HISTOLOGY AS BIOMARKERS: IN MOUSE BRAIN TREATED WITH RADIATION, CADMIUM AND THERAPEUTIC AGENT (ALOE VERA)

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Abstract: There are two different types of radiation, energetic particles and electromagnetic waves. These two types can penetrate into living tissue or cell and result in transduction of radiation energy to biological materials. The absorbed energy of ionizing radiation can break chemical bonds and cause ionization of different molecules including water and different biological essential macromolecules of DNA, membrane lipids and protein. Many types of DNA lesions are produced in cell by ionizing radiation and chemicals during cancer therapy. Cadmium is known to deplete glutathione and protein bound sulfhydryl groups which results in enhance production of reactive oxygen species (ROS). The reactions of these ROS with cellular biomolecules have been shown to lead to lipid per-oxidation. Aloe vera is dietary antioxidant that play an important role in controlling oxidative stress. For this purpose, six to eight weeks old male Swiss albino mice (Mus musculus) were randomly divided into seven groups on the basis of radiation, cadmium, combined treatment and Aloe treated groups. The animals were sacrificed at each post-treatment intervals of 1, 2, 4, 7, 14 and 28 days. The brain were taken out and weighed to the analytical balance and fixed for 24 hours in alcoholic Bouin’s fixative. A pinch of lithium carbonate was added to remove excess picric acid in the fixative. Histological studies were carried out using the standard techniques of haematoxyline and eosin staining. After routine procedure of microtomy the sections were stained with Harris haematoxylene and eosin in alcohol, dehydrated in graded series of alcohol, cleared in xylene, mounted in DPX and examined microscopically. The histological changes observed were, pycnotic nuclei and crenated cells with condensation of nuclear material resulting into hyperchromatic cells. Hydrocephaly with enlarged lateral ventricles was also noted and corpus callosum was seen malformed. Thickenened meninges and venous congestion were also noticed. In the irradiated brains cytoarchitectnic layers were reduced in depth and showed some degree of intermixing of cells of various laminae. Hematoma was present between the cortex and medulla with numerous pycnotic and necrotic nuclei. Disarray of the cortical tissue with disorientation of cell processes was also evident. Damage in the cortex was noticed in the form of karyolysis, pycnosis and spongy degeneration of the connective tissue with thickening of meninges. Dilation of blood vessel was also observed at certain palces. After combined treatment of radiation and cadmium chloride synergistic changes were observed. These changes were less severe in the Aloe vera treated brain which may be due to the protection provided by drug.

Keywords: Mice, Aloe vera, Radiation, Cadmium, Brain
INTRODUCTION

Radiation is beneficial for therapeutic purposes but high dose of continuous exposure can cause disasters consequences by damaging cell membrane and causing radiation syndrome. Damage to the biological systems by ionizing radiation is caused primarily due to macromolecular lesions by direct interaction of radiation with macromolecules as well as indirectly through reactive oxygen and nitrogen species generated during the radiolysis of water forms a variety of free radicals that are hydroxyl radical, superoxide radical and organic radicals. They are highly unstable species and damage critical cellular structures, including nucleic acids, membrane lipids and proteins. Metals have a special place among the toxic substances as they are neither created nor destroyed by man’s body. Cadmium is a heavy metal that has received considerable concern environmentally and occupationally. It has been demonstrated that cadmium stimulates free radical production, resulting in oxidative deterioration of lipids, proteins and DNA and initiating various pathological conditions in humans and animals\(^1\). Cadmium has a long biological half-life mainly due to its low rate of excretion from the body. Thus, prolonged exposure to cadmium will cause toxic effect due to its accumulation over time in a variety of tissues, including kidneys, liver, central nervous system (CNS), and peripheral neuronal systems. Cadmium can be uptaken from the nasal mucosa or olfactory pathways into the peripheral and central neurons; for the latter, cadmium can increase the blood brain barrier (BBB) permeability. However, mechanisms underlying Cadmium neurotoxicity remain not completely understood. Effect of cadmium neurotransmitter, oxidative damage, interaction with other metals such as cobalt and zinc, estrogen-like, effect and epigenetic modification may all be the underlying mechanisms.

