Effects of Radiation on the Spinal Cord of Wistar Rats Exposed to X-Ray.

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ABSTRACT

The spinal cord is a major dose limiting organ in radiotherapy and it is unavoidably included in the treatment fields, injury to the spinal cord results in the destruction or impairment of its function which manifest from one to several years after treatment. It is an animal experimental study with a total of 20 adult albino Wistar rat weighing between 150g to 200g were used for this study. The animals were acquired from animal's house at University of Port Harcourt and acclimatize for two weeks. In this results from this research work, there were notable damage to the spinal cord after being exposed to radiation at a given time. These damages includes; degenerations and reduction in number of motor neutrons, degeneration of pyramidal cells, depletion of cell cytoplasm, vacuolation were also observed in this rats belonging to group C and less motor neurons were seen. The exposure of high amount of radiation continually to the spinal cord damages the motor neurons of the corticospinal pathways where it caused cells degeneration leading to cell death. The death of the motor neurons would mean an impairment in the locomotive functions of the animal.

Keywords: Spinal Cord, pyramidal cells, exposed adult rats, Radiation, Degenerative motor neurons, Cytoplasm, Vacuolation.

INTRODUCTION

Ionizing radiation is a ubiquitous phenomenon that has important ramifications for biological systems. It is characterized by its ability to extract tightly bonded electrons from atoms, leading to their ionization. Its sources include radiation from the natural environment as well as man-made operations including industrial processes, nuclear energy generation, and medical imaging. Ionizing radiation has several known therapeutic uses, most notably in the treatment of cancer. However, there is significant worry about possible negative effects on healthy tissues, particularly those with high radio sensitivity.

One of the spinal cord's primary roles in the central nervous system is to transmit nerve impulses from the brain to the body's other organs. Because the spinal cord (SC) is crucial to the preservation of physiological processes, any injury to it can result in severe neurological abnormalities such as paralysis, loss of sensation, and impaired motor function. It has been previously demonstrated that ionizing radiation may induce a range of pathological alterations in the spinal cord, including demyelination, necrosis, and vascular damage. Radiation myelopathy is a condition that is often progressive and delayed and may be permanent. These changes may eventually cause this disorder.

The model organism known as Wistar rats, which is frequently employed in biomedical research, has shown to be extremely helpful in comprehending the biological impacts of a variety of external conditions, such as ionizing radiation. Their physiological similarities to humans, well-characterized genetics, and simplicity of handling make them a perfect candidate for researching the processes behind spinal cord injury and radiation-induced damage.

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This study aims to examine the effects of ionizing radiation on the spinal cord of Wistar rats, with particular attention to the post-exposure histological, molecular, and functional alterations. Through clarifying these impacts, the study aims to enhance comprehension of radiation-induced spinal cord injury and offer valuable perspectives that may guide the creation of preventative measures or remedial actions to lessen such harm in medical environments.

MATERIALS AND METHODS

The research is an animal experimental study. The animals were acquired from the animal house, Department of Pharmacology, University of Port Harcourt, and allowed to acclimatize for two weeks. The animals were fed with rat feed and water. The animals were divided into four groups with five (5) rats in each group. Group A was the control group, group B was exposed for three (3) days, group C was exposed for seven (7) days and group D was exposed for fourteen days (14 days which is 2 weeks).

The rats were sacrificed at the end of each group experiment and the spinal cords were extracted and fixed in 10% formalin for 24hrs before a histolopathological study was carried out on them.

Anesthesia was achieved by inhalation of anesthesia containing 0.001percent of diphenylamine which resulted in mild anesthesia. 5ml of anesthetic was used to soak sterile cotton wool balls and the balls were placed into a glass desiccator. The rats were placed individually into the desiccator and they were thoroughly monitored until they were anesthetized.

Radiation Technique

The amount of time for exposure of the test animals was two weeks; the amount of X-radiation dose was constant across all the groups except for the control group which was not irradiated at all. The difference among the group was the total number of days used for exposure; a mobile x-ray machine was used. The anesthetized rat was placed centrally on a bench and the distance between the animal and the x-ray machine was 10cm. The centering point of the x-ray were focused mainly on the head [brain] and spinal cord.

Figure 1: An exposed rat during the experiment.

Group A: This was the control group and no radiation was administered to this group.

Group B: was exposed to radiation for three days and sacrificed for histopathology.

Group C: was exposed to radiation for seven days and sacrificed for histopathology

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Group D: was exposed to radiation for fourteen days and sacrificed for histopathology.

Exposure Factor and time: the exposure factor used on the rats are 85kV and 20m as. The rats were exposedto a 0.0026-Gy dose of x-radiation at 0.02 seconds using a distance of 100cm. Each rat is exposed to the same amount of radiation three times daily.

