2013 International Nuclear Atlantic Conference - INAC 2013
Recife, PE, Brazil, November 24-29, 2013
ASSOCIAÇÃO BRASILEIRA DE ENERGIA NUCLEAR - ABEN
ISBN: **978-85-99141-05-2**

AN ATHYMIC MOUSE MODEL TO MIMIC COBALT-60 CUTANEOUS RADIATION INJURY.

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ABSTRACT

Propose: Cutaneous wound from irradiation is the most common complication in radiotherapy treatment, and can be lead to mortality. We describe an athymic mouse model to mimic cutaneous radiation injury by Cobalt-60.
Methods: A protocol was including dosimetry with silicon diodes,10x10x5 cm arrangement made by four lead bricks and PVC pipe designed to immobilize the athymic mouse in order to irradiate one clamped back skin point that was subdivided in four parts. To get the measurements of dose rates on the arrangement in Panoramic Irradiator, it was used a silicon diode encased in an opaque protection for ambient light and connected to an electric cable, forming a dosing probe. The currents generated in diode sensitive volume as a function of time of exposure to gamma radiation coming from the radiator, with dose rate of 0,015 Gy/min in positions 1, 0,021 Gy/min in position 2, 0,55 Gy/min in position 3 and 1,45 Gy/min in position four. After the dosimetry, each athymic mouse was anesthetized using Xylazine and Ketamine dilution and entered into a PVC pipe and a small portion of skin (1 cm3) was clamped. This tube was then fixed to arrangement and the athymic mouse was irradiate for 60 min, than it was being returned to its cage.
Results: The wound was visualized in all animals and photographed after 5 days of irradiation, with the emergence of ulceration after 9 days. No systemic or lethal sequelae occurred or visualized in any animals. Late clinical signs included a wound healing after 22 days.
Conclusion: While still being a baseline study, we created a new functional preclinical animal model that can be used for new therapies and may improve radiotherapy management.

Key Words – Cutaneous Wounds, Cobalt-60, Radiotherapy

1. INTRODUCTION

According to report by the International Agency for Research on Cancer (IARC) / WHO (2008-2010), the global impact of cancer more than doubled in 30 years. In this report, it was estimated that occurred about 12 million new cancer cases and 7 million deaths. This impact will fall primarily on the countries of medium and low development where, according to IARC / WHO, will be half of new cases and about two thirds of cancer deaths [1].

Among the possibilities to cancer treatment, radiotherapy is one of the treatment modalities used during the period of its evolution. Treatment with radiotherapy can be divided into two methods, brachytherapy and external beam radiation. External radiation therapy uses different sources of radiation such as X-rays and photons emission (linear accelerators, and Cobalt-60) [2]. The purpose of applying ionizing radiation is in the region where we want to treat the cancer by increasing cell death and thus eliminating the existing

tumor mass. However, in addition to the therapeutic effects of radiation, there are specific complications in different regions of the human body that are related to the response of tissues to radiation and the cell's ability to repair or not radiation-induced lesions. The rapid response of tissues are those with clinical manifestations of injury, after irradiation, in a short period of time, such as skin, mucous membranes, hematopoietic tissue, lymphoid tissue and certain tumors. The slow responses of tissues are those with more delayed. Examples are bone tissue, connective, muscular and nervous systems, which have low proliferative activity [3].

Treating chronic non-healing ulcerations post radiation involves implementing wound-healing principles. Providing an optimal wound environment to promote granulation utilizing the most appropriate dressing is the first step in addressing these ulcerations. Pain often accompanies these ulcerations, therefore pain management is a crucial component of the treatment plan. The pain associated with these ulcerations is often the reason a patient seeks treatment in the first place. Dressing choices to reduce the pain associated with these ulcerations must be incorporated. Often, dressings that need to be changed every few days or even longer may be selected to minimize the pain associated with dressing changes. Use of a hydrogel in dry wound beds will promote wound healing as well as decrease the pain. Other dressing considerations include anatomical locations of the ulceration, topical antibacterial control, and protection of the fragile peri-wound skin areas [4,5].

Surgical options in the treatment of irradiated tissue include removal of the non-viable tissue and bone and reconstructive repair. Surgical procedures should be performed once past the acute or inflammatory phase. Wound debridement to healthy bleeding tissue may not be adequate as fibrosis may prevent contracture of the arterioles. Debridement to tissue exceeding the wound margins may be necessary to promote optimal wound healing in post-radiated ulcerations [4,5].