The available finding indicates the neurotoxic effects of Cadmium that was associated with both biochemical changes of the cell and functional changes of central nervous system, suggesting that neurotoxic effects may play a role in the systemic toxic effects of the exposure to Cadmium, particularly the long-term exposure. \textit{Aloe vera} has been claimed to contain several important therapeutic properties, including anticancer effects\(^2\). Having these unique properties, \textit{Aloe vera} could be used in the clinical field as a protector against radiation and heavy metal toxicity in human beings. The most important constituents of aloes are the two Aloins, Barbaloin and Isobarbaloin, which constitute the so called 'crystalline' Alon, present in the drug at from 10 to 30 per cent. Other constituents are amorphous Aloin, resin and Aloe-emodin. The proportion in which the Aloins are present in the respective Aloes is not accurately known.

Hence, in the present study an attempt has been made to investigate the possible prophylactic role of \textit{Aloe vera} against radiation and cadmium induced changes in the brain of Swiss albino mice. Brain is considered abnormally sensitive to oxidative damage and in fast early studies
demonstrating the case of peroxidation of brain membranes. Brain is enriched in the more easily oxidizable polyunsaturated fatty acids such as docosahexaenoic acid and eicosapentaenoic acid as it has a limited ability to perform aerobic glycolysis. Brain is not enriched in antioxidant defences. It contains relatively low levels of superoxide dismutase, catalase and glutathione peroxidase.

MATERIALS AND METHODS

PROCUREMENT OF ANIMALS

Six to eight weeks old male Swiss albino mice were obtained from animal house of Lala Lajpat Rai University of Veterinary and Animal sciences, Hissar (India) and maintained in an air cooled room under controlled conditions of temperature and light. The animals were given standard mice feed procured from Brook Bond Lipton India Limited, Kolkata. Occasionally germinated gram was also given along with tap water ad libitum. In addition, tetracycline water, once in a fortnight, was also provided orally as a precautionary against infection.

CADMIUM CHLORIDE TREATMENT

Cadmium, in the form of cadmium chloride was administered orally in drinking water. Cadmium chloride was procured from S.D. Fine Chemicals Private Limited, Boisar (Mumbai).

SOURCE OF IRRADIATION

The animals used in the experiment were irradiated at the Radiotherapy Department of Prince Bijay Singh Memorial Hospital, Bikaner (Rajasthan) by Theratron, a Cobalt-60 beam therapy unit, procured from Atomic Energy Agency Limited, Canada.

MODE OF IRRADIATION

All the mice were exposed to Co60 γ-radiation simultaneously in a well ventilated wooden box of size 30 cm x 30 cm x 5cm having a glass lid. The box was placed at a distance of 75cms from the radiation source.

During experimentation, the dose rate varied from 0.69 Gy/min to 1.35 Gy/min. The dose was calculated at the midpoint by multiplying dose rate and tissue air ratio. The tissues of Swiss albino mice were assumed to be equivalent to human soft tissues.

Aloe vera

The fresh shade dried leaves of Aloe vera were powdered and refluxed with double distilled water for 36 hours at 40°C and vacuum evaporated, resulting in a powdered extract. The powdered extract was dissolved in DDW just before oral administration. Dose selection of Aloe
Aloe vera was done on our previously conducted animal survival study. The extract of Aloe vera was fed orally at the dose of 1000 mg/kg/b.wt./animal/day seven days prior to irradiation or cadmium chloride treatment.

**EXPERIMENTAL DESIGN**

In the present study, the animals were grouped as under:

GROUP-I - Sham Irradiated Animals - Normal

GROUP-II - Cadmium chloride treated animals

GROUP-III- Only irradiated animals

  Sub group IIIa : 3.5 Gy
  Sub group IIIb : 7.0 Gy

GROUP IV- Animals treated with radiation and cadmium chloride

  Sub group IVa : 3.5 Gy + CdCl₂
  Sub group IVb : 7.0 Gy + CdCl₂

GROUP V- Cadmium chloride and Aloe vera treated animals

GROUP VI - Radiation and Aloe vera treated animals

  Sub group VIa : 3.5 Gy + Aloe vera
  Sub group VIb : 7.0 Gy + Aloe vera

GROUP VI-I Radiation, Cadmium chloride and drug treated animals

  Sub group VIIa : 3.5 Gy + CdCl₂ + Aloe vera
  Sub group VIIb : 7.0 Gy + CdCl₂ + Aloe vera