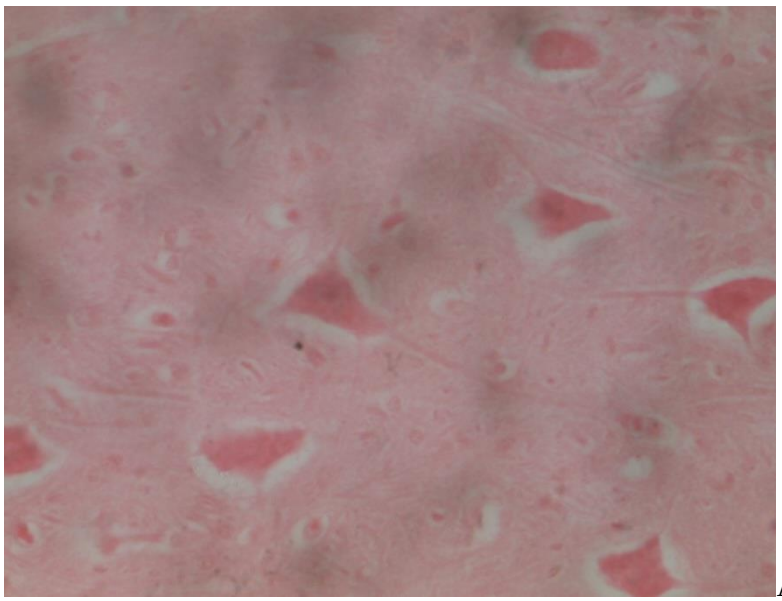
Organ Harvesting

The rats were sacrificed humanely, the brain was harvested and fixed in 10% formalin for 24 hours before subsequent tissue processing.

Histopathological Procedure

The Spinal cord was fixed in formalin, dehydrated with alcohol; it was cleared with xylene and infiltrated with paraffin wax. The tissue got embedded and thin sections were cut using a microtome. Haematoxylin and Eosin stains were incorporated on the sections to show the cytoarchitecture (microscopic structure). The slides were viewed under a light microscope.

RESULTS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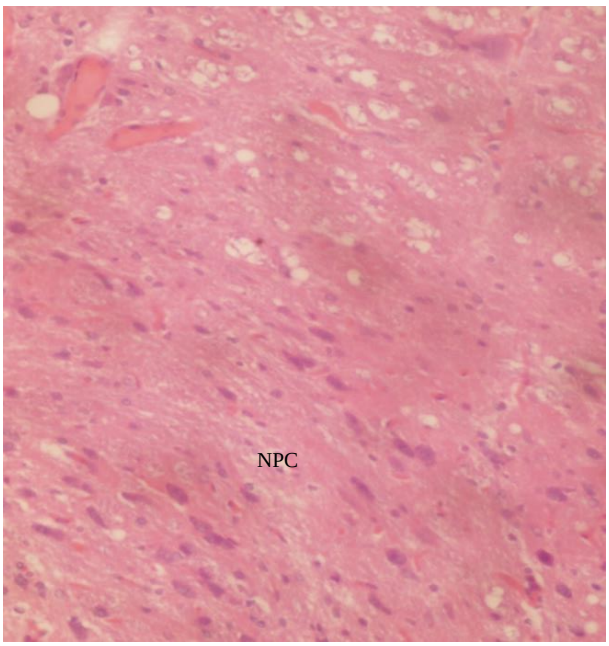

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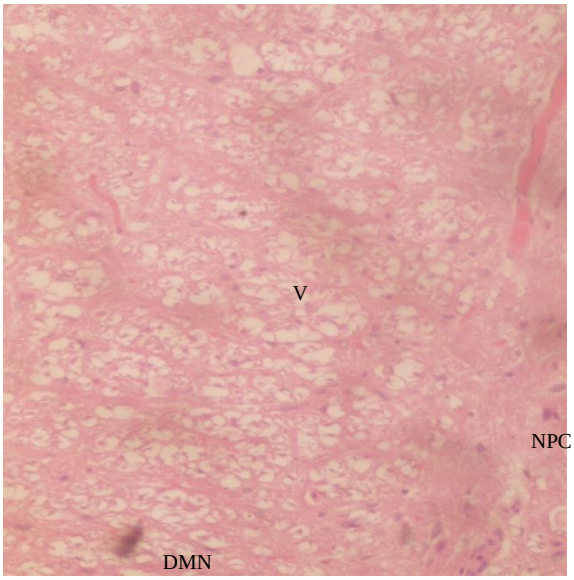