Current researches for cutaneous radiation damage are limited, but future discoveries may provide therapies which revitalize affected tissue and ameliorate the progressive deterioration of skin [6]. In order to reproduce the side effects on radiotherapy our aim was to provide a new design of a novel protocol to apply Cobalt-60 radiation to the skin of athymic mouse in a single dose, and furthermore to be used to test human tissue-based therapies in the setting of cutaneous radiation injury.

2. MATERIALS AND METHODS:

2.1. Dosimetry for Cobalt-60 source using silicon diode probe

The experiment was performed on Panoramic Irradiator on Technology Radiation Center of IPEN/CNEN-SP at 6/22/2012. This irradiator has a metal table with 1.20 m height and 1 m square head with edge. The table has a hole in the center in which crosses a metal shaft with 10 cm diameter, that directs the source of Cobalt-60 (20 cm high) from the center of the table until the roof of the irradiation chamber.

Four lead bricks blindage with dimensions of 10 x 10 x 5 cm were positioned at 10 cm at this shaft to minimize the effects of Cobalt-6o radiation on athymic mouse. This mouse will be inserting on 7 cm height PVC arrangement closed at the ends, which secures the athymic mouse in blindage position, as show on **Figure 1** and **2**.

To get the measurements of dose rates it was used a silicon diode encased in an opaque protection for ambient light and connected to an electric cable, forming a dosing probe. This probe was connected at the entrance of the electrometer Keithley® model 617.

The GPIB connection passes the data from the electrometer to laptop with the aid of a data acquisition program developed in LabVIEW™.

Initially the silicon diode was positioned above the table of 10 cm and 10 cm of irradiation distant central axis of the shaft were then carried out the connections between equipment. With all equipment plugged in and turned on, waiting 10 minutes to stabilize the system. After this time, fired up the data acquisition program to measure the leakage current for 10 minutes. With the heater on and the source exposed on the table, was held the measure of dose rate. This procedure was repeated ten times for stabilization of the response from dosimeter and checking the accuracy of the same, as the protocol of the International Atomic Energy Agency.

Before measure radiation rates in the PVC arrangement, it is necessary to produce the sensitivity curve of the probe, radiating the diode at different dose rates. To quantify the different rates of doses in the arrangement was mounted a calibration curve of diode, current measures certified Panoramic positions radiator. Current measures were held, positions: 10; 20; 30 and 40 cm.

After the calibration curve of diode, it was inserting into PVC arrangement in four different positions, whereas the outermost position (**Figure 1A** - item 4) to receive the highest dose of radiation and on the other three subsequent positions (**Figure 1A** - item 1 to 3) the dose can be smaller, thus avoiding direct exposition of mouse to gamma radiation.

2.2. Animal preparation and irradiation

All studies were carried out in accordance with the Care and Use of Laboratory Animals. Three nude mice aged 8 weeks, male, weighing 20 ± 5 g and bred and fed in the Animal Center of Biotechnology Center at IPEN/CNEN – SP. The mice used were approved by Committee of Ethics and Research for Animals (IPEN/CNEN – SP). The protocol was designed to irradiate the back skin of athymic mouse and avoid the body and internal organs from significant radiation damage.

Before any animals procedures, it was anaesthetized using 0,3 mL intramuscular injection of ketamine and xylazine in solution with physiologic serum (1,0 mL ketamine + 0,5 mL xilazine + 8,5mL physiologic serum).

The athymic mouse was inserting inside a PVC arrangement which was subsequently closed at the ends. There is a hole at this PVC arrangement which was added on top a circular shape accessory made of quartz glass, with 1 cm high and 2 cm wide. Into this hole the animal skin will be clamped and stretched in such a way that it is possible to insert a strip needle, which serve as support for the skin is stretched. Following the results of dosimetry, the PVC arrangement contending the athymic mouse was positioned behind the leads bricks, and for 1 hour the animals was irradiated as showing in **Figure 1B** and **2B**.