**AUTOPSY:**

The Govt. Dungar College, Bikaner is registered under CPCSEA, Chennai (registration no. 1066/ac/07/CPCSEA) and has its own Institutional Animal Ethics Committee (IAEC). The present experiments were conducted under the supervision of IAEC of the College.
Five animals of each group (groups II to VII) were autopsied after cervical dislocation at each post-treatment interval of 1, 2, 4, 7, 14 and 28 days. In addition, five sham-irradiated (normal) mice were also autopsied in a similar manner.

Immediately after the autopsy, the brain was taken out and weighed. Later on, the width and length of brain were also recorded. Afterwards, part of brain was kept at -20°C for biochemical investigation and the rest of brain was fixed in Bouin’s Fluid for histological studies.

HISTOPATHOLOGICAL STUDIES:

The pieces of cerebral cortex were fixed in Bouin’s fluid for 24 hours and after fixation, the brain tissue were processed in a series of graded ethanols and xylene and then embedded in paraffin wax. 5 μm thick sections were cut by microtome and stained with Harris Hematoxylin and eosin.

RESULTS

In the present experiment histopathological changes found dose dependent in the brain of Swiss albino mice. The more severe changes seen in the brain architecture after exposure to 3.5 and 7.0 Gy of gamma rays exhibiting vacuolation in the neuropil and decreased number of picnotic nuclei, at certain places, karyolysis and condensation of nuclear material are also seen. In the irradiated brains represents a completely distorted architecture of cerebral cortex. Disarray of the cortical tissues with disorientation of cells evident and enucleated cells are also visible. (Figs.1-10)

In the combined treatment of gamma rays and cadmium chloride displaying disorganized cerebral cortex, pycnotic nuclei, shrinkage and fragmented nuclei were observed. Fusiform and granular cells are clearly evident.

In the drug treated animals representing recovered architecture of cerebral cortex was noticed.

![Image](image.png)

Showing normal brain architecture, cytoplasmic and nuclear structures
After 7 days of cadmium chloride treatment depicting disorientation of cells in sub molecular cortex. Some disintegrating neurons and a few abnormal nuclei are visible.

After 14-days of gamma ray exposure (3.5 Gy) exhibiting vacuolation in the neuropil and decreased number of pycnotic nuclei. At certain places, karyolysis and condensation of nuclear material are also seen.

After 4-days of gamma irradiation (7.0 Gy) representing a completely distorted architecture of
cerebral cortex. Disarray of the cortical tissue with disorientation of cells is evident. Enucleated cells are also visible.

After 4-days of combined treatment of gamma rays (3.5 Gy) and cadmium chloride displaying numerous pycnotic nuclei and necrotic cells in the cortex. Karyolysis and karyorrhexis are also evident. Spongy degeneration of the tissue is clearly evident.

After 4-days of combined treatment of gamma rays (7.0 Gy) and cadmium chloride displaying disorganized cerebral cortex, pycnotic nuclei. Shrinkage and fragmented nuclei are visible. Fusiform and granular cells are clearly evident.
After 28-days of cadmium chloride intoxication in the presence of *Aloe vera* displaying better arranged cerebral cortex with almost normal condition of the cells.

After 2-days of combined treatment of gamma rays (3.5 Gy) and cadmium chloride in the presence of *Aloe vera* depicting distortion in cerebral cortex tissue, pycnosis, karyolysis and enucleation.
After 1-day of combined treatment of gamma rays (7.0 Gy) and cadmium chloride with prior administration of *Aloe vera* showing karyolysis and karyorrhexis.

After 14-days of combined treatment of gamma rays (7.0 Gy) and cadmium chloride with prior administration of *Aloe vera* representing recovered architecture of cerebral cortex. A few cells are seen without nuclei.

**DISCUSSION**

The toxic action of cadmium on nervous sensory ganglia is specific\(^5\). The same conclusion concerning its toxicity for the central nervous system of newborn animals cannot be attained, because essentially similar lesions can be produced by other metals. However, cadmium appeared to be the most potent poison affecting the cerebrum.

