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Evaluation of induced biological effects in rats by continuous and natural gamma radiation using a physical simulator

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ABSTRACT

Purpose: The effects of radioactivity on human health have been debated for many years but there are still important gaps that need to be addressed especially related to the effects of high natural background radiation on the local population. The beach of Meaípe, in the city of Guarapari (Brazil), emits natural gamma radiation due to the presence of monazite sands. We aimed to investigate the effects of gamma radiation doses on the biological system of wistar rats using a physical simulator of gamma radiation designed using Meaípe monazite sands. **Methods:** Female Wistar rats were divided into three groups, submitted to no radiation (control group) and to continuous radiation levels, one of very high level ($20 \mu\text{Sv h}^{-1}$) and another of high level ($3.6 \mu\text{Sv h}^{-1}$). The three group of animals were monitored weekly for 3 months and at the end of the study the animals were sacrificed, and the organs were extracted and weighed for anthropometric, oxidative stress and histological evaluations. **Results:** Exposure to radiation released by the monazite sands did not cause anthropometric alterations or blood pressure change in the animals. Similarly, there was no change in the quantification of ovarian follicles between the radiation groups and the control group. There was no difference in the oxidative stress quantification by the thiobarbituric acid reactive substance and advanced oxidation protein product methods in the ovaries. There were no evidenced damages in the structure of the renal tissue. It was observed the presence of granulomas in the hepatic tissue and alterations in the nuclei of the hepatocytes. **Conclusions:** Our results suggest that the continuous exposure of females rats to 3.6 and $20 \mu\text{Sv h}^{-1}$ doses of gamma radiation slightly affected the hepatic tissue, but did not alter the histological parameters in the kidneys and ovaries and oxidative stress.

Keywords: natural gamma radiation; oxidative stress; inflammation, biological tissues.

1. Introduction

The effects of ionizing radiation on human and animal health have been studied and debated for several decades. However, there are still issues about radiation with no conclusive answers, particularly related to the low-dose rate of ionizing radiation in areas of high natural background radiation. Many recent studies have indicated beneficial effects of low-dose rate (Pollycove and Feinendegen, 2001; Luckey, 2006; Jain et al., 2016; Shibamoto and Nakamura, 2018). According to Scott and Tharmalingam (2019), the natural defenses against cancer are enhanced by low doses and dose rates of ionizing radiation, which has aided in the continuation of human life over many generations. However, other studies suggest harmful effects even at low doses (Møller and Mousseau, 2013).

The responses to low doses and dose rates, at cell and molecular level, are very different from those following acute high doses, implying that different mechanisms of action and different metabolic pathways are activated by high and low doses of radiation (Brooks 2019).

The radiative source of the present work is monazitic sand and its natural radioactivity comes from ^{232}Th . The maximum radiation rate used in this work is $20\text{ }\mu\text{Sv h}^{-1}$. Similar sources exist in the literature, such as ^{57}Co (Seawright et al. 2017) and ^{137}Cs (Graupner et al. 2016), among others. However, the sources of the other works, as far as we know, have higher irradiation rates, for example, $100\text{ }\mu\text{Sv h}^{-1}$ (Seawright et al. 2017), $200\text{ }\mu\text{Sv h}^{-1}$ (Gridley et al. 2009), $834\text{ }\mu\text{Sv h}^{-1}$ (Takai et al. 2019); $1200\text{ }\mu\text{Sv h}^{-1}$ (Otsuka and Sakai 2005), $1400\text{ }\mu\text{Sv h}^{-1}$ (Graupner et al. 2016), preventing a direct comparison between the effects on health of these different sources of acute irradiation.

There is controversy as to the threshold and linearity of the biological effects of gamma radiation, and most experimental studies have used nuclear reactors to obtain their results, frequently using doses above those found in a natural environment (Durović et al. 2008; Pollycove 2007; Suit et al. 2007; Veiga and Koifman 2005). Radiation doses considered high ($>3\text{ mSv y}^{-1}$) are correlated in the literature with tumorigenesis and carcinogenesis, in addition to other biological damage (Lou et al. 2018; Pollycove and