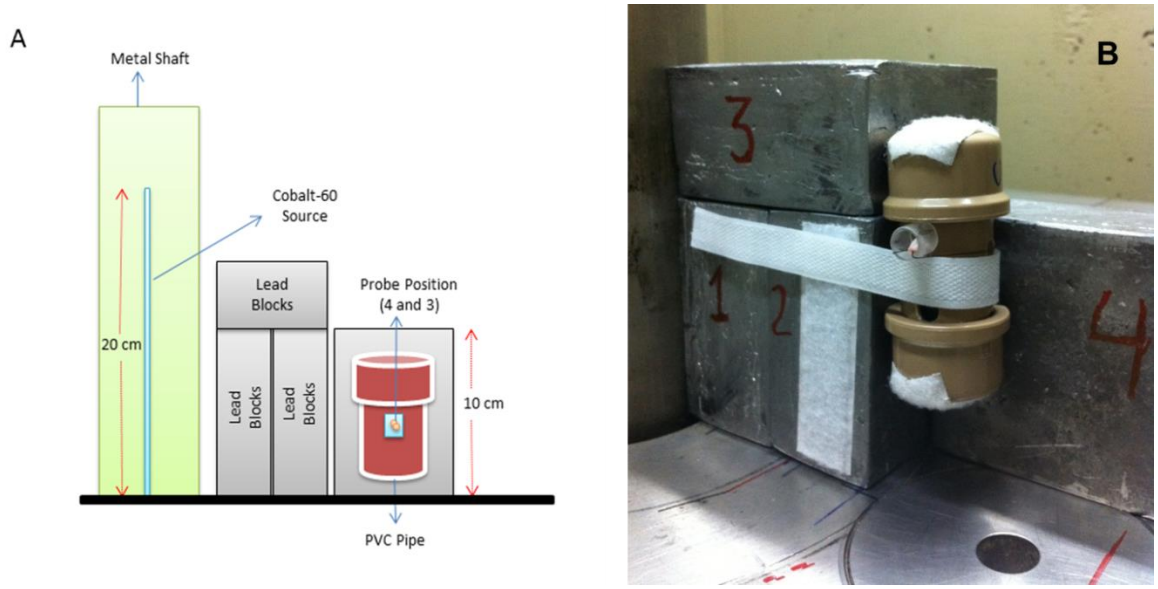

Figure 2 - Side view of probe arrangement lead blindage with positions 3 and 4 (A) and with of athymic mice in position (B).

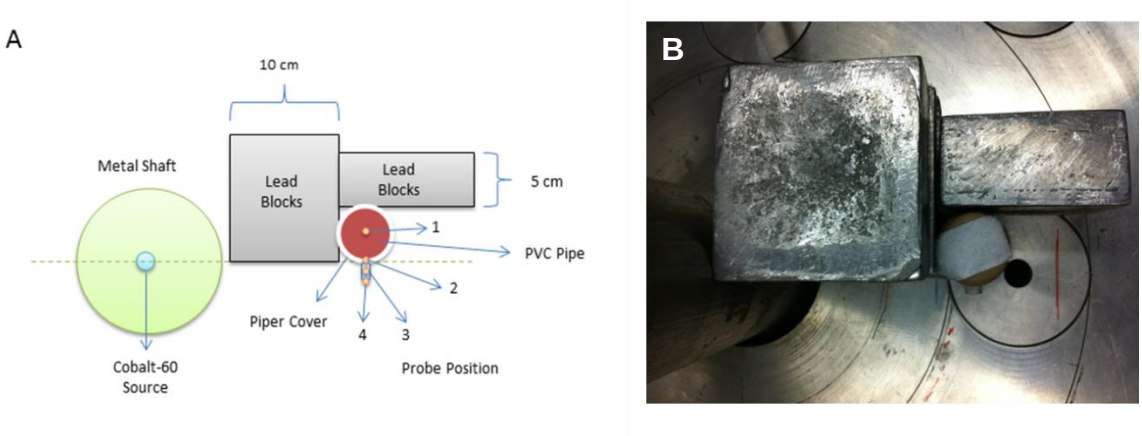

Figure 1 - Top view of probe arrangement lead blindage with positions 1, 2, 3 and 4 (A) and the athymic mice position (B).

3. RESULTS

3.1. Stabilization of the probe response and dosing accuracy

The currents generated in diode sensitive volume as a function of time of exposure to gamma radiation coming from the radiator, with dose rate of 2.18 Gy/min, is illustrated in **Figure 3**. The results obtained show that the observed current signals are stable throughout the irradiation time, with an accuracy of 98%.