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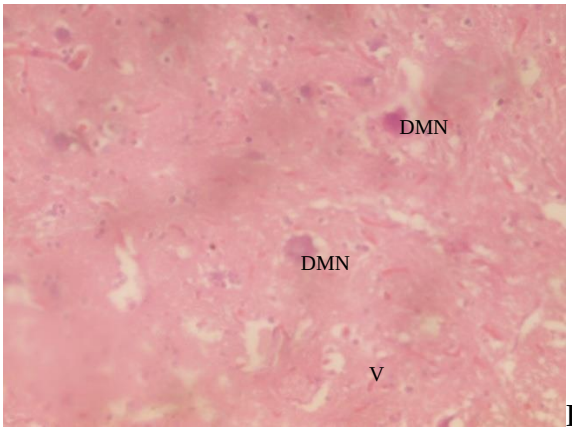

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Figures 2. Shows the photomicrograph of 400x magnification of (A) control rats (B) Cytoarchitecture of the spinal cord in 3 days X-ray exposed adult rats (C) Cytoarchitecture of the spinal cord in 7 days X-ray exposed adult rats (D) Cytoarchitecture of the spinal cord in 14days X-ray exposed adult rats.DMN- Degenerative Motor Neurons NPC- Normal Pyramidal Cell, V=Vacoulation.

DISCUSSION

Previous research work that has been done on the effect of radiation on the spinal cords have based most of their research work on functions of the nervous system, behavioral studies, biochemical and Histopathological changes. This work focuses on the changes noted histopathologically on the rat's spinal cord after irradiation.

In the results from this research work, there was notable damage to the spinal cord after being exposed to radiation at a given time. These damages include;

1. Degenerations and reduction in number of motor neurons.
2. Degeneration of pyramidal cells.
3. Depletion of cell cytoplasm.
4. Vacuolation were also observed in this rats belonging to group C. Less motor neurons were seen.

After 14days X-ray exposure in the rats, it was observed that there was degeneration of pyramidal cells. Further degenerative motor neurons (DN), Depleted cytoplasm as well as vacuolation were also observed in these rats and less motor neurons were seen. From this findings, it can be concluded that exposure to X-ray have indeed a negative effect on the spinal cord, which could lead to loss of motor functions and paralysis. This finding is similar with Wong et al., (1995) research on the response of rat spinal cord to very small dose per fraction. In the research, the rat spinal cord was irradiated with top-up dose of three daily doses of

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10.25Gy, followed by graded single doses or fractionated doses. The endpoint was forelimb paralysis which was secondary to white matter necrosis confirmed histopathologically.

Wong et al., (1993) while carrying out an experiment on re-irradiation tolerance of the rat's spinal cord to fractionated X-ray doses, noted a forelimb paralysis from a latent period of which ranges from 189 to 245days from the initial days of treatment.

Similarly, Wong and Hao (1997) aimed at accessing the influence of the level of initial injury on the long term recovery kinetics of radiation damage in the central nervous system using rat spinal cord model and the result was forelimb paralysis caused by white matter necrosis.

Van der Kogel and Barendsen (1974) found out that in the cervical cord, the oligodendrocyte and vascular endothelial populations rather than the Schwann cells appear to be the most vulnerable to irradiation. It also shows that sections of the cervical cord from the irradiated animals showed necrosis of the white matter which is similar to findings of this research.

Ann and Shirley (1978) while studying radiation damage to the rat's spinal cord noted that considerable sparing of the spinal cord can be achieved by using a large number of fractions.

li et al.,(1996) Alkinsonet al., (2003) reported that irradiation of the rat's spinal cord with a single dose exposure of 1-30Gy, led to 24Hrs after treatment, dramatic increase of oligodendroglial apoptosis and concomitant disease of 0.2A cells and mature oligodendrocystes. A similar radiation induced oligodendroglial apoptosis was observed after whole brain irradiation with doses of 10 – 22Gy in the SVZ, and SGZ of the hippocampal DG and corpus callosum (Sasaki et al., (2000), Chow et al., (2000) and Kurita et al., (2001)).

This finding aligns with previous reports on the effects of irradiation on the spinal cord.

CONCLUSION

The use of X-rays irradiation in the medical field is of great importance but it must be utilized with good caution since radiation exposure can evoke serious health consequences, as pointed out in spinal cord exposure in this work. The use of radiation excessively could cause degenerations and reduction in number of motor neurons, degeneration of pyramidal cells, depletion of cell cytoplasm, vacuolation and as such, doses of radiation-induced on individuals should be calculated and the ALARA (as low as reasonable achievable) principle must be strictly adhered to.

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