tested body organs.[6] The aim of this particular investigation was to observe the effects of *Moringa oleifera* leaf extract on the histological architecture on liver of mice.

Review of Literature

Liver is the largest gland in the body and it occupies an important place among vital organs. It was considered earlier relatively resistant to gamma radiations but found it moderately sensitive to radiations and to lower doses also. Liver is an organ which suffers from direct and indirect both the types of damage. It mainly contains hepatocytes, blood vessels, bile ducts and reticulo-endothelial system. It is a capsular organ. It is responsible for detoxification of toxins produced in the body.[7]

The rats were irradiated with 1000 R x-rays and it was concluded that radio-sensitivity changes with the age of the animal.[8] This alteration of radio-sensitivity of rat liver may correspond to variation of DNA amount of nucleus of liver cells due to growth. After whole body x-irradiation of mice, it was also observed that hepatic changes become more pronounced with age.[9] A decrease in nuclear content was observed after treating the rat liver with 650 R of x-irradiation.[10]

The *Channa punctatus* (fish) were exposed to different doses (i.e. 2.25, 4.50 and 9.0 Gy) of gamma radiation from a ^{60}Co source. They observed radio-lesions in liver, which was dose dependent. The lesions observed were edema, cytoplasmic degranulation and vacuolation, pycnosis and distortion of hepatic architecture. Onset of recovery was seen on day 7 after irradiation in 2.5 Gy dose group. Liver exhibited normal picture on day 14 in 2.25 and 4.5 Gy dose group but in 9.0 Gy dose groups the lesions persisted. The radiation effects were found dose dependent.[11]

The protective role of *Aloe vera* against radiation and cadmium induced histopathological changes in the liver of Swiss albino mice have been investigated in a study.[12] They planned to evaluate the protective effect of *Aloe vera* (a herbal drug) against radiation and cadmium induced histopathological alterations in the liver of Swiss albino mice. The animals were exposed to 5.0 Gy of gamma rays with or without cadmium chloride treatment. The *Aloe vera* juice was administered since seven days prior to irradiation or cadmium chloride treatment and up to the last autopsy interval. The animals from all the experimental groups were sacrificed by cervical dislocation at each post-treatment interval of 1, 2, 4,7,14 and 28 days. After sacrificing the animals, pieces of the liver were cut and immediately fixed in Bouin's fluid for histological observations after routine processing. The changes observed were distortion of hepatic architecture, intracellular oedema, narrower sinusoids, cytoplasmic degranulation, vacuolation, hyperaemia, pycnotic and crenated nuclei. After combined treatment of radiation and cadmium synergistic effects were noted. The *Aloe vera* pretreated mice showed less severe changes in comparison to the non drug treated animals at all the corresponding intervals. An early and fast recovery was also observed in *Aloe vera* treated groups. Thus it appears the *Aloe vera* is potent enough to check radiation and cadmium induced hepatic lesions in Swiss albino mice.[12] Mercury, identified thousands of years ago is one of the oldest toxicants known.[13] Although in recent years, environmental and occupational exposures to mercury have been greatly reduced, this metal still remains a threat to human health from multiple sources: air, water and food.[14] Once absorbed, mercury distributes widely to all tissues. The principal target organs of the inorganic mercury are kidney and liver.[15] Previous studies have revealed that mercuric chloride caused histopathological and ultrastructural lesions in the liver evidenced by peri-portal fatty degeneration and cell necrosis.[16]

In the present study, the liver of mercury-treated rats showed congestion of hepatoportal blood vessels, congestion of central vein, edema in the portal tract and fatty changes indicating the toxic effect of mercuric chloride. It has been shown that in chronically diseased liver, some cells are activated by factors released by the liver hepatocytes and Kupffer cells, proliferate, and acquire the features of myofibroblasts, with or without the lipid droplets.[17]