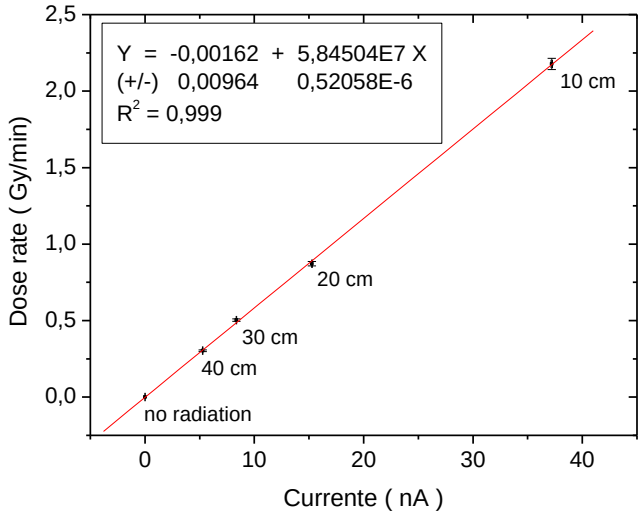

Figure 3 - Calibration curve diode (dose rate / current) with respect to source distance (10, 20, 30 and 40 cm). Doses rates certified by the IAEA.

3.2. Sensitivity curve and dose rate measures in PVC arrangement for irradiation of athymic mouse

Current measures were held, positions: 10; 20; 30 and 40 cm, with guaranteed doses of 2.17; 0.87; 0.30 and 0.50 Gy/min respectively. Registered by the diode current as a function of dose rates, linear behavior presents. With the chart, response curve measures the dose rates were in the arrangement of the athymic mouse. The **Table 1** presents a summary of dose rate values obtained in positions 1, 2, 3 and 4 in PVC arrangement.

Table 1 – Dose and dose rate of radiation in an hour arrangement obtained with diode, for irradiating athymic mouse.

Position	**1**	**2**	**3**	**4**
Dose Rate (Gy/min)	0,015	0,021	0,55	1,45
1 hour dose radiation (Gy/h)	0,9	1,3	32,9	86,8

3.3. Athymic mouse wounds

No systemic or lethal sequelae occurred in any study animals. All irradiated skin area underwent significant ulceration. The total dose was 86 Gy and the first sign of wound showed after 9 days of single dose irradiation **(Figure 4A)**. The wounds had undergone dry desquamation surrounded by intense crusting at the borders. After 15 days, the wounds reached its maximum degree of severity without compromising the health of athymic mouse **(Figure 4B).** Later than, it's possible to see evidences of scabbing, crusting, and signs of healing throughout wounds and 22 days afterwards the wound self-healing as showing on **Figure 4C**.