In general, the cerebral structure that emerged most recently during phylogenesis (neopallium) is more sensitive to irradiation than those of older derivation (archipallium and paleopallium). Also, more peripheral cerebral structures, which are formed by repeated waves of dividing and
migrating neuroblasts, have the longest period of high sensitivity to ionizing radiation. The neocortex is a prime example of such a structure.

In the present investigation, qualitative changes reflect the thickening of the meninges, hyperaemia, pycnotic nuclei and developmental retardation of the individual neurons in terms of size and shape, degeneration of connective tissue, deviation from the normal cytoarchitecture of the neocortex, poorly delineated laminations and an irregular arrangement of neurons. The histological changes produced differ in the degree of severity with the dose. After combined treatment the changes were more severe showing synergistic effect of radiation and cadmium chloride. In the Aloe vera treated animals the changes were less severe and an early recovery was also observed which may be due to the protection provided by the drug. These histopathological changes are in conformation with the findings of.

It was reported that offspring of mice after whole body X-irradiation at a dose 10, 25 and 100 rads on days 13, 15 and 17 p.c. for brain weight, cortical thickness, cell packing density in the cortex and dendritic arborization of pyramidal cells in the layer V at 6 and 12 weeks of age. Brain weight, cortical thickness and cell packing density were reduced by 100 rad, but not by 25 rad and 10 rad of X-ray. These results indicated that prenatal radiation with 25R did not cause a deficit of cortical neurons in the adult mice, but obviously caused an irreversible developmental abnormality of cortical neurons in terms of dendritic arborization. X-irradiation of as low as 25 rad in the early stage of cortical histogenesis affected the proper migration of young neurons and brought about a disorder of cerebral architecture in mice. These results are in conformation with our present findings after 3.5 and 7.0 of gamma irradiation with or without cadmium chloride in the cerebral cortex of Swiss albino mice.

The mechanism of deviated positioning of cortical neurons and its functional consequences are not known at present. It should be noted that the critical period for radiation induced histogenetic disorders of the cerebral cortex was the period when the telencephalic matrix cells started to differentiate into cortical neurons and the cortical plate began to form, not only for the immediate pathological changes but for the long-term developmental disorders.

It was reported that the oral dosing of experimental animals with lead induced necrosis, hemorrhage and degenerative changes which are in general agreement with the present study. Congestion, degenerated neurons, satellitosis, neuronophagia, encephalomalacia, coagulative necrosis of Purkinje cell of cerebellar white matter were observed. These changes were supported by the morphometric results which revealed a significant reduction in the mean thickness of the Purkinje cell layer as compared to control rats. Similar results were also observed. These results are in conformation with our present histopathological observations with radiation and cadmium induced toxicity.
Present study also reflected that with both the types of doses, with or without cadmium chloride the outer layers of the cortex gets more affected than lowermost layer of the cortex as was evident by the presence of large number of pynotic and necrotic cells. With the advancing age the degree of damage was decreased.

**PROTECTIVE MECHANISM OF ALOE VERA**

1. It has been shown that the exogenous application of *Aloe vera* increases glutathione levels in the tissues on one hand and maintains -SH groups and increases protein synthesis on the other hand.

2. The protection offered by *Aloe vera* has been explained by scavenging or oxidizing free-radicals Thus it can be concluded that *Aloe vera* may inhibit the Lipid peroxidation by:

(i) Reducing the formation of free radicals,

(ii) Scavenging of free radicals\(^{(13)}\) (antioxidant mechanisms).

(iii) Exudating the repair mechanism of damaged cell membrane.

(iv) Delay of cellular division and inducing hypoxia in the tissues\(^{(14)}\).

(v) *Aloe vera* provides powerful anti-oxidant action, due to amongst to other properties, its vitamin content, especially vitamin A, E and C\(^{(15)}\). In addition to its innate anti-oxidant properties and constituents, *Aloe vera* has the ability to stimulate the body’s own anti-oxidant activities. This results in reduced oxidative stress, which has been shown to play “play an important role in age related disease”\(^{(16)}\).

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