An attempt has been made to investigate the effects of ethanolic *Moringa oleifera* leaf extract on the histology of vital body tissues. The rationale is that histological observations would provide a more reliable and consistent picture of the effects produced by the interactions of the phytochemicals with the body cells and tissue. It may be helpful in observing the possible toxicological effects on body tissues or on the other hand, the positive effects on the body tissues. A total of twelve Wistar rats (n=12) were used for the investigation; divided in two groups of Control (A) and Treated (B). A daily dosage of 200mg/kg body weight of ethanolic *moringa* leaf extract was administered orally to the treated Group B for 28 days. Analysis of each tissue's histo-morphology, general histo-architecture and cytological structures was critically done. The basis of analyses and inferences was clearly defined: whether *Moringa oleifera* leaf extract produced any observable deleterious effects on the tissue [toxicological evaluation]; or whether its effects would improve the tissue's histological architecture especially in manners that can produce improvement in physiological conditions of the individual tissue or general body health [medicinal and nutritional properties]. Extract produced positive effects in the liver.[18]

MATERIALS AND METHODS

The adult healthy male Swiss albino mice (6-8 weeks old) were procured from Lala Lajpat Rai University of Veterinary and Animal Sciences, Hissar. The Govt. Dungar College, Bikaner is registered under CPCSEA, Chennai (registration no. 1066/GO/RE/S/07/CPSEA) and has its own Institutional Animal Ethics Committee (IAEC). In view of the above, the present experiments were conducted under the supervision of IAEC of the College. The animals were housed in polypropylene cages and maintained on balanced mice feed and tap water *ad libitum*. The 0.5 ppm aqueous solution of mercuric chloride was prepared and then administered orally in drinking water. The animals were exposed by the Cobalt-60 gamma radiotherapy source (Theratron) of AECL make, obtained from Canada. This facility was provided by the Radiotherapy Department of Prince Bijay Singh Memorial Hospital, Bikaner (Rajasthan). The animals were irradiated at the dose rate ranging from 0.95 Gy/min to 1.97 Gy/min. The dried powder of *Moringa oleifera* was procured from the Umalaxmi organics private limited, Jodhpur (India) and aqueous extract of the same was obtained in the department. The plant extract of *Moringa* was fed orally at the dose of 150 mg/kg body weight. The *Moringa* extract was given daily from seven days prior to individual or combined treatment of mercuric chloride and radiation and continued up to the last autopsy interval.

Design of experimentation

Group – I The animals of this group were sham-irradiated and served as control (normal) group.

Group – II All the animals of this group were orally fed with mercuric chloride solution at the dose of 0.5 ppm *ad libitum* in drinking water continuously till the end of experiment.

Group – III The animals of this group were exposed to 5.0 Gy of gamma radiation from Cobalt-60 source.

Group – IV All the animals of this group were orally fed with mercuric chloride solution (0.5 ppm) and also exposed to 5.0 of gamma radiation.

Group – V The animals of this group were orally fed with mercuric chloride (0.5 ppm) and also received *Moringa oleifera* orally for seven days at a dose of 150 mg/kg body wt./animal/ day prior to mercuric chloride treatment and continued up to the last autopsy interval.

Group – VI The animals of this group were exposed to 5.0 Gy of gamma radiation from Co^{60} source. The *Moringa oleifera* was given seven days prior to irradiation and continued up to last autopsy interval.

Group – VII The animals of this group were orally fed with mercuric chloride solution at the dose of 0.5 ppm and received *Moringa oleifera* orally (150 mg/kg/b.wt./animal/day) for seven days prior to 5.0 Gy irradiation and mercuric chloride till the last autopsy day of experiment.

Autopsy

A minimum of five animals from groups II to VII were sacrificed by cervical dislocation and autopsied at each post-treatment intervals of 1, 2, 4, 7, 14 and 28 days. Five sham-irradiated mice were also sacrificed in the similar manner.

Histological studies

After sacrificing the animals, pieces of the liver were fixed in Bouin's fixative for 24 hours. The tissues were washed in water to remove excessive of fixative, dehydrated in graded series of alcohol, cleared in xylene and embedded in paraffin wax. Sections were cut at 5 µm and stained in Harris haematoxyline and alcoholic eosin.

OBSERVATIONS

In the present experiments histopathological changes were noticed in the liver of Swiss albino mice exposed to 5.0 Gy gamma rays with or without mercuric chloride treatment. The changes observed on day-1 after exposure to 5.0 Gy were distortion of hepatic architecture, intracellular oedema, narrower sinusoids, kupffer cells, cytoplasmic degranulation, vacuolation and pycnotic nuclei. Binucleated cells, necrosis, hyperaemia of blood vessels and lecucocytic infiltration were also noticed. The changes were more marked on day-4 and continued up to day-14. But on day-28 the signs of recovery were observed. In the combined treatment of radiation and mercuric chloride synergistic effects were observed. The liver of *Moringa oleifera* treated animals exhibited less severe damage as compared to non-drug treated animals at all the corresponding intervals. An early and fast recovery was also noticed in *Moringa oleifera* pretreated animals(Figs 1-8).

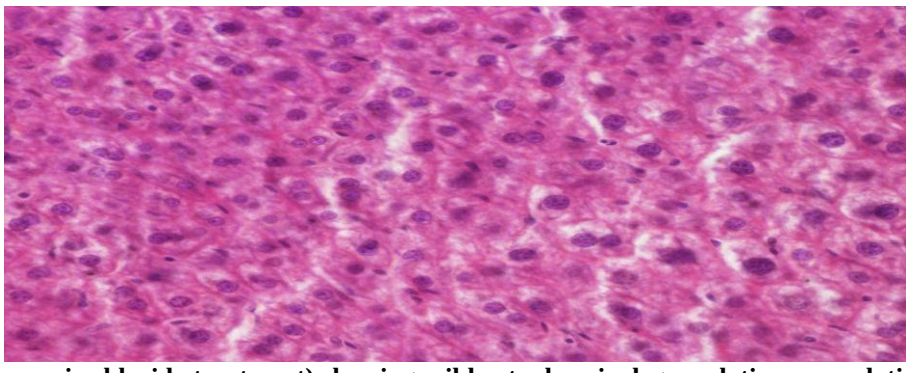

Fig 1: (Day-4, mercuric chloride treatment) showing mild cytoplasmic degranulation, vacuolation, kupffer cells, pycnotic and binucleated cells.

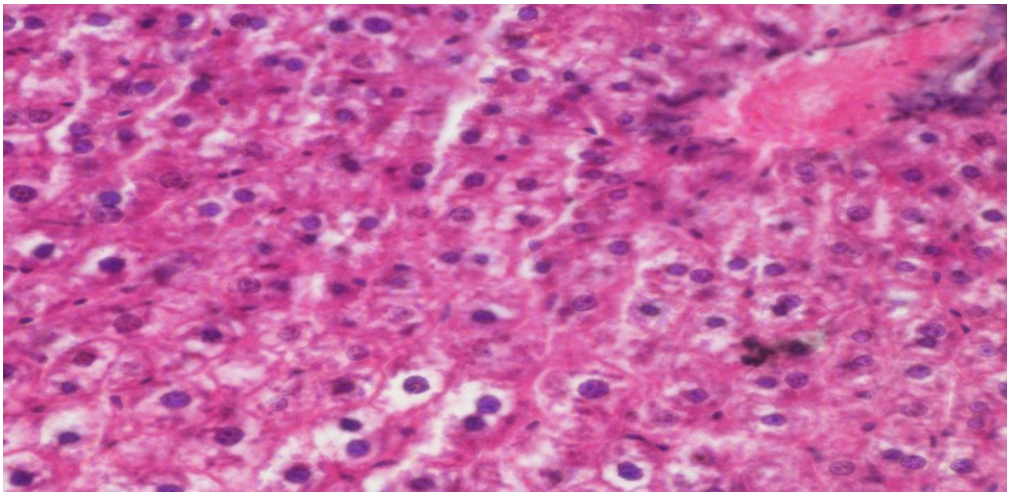

Fig 2: (Day 7, gamma irradiation) Depicting hyperemia of blood vessels, giant cells, fusion of nuclei and leucocytic infiltration.

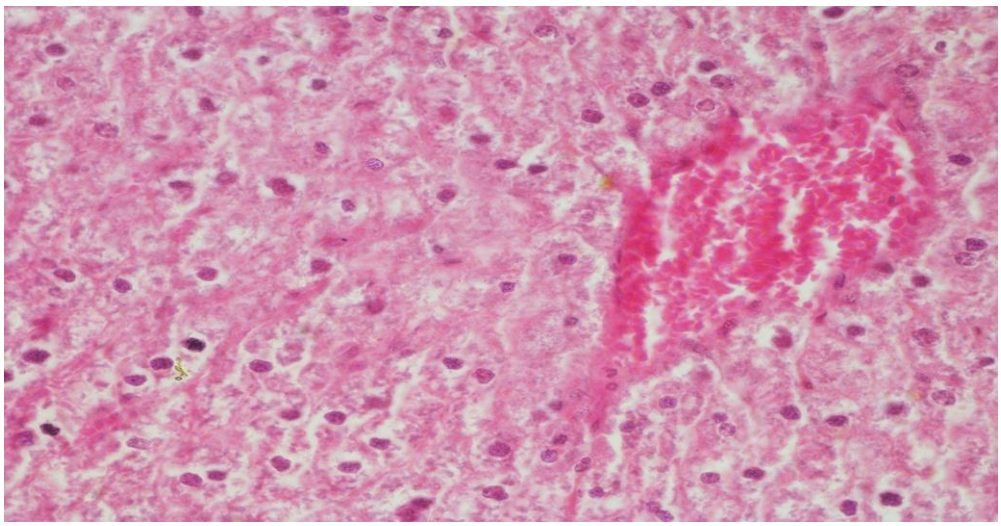

Fig. 3. (Day 2, mercuric chloride + gamma radiation) illustrating enulceation, hyperemia, cytoplasmic degranulation and vacuolation.

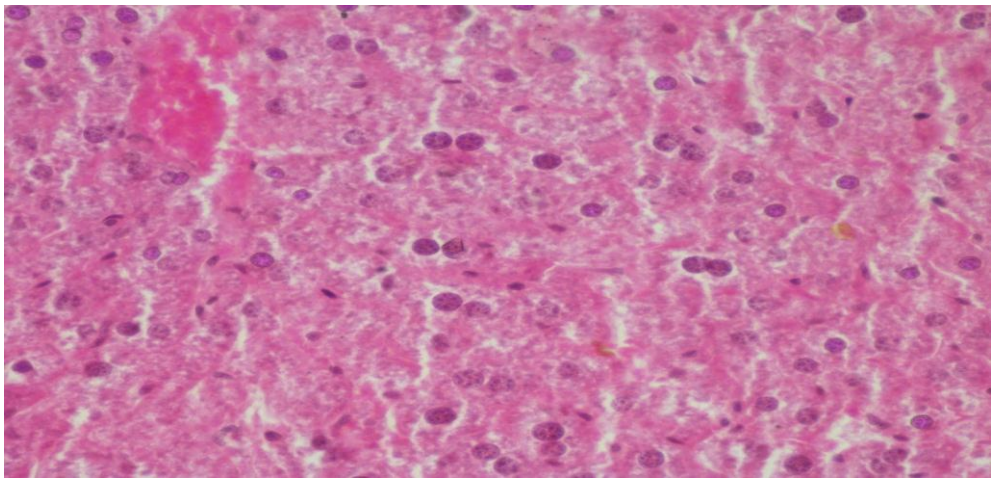

Fig. 4. (Day-14, 5.0 Gy radiation + mercuric chloride) exhibiting binulceated cells, fusion of nuclei and kupffer cells.

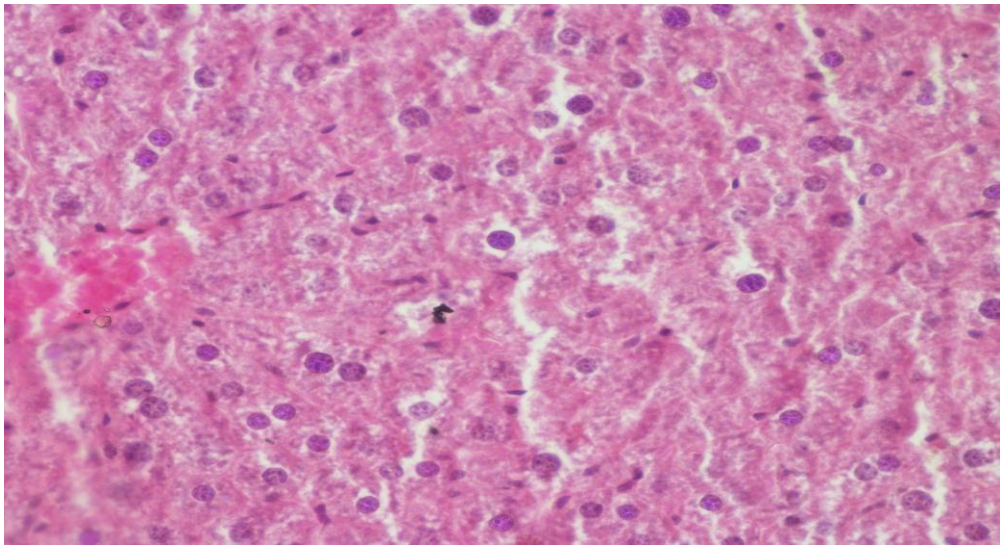

Fig. 5. (Day-2, Mercuric chloride + *Moringa*) depicting comparative better hepatocytes, lecucytic infiltration, fusion of nuclei and hyperemia.

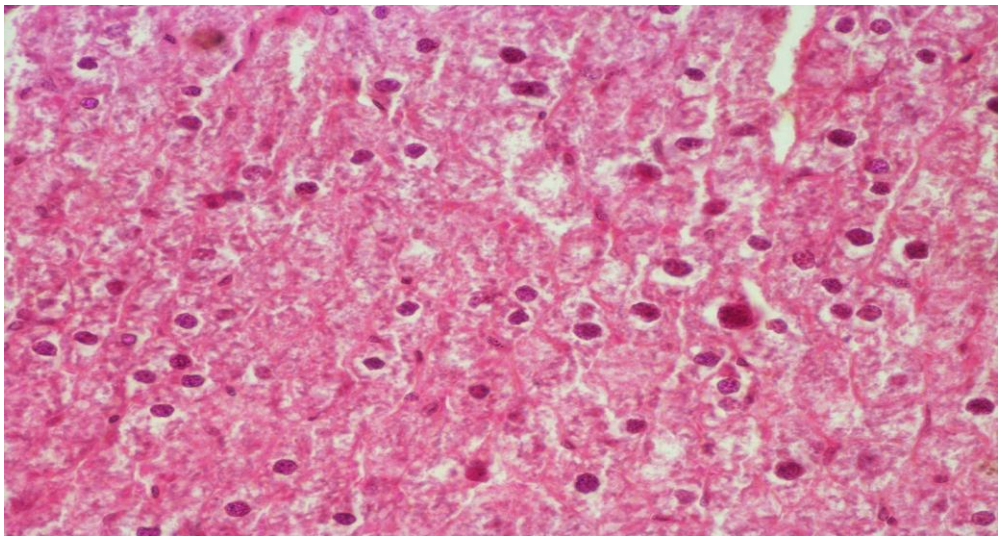

Fig. 6 (Day-7, Gamma radiation + *Moringa*) showing a few giant cells, better condition of nuclei, cytoplasmic degranulation and vacuolation).

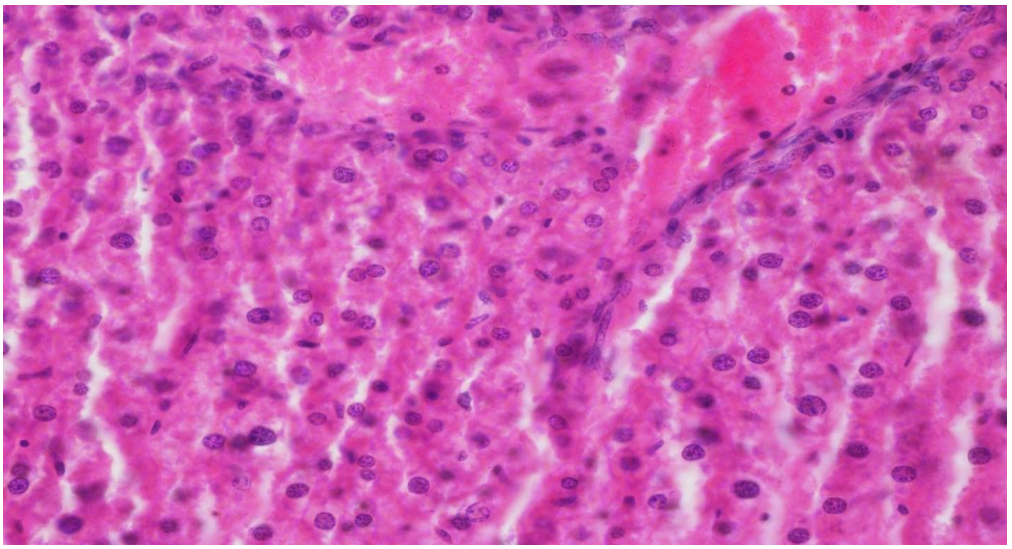

Fig. 7: (Day 2, Radiation + Mercuric chloride + *Moringa*) illustrating hyperemia, leucocytic infiltration hyperemia and kupffer cells).

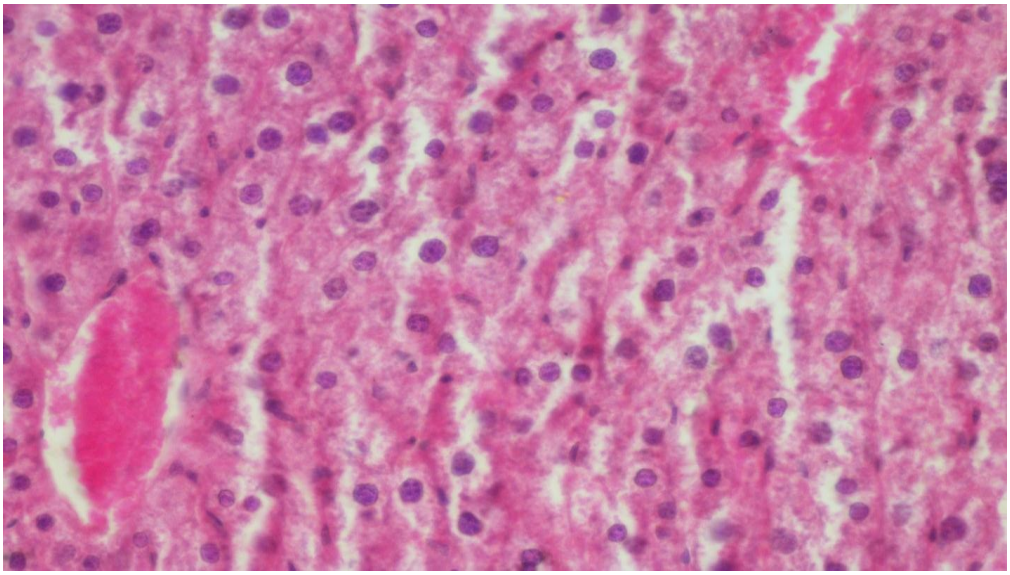

Fig 8: (Day 14, Radiation + Mercuric chloride + *Moringa*) illustrating hyperemia, better nuclear condition, hyperemia and kupffer cells).

DISCUSSION

Over the last decade evidence has accumulation for a role of reactive oxygen animal models of toxicity including mercuric chloride. The prooxidant properties of mercury are well established.[19] It has also been investigated the aetiology of mercury induced porphyrinuria under *in vitro* conditions; their findings support the view that mercuric chloride ions both compromise the antioxidant potential of GSH and promote formation of reactive species via thiol complexation.[20] The induction of lipid peroxidation associated with mercuric chloride treatment of isolated rat hepatocytes has been reported and suggested a causative role of oxidative stress in mercury cytotoxicity.[21]

The mechanisms involved in radiation-induced cellular injury and death remains incompletely understood. In addition to the direct formation of highly reactive hydroxyl radicals ($HO^{\cdot}$) by radiolysis of water, oxidative stress events in the cytoplasm due to formation of H_2O_2 may also be important. Since the major pool of low-mass redox-active intracellular iron seems to reside within lysosomes, arising from the continuous intra lysosomal autophagocytotic degradation of ferruginous materials, formation of H_2O_2 inside and outside these organelles may cause lysosomal labilization with release to the cytosol of lytic enzymes and low-mass iron. If of limited magnitude, such release may induce 'reparative autophagocytosis', causing additional accumulation of redox-active iron within the lysosomal compartment. The radio-resistant histiocytic lymphoma (J774) cells to assess the importance of intra lysosomal iron and lysosomal rupture in radiation-induced cellular injury have been used. It was found that a 40 Gy radiation dose increased the 'loose' iron content of the (still viable) cells approx. 5-fold when assayed 24 h later. Cytochemical staining revealed that most redox-active iron was within the lysosomes. The increase of intra lysosomal iron was associated with 'reparative autophagocytosis', and sensitized cells to lysosomal rupture and consequent apoptotic/necrotic death following a second, much lower dose of radiation (20 Gy) 24 h after the first one. A high-molecular-mass derivative of desferrioxamine, which specifically localizes intra lysosomally following endocytic uptake, added to the culture medium before either the first or the second dose of radiation, stabilized lysosomes and largely prevented cell death. These observations may provide a biological rationale for fractionated radiation.[22]

The liver tissue of the control group is being illustrated at various suitable magnifications in photomicrographs. The lowest magnification shows a normally organized liver tissue with the plates of hepatocytes being separated by sinusoids. The central vein is also observable. At the higher magnifications the hepatocytes are observable, arranged in plates as well as the sinusoids separating them. A few Kupfer cells are also observable. The portal triad- artery, vein and bile duct branches are also observable. All the basic features of a normal liver tissue as found in the control are present. The hepatocytes are however quite prominent and they appear better defined. While these observations show that the administered *Moringa* leaf extract would not produce deleterious effects on the liver tissues; it could also suggest that it could stimulate better state of health and functional status of the hepatocytes; and consequently, improve the functions. It should be noted that hepatocytes acute states of health depend greatly on their functional response to systemic bio- and chemo-assaults. *Moringa* could therefore have produced synergistic anti-toxicity or effects to either complement the liver's similar functions, provide prophylactic effects against the consequences of cellular (hepatocytes) activities or help the liver cells improve their state of health. It is important to note that a number of murine model investigations have reported the

potency *Moringa oleifera* leaf extracts in protecting the liver from chemical toxicity and damage.[23,24]

CONCLUSION

It can be concluded from the present investigation that if herbal drugs like *Moringa oleifera* is given to cancer patient during or prior to radiotherapy it could minimize the side effects caused by the radiation.

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