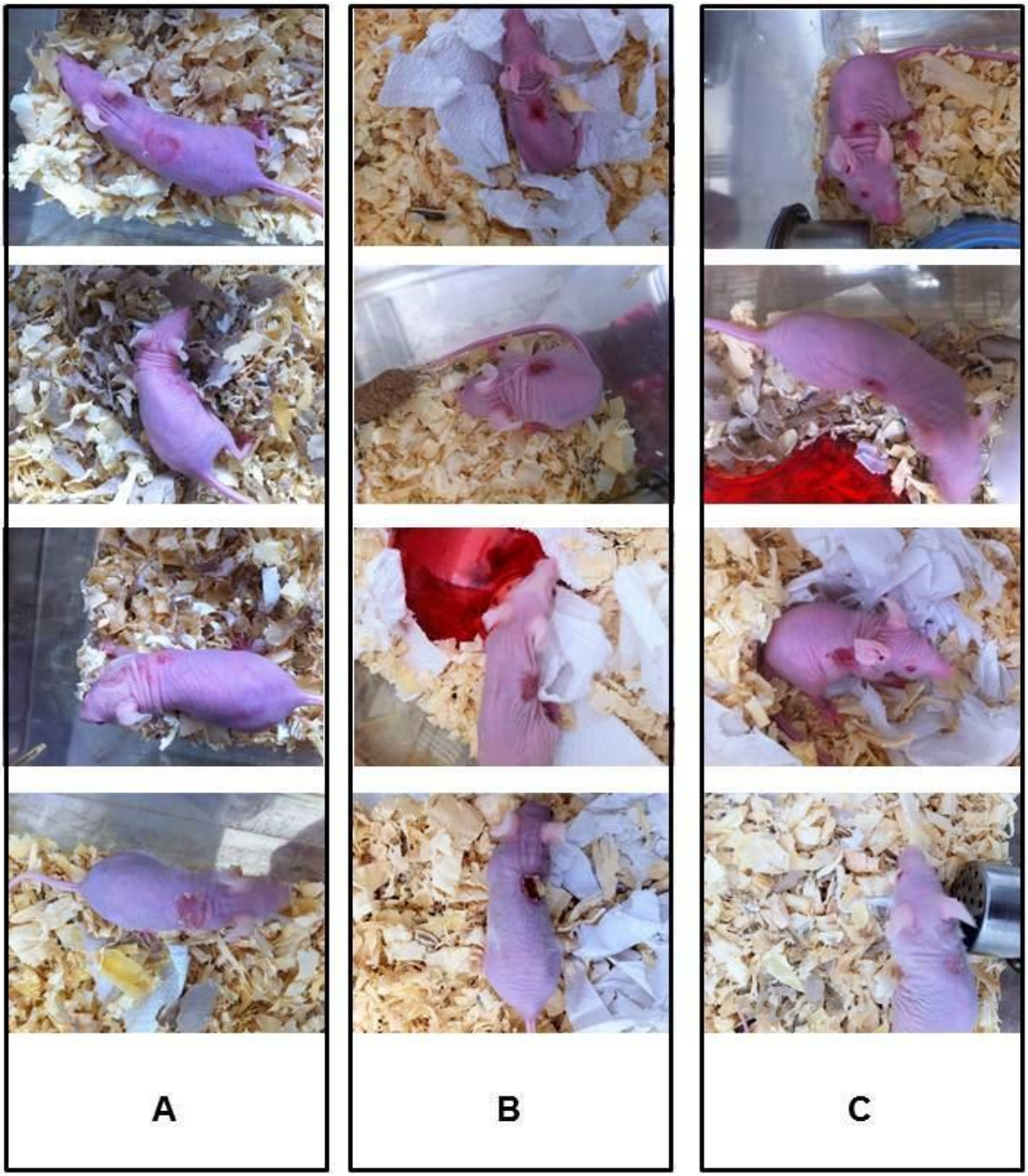

Figure 4 - First sign of wound showed after 9 days of single dose irradiation. The wounds had undergone dry desquamation surrounded by intense crusting at the borders **(A);** after 15 days, the wounds reached its maximum degree of severity without compromising the health of athymic mouse **(B).** Later than, it's possible to see evidences of scabbing, crusting, and signs of healing throughout wounds and 22 days afterwards the wound self-healing **(C)**.

4. DISCUSSION

The radiation dose thresholds show a wide variation. On average the given dose thresholds from the bibliography are: 2 Gy for transient erythema, 6 Gy for main erythema, 10 Gy for dry desquamatation, 15 Gy for moist desquamatation, telengiectasia and chronic erythema and 18 Gy for skin necrosis and ulceration [7]. Based on our dosimetry (**Table 1**) we have tested different doses in five different athymic mouse (43,5; 58; 72,5; 86,8; 101,5

Gy) and could produce a radiodermatitis with radionecrosis from 86,8 Gy and up in single dose (**Figure 4A**). Lower doses don't produce signals of radiodermititis and/or radionecrosis even after 5 months (**Figure 5A**) and 86,8 Gy to up, unfortunately producing unviable proportions of radiodermatitis and radionecrosis reducing severely the animal life quality (**Figure 5B**).

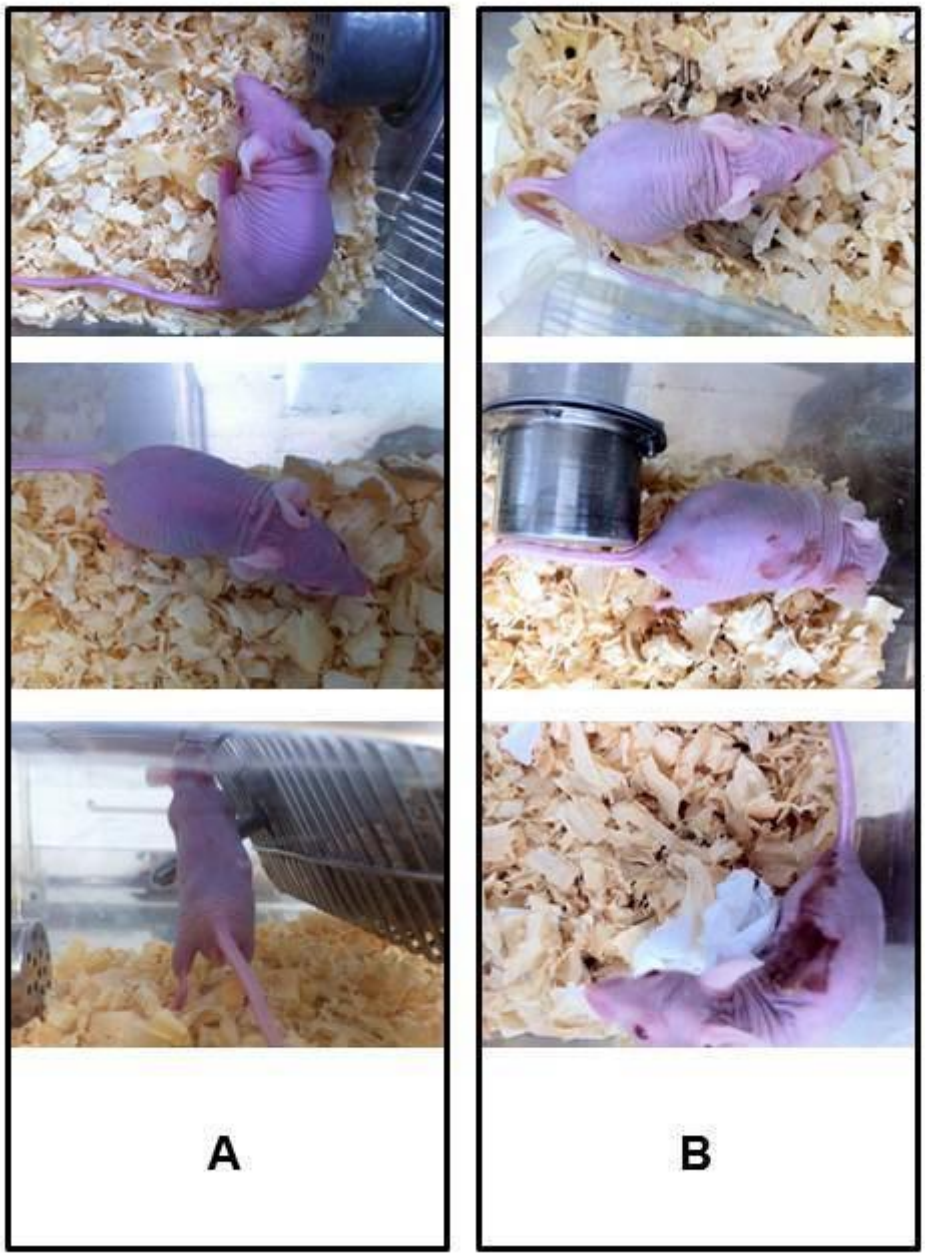

Figure 5 - Lower doses (1 - 43,5 Gy; 2 – 58 Gy; 3 – 72,5 Gy) as showing on **A** don't produce any signals of radiodermatitis and/or radionecrosis on athymic mice even after 9 days, differently as we can observe in the 86,8 Gy single dose animals, although we can observe irregular desquamation on athymic mouse 2 and transient erythema on 3. This sings don't have envolved to radiodermatitis and/or radionecrosis. 86,8 Gy single dose and up producing radiodermatitis and radionecrosis however 101,5 Gy single dose unfortunately producing unviable proportions of radiodermatitis and radionecrosis reducing severely the animal life quality **(B** – Top to bottom **–** After 8 days, 9 days and 12 days**).**

The effect of radiation on wound healing has been reported on many occasions, including various aspects of mechanism of wound healing delay, such as the dosage of irradiation and the degree delay. There has, however, been little research dealing with the relation between the dosage of irradiation and delay of healing and also the time-effect of this phenomenon [7]. In spite of using an animal model and different conditions of a radiotherapy treatment with a single dose of 86 Gy we observe the appearance of a radionecrosis from the ninth day and total self-healing after 22 days of experiment, they had no physiological alterations in animal, even after 5 months. These results, although they differ from previous studies of radiodermatitis and radionecrosis caused by radiotherapy, encourages a new need for research involving wounds caused mainly by gamma radiation.

Other studies using an x-ray image guided stereotactic irradiator [6] demonstrate that the 30.4 Gy dose caused mild injuries which had the capacity to resolve as early as 20 days postirradiation without intervention. We can observe that high doses had been capacity to resolve as itself, however with more few days. Above 95 Gy we observed that the injuries caused by irradiation reached a considerable depth to the extent of not being possible any restorative treatment (**Figure 5B**).

5. CONCLUSION

The present study aimed to establish an animal model of radiodermatitis and radionecrosis. We present a protocol using gamma radiation (Cobalt-60) with an 86,8 Gy single dose, able to create these injuries without causing any physiological and / or systemically alteration in Nude mice so that others experiments can be made to treating this advent.

Acknowledgments:

This experiment was prepared with financial support from the IAEA through the RC project (Research Contract No. 15333), CNPq Universal Project - 480390/2010-0 and CNEN.

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