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Mitigation of radiation and mercury induced histological changes in the brain of Swiss albino mice by *Moringa oliefera*

Manisha **Agarwal***, BirbalRam, Archana **Purohit**, Aruna **Chakrawarti** and R.K. **Purohit**

Radiation Biology Laboratory, Govt. Dungar College Bikaner (Raj.)
*Corresponding email:visheshagarwal003@gmail.com

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ABSTRACT

For the purpose of study male Swiss albino mice were divided into seven groups. The mice were exposed to gamma radiation (2.5 Gy and 5.0 Gy) and also provided mercuric chloride (0.5 ppm) both separately and combined. The herbal radioprotector Moringa was given to experimental groups after irradiation and mercury treatment. Animals were autopsied at post treatment interval of 1,2,4,7,14 and 28 days. After sacrificing the animal's histopathology of Brain was observed. In the present investigation, qualitative changes reflect the thickening of menninges, hyperaemia, pycnotic nuclei and developmental retardation of the individual neurons in terms of shape and size, degeneration of connective tissue, deviation from normal cytoarchitecture of the neocortex and irregular arrangements of neurons. The histological changes differ in degree of severity with dose and changes were more pronounced in combined treatment groups. Moringa treated groups demonstrated early and fast onset of recovery in comparison to non-Moringa treated groups. The presence of various bioactive compound in MOE are efficacious in protection and prevention of damage in antioxidant defense system of brain.

Keywords: radiation. Mercury. mice, moringa, brain

INTRODUCTION

In search for herbal radioprotection that could be effective and reliable in cases of radiation exposure, with the focus on the brain tissue, there is a need for neuroprotection against ionizing radiation. Estimated 70 percent of tissue damage that happen during irradiation is due to free radicals' generation, therefore it is necessary to find agents that could neutralize or eliminate free radicals. Antioxidants can stop or slow down oxidative stress in tissue and so reduce DNA damage that results from ionizing radiation. Most important endogenous antioxidant enzymes in human body are superoxide dismutase, catalase and glutathione peroxidase (SOD, CAT, GSH-Px). Radioprotective agents could generate endogenous neuroprotection, influence DNA repair, lower inflammatory response, and slow down

cellular division. The use of neuroprotective substances before or during radiation exposure could be a possible option to reduce radiation-induced tissue destruction.

Moringa oleifera (MO) is a highly valued plant in tropic and subtropical countries where it is mostly cultivated. The leaves are highly nutritious, being a good source of protein, β-carotene, vitamins A, B, C and E, riboflavin, nicotinic acid, folic acid, pyridoxine, amino acids, minerals and various phenolic compounds. MO leaves are highly nutritious, being a significant source of beta-carotene, vitamins, protein, minerals, amino acids, sterols, glycosides, alkaloids, flavonoids and phenolic. Almost all the parts of these plants have been used for various ailments in the indigenous medicine of South Asia, including the treatment of inflammation and infectious diseases along with cardiovascular, gastrointestinal, haematological and hepatorenal disorders. MO possesses antitumor, anti-inflammatory, antihypertensive, cholesterol lowering, antioxidant, antidiabetic and hepatoprotective activities.

Natural sources of mercury include volcanic activity, earthquakes, erosion, and the volatilization of mercury present in the marine environment and vegetation. Mercury emitted both naturally or as a result of human activity is primarily found as inorganic metal vapor (Hg^0). Among the natural sources of mercury, the largest emissions are from the degassing of the earth's crust. More than five tons of mercury is estimated to be released into the sea every year as a result of erosion and geochemical cycles.

Therefore, present study was done to evaluate the histopathological effect of radiation and mercury on brain and its mitigation by *Moringa oliefera.*

MATERIAL AND METHODS

The adult healthy Swiss albino mice were procured and maintained at animal house of Govt. Dungar College Bikaner (registration no.1066/go/re/s/07/ CPSEA). and provided balanced mice feed and water *ad libitum*. The animals were divided into seven groups to investigate protective effect of *Moringa oliefera* in mice brain against radiation (3.0Gy and 6.0 Gy) and mercuric chloride (0.5 ppm) alone and in combination also. The experimental animals also provided oral doses of *Moringa oleifera* extract(150 mg/kg body weight)seven days prior to irradiation. A minimum of five animals from each group were sacrificed by cervical dislocation and autopsied at each post treatment intervals of 1,2,4,7,14,28 days. After sacrificing the animals, brain was fixed in Bouin's fixative for 24 hours. The tissues were washed in water to remove excess of fixative, dehydrated in graded alcohol series, cleared in xylene and embedded in paraffin wax., Sections were cut at 5µm and stained in Harris hematoxylin and alcoholic eosin.

RESULTS

Cerebo cytoarchitecture was prominently damaged by the radiation treatment with both doses. A reduced total cell and neuron packing density has been noticed in cerebral cortex of mice at all the autopsy interval. Submenengial hemorrhages, neuronal degeneration perineuronal vacuolation ,atrophy of nerve cell were more prominent in mercury treated groups. Higher dose of radiation manifest greater degree of radio lesions. which showed in brain histology Cytoplasmic degranulation, vacuolation were observed.

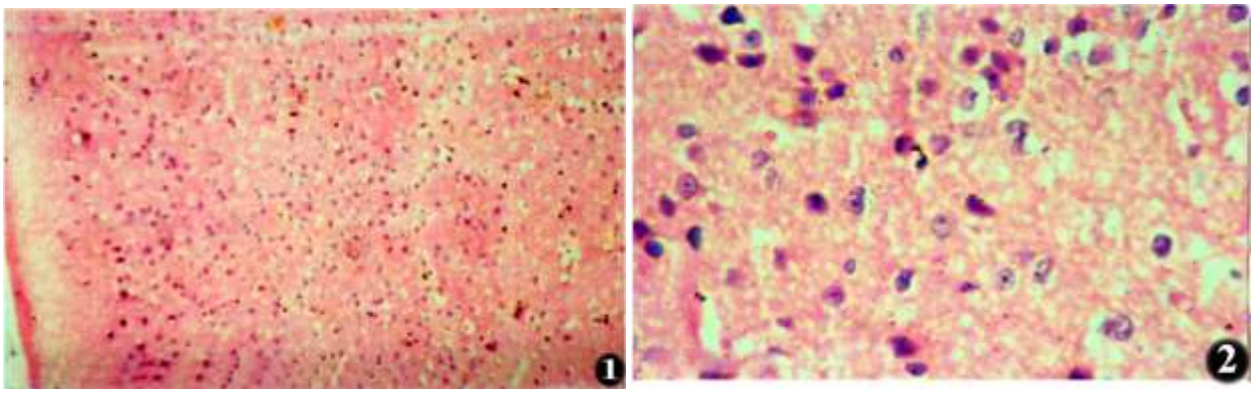

Figure 1: After 4 days of Mercuric Chloride treatment showing distorted architecture of \cerebral cortex, pycnotic nuclei and fatty degeneration

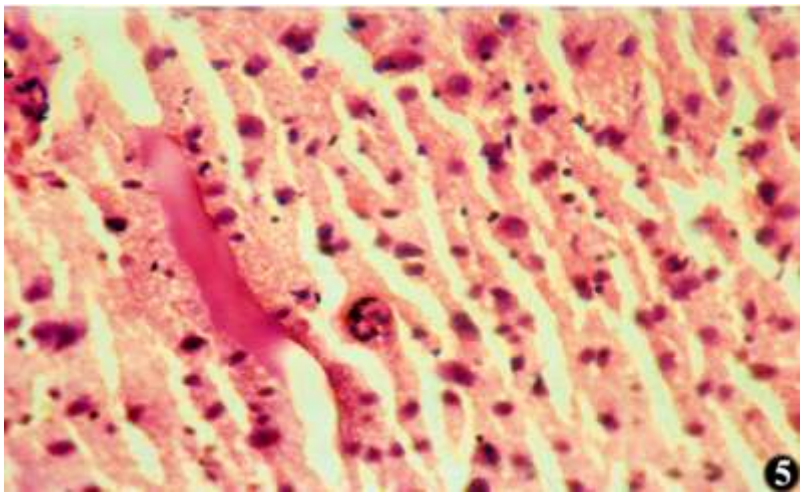

Figure 2: After 7 days (2.0 Gy + Mercuric chloride) showing completely disorganized architecture of cerebral cortex, degranulation of cytoplasm, cellular and fluid infiltration.

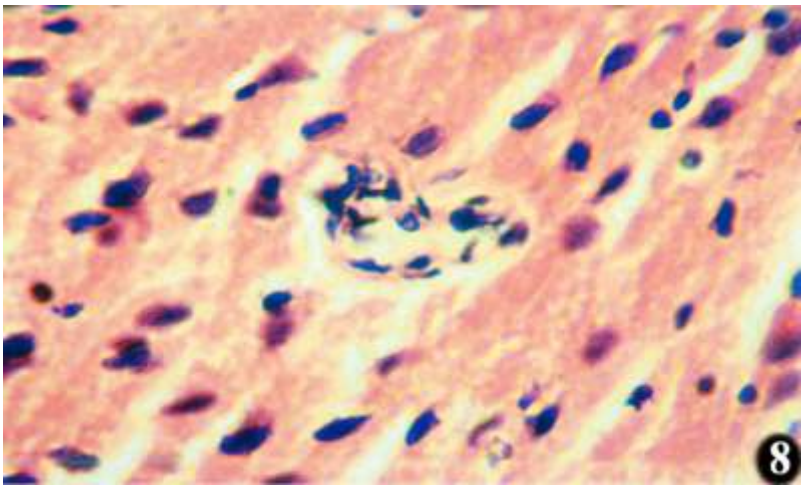

Figure 3: After 14-days (Hg_2Cl_2+ *Moringa*) showing better arranged cerelral cortex, mild cytoplasmic vacuolation and pycnotic nuclei. Clustering of neurons is visible.

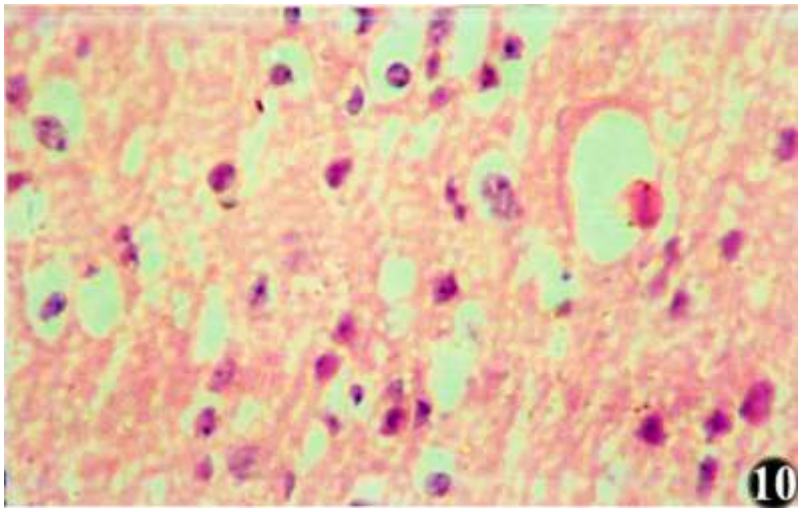

Figure 4: After 2-days (4.0 Gy + *Moringa*) displaying distortion and displacement of nuclei, cytoplasmic degranulation, vacuolation and spongy degeneration.

The pycnotic nuclei increase in number, Hematoma were present between the cortex and medulla with numerous pycnoyic and necrotic nuclei. Combined treatment group of radiation and mercury showed marked increase in severity of damage. The moringa treated groups showed early recovery On day-14 autopsy of the moringa treated group signs of recovery were appeared to reached at almost normal. Moringa provide protection from oxidative damage and causes fast recovery in comparison to non-drug treated group shows the radioprotective efficacy of Moringa.

DISCUSSION

Cellular response to radiation injury in the brain involves multiple cell types including astrocytes, microglia, oligodendrocytes, endothelial cells and neurons that initiates and respond to inflammatory cascade and contributes to progressive neuronal damage. (Tofilon and Fike, 2000; MOudler and Cohen, 2007).

The blood brain barrier functions to restrict the passage of most soluble molecules found in systemic circulation into the CNS. radiation results in destablization of the plasma membrane of vascular endothelial cells of BBB and changes in endothelial morphology are observed including basal lamina thickening, cytoplasmic vacuolization and cell swelling (Baker and Krochak, 1989).

Mice exhibiting reduced neurogenesis following 10 Gy of intracranial radiation also had reduced cognitive performance in maze test (Raber *et al*., 2004).

The beneficial functions of *M. oleifera* are strongly associated with its phytochemicals such as flavonoids or isothiocyanates with bioactivity. *M. oleifera* is beneficial in the prevention and treatment of a series of chronic diseases—including inflammatory diseases, neuro-dysfunctional diseases, diabetes, and cancers.
Natural compounds rich in polyphenols have strong antioxidant properties and can decrease oxidative damage in tissues by scavenging free radical (Niedzwiecki *et al.* 2016; Thapa *et al.* 2017; Zhang *et al.* 2014).

The methanol extract of *M. oleifera* leaves contains chlorogenic acid, rutin, quercetin glucoside, and kaempferol rhamnoglucoside, whereas in the root and stem barks, several procyanidin peaks are detected (Atawodi 2018). Similarly, the Moringa genus has high antioxidant activity mainly due to its high content of bioactive polyphenols (Verma *et al.* 2018) (Sreelatha and Padma, 2009).

The pre-administration of the hydro-ethanolic extract of *M. oleifera* before oral administration of paracetamol at the dose of 3 g/kg to male Sprague Dawley rats results in a significant reduction of lipid peroxidation; interestingly, the levels of glutathione-S transferase (GST), glutathione peroxidase (GPx), and glutathione reductase (GR) are restored to the normal levels in the group subjected to the pre-administration of *M. oleifera* extract. (Uma *et al*. 2010).

Fortunately, as a medicinal plant, *M. oleifera* extracts from both mature and tender leaves exhibit strong antioxidant activity against free radicals and prevent oxidative damage due to the enrichment of polyphenols. Lipid peroxidation (LPO) plays an important role in the metabolism of the body, which can lead to cell lesion and nerve damage if internal and external balances are broken. In a radiation-induced Swiss albino mouse model with oxidative stress, the pre-treatment with *M. oleifera* leaf extract for 15 consecutive days can effectively restore glutathione (GSH) level and prevent lipid peroxidation in liver. This protective effect may be related to a variety of phytochemicals such as ascorbic acid and phenols (catechin, epicatechin, ferulic acid, ellagic acid, and myricetin) through scavenging radiation-induced free radicals. (Sinha *et al.* 2011; Sinha *et al.* 2012)

CONCLUSION

Oxidative damage to organs and tissues by radiation can be mitigate by use of herbal drug may be beneficial during exposure to radiation for therapy ,occupational or accidental .M.oliefera is one of that herbal drug extract of which is proved to accelerate the recovery of damaged cells during this experiment. Thus the drug may have many bioactive compounds which could reduce damage caused by radiation and in future might be effective as radiation countermeasures.

Conflict of Interest:
None of the authors have any conflicts of interest to disclose. All the authors approved the final version of the manuscript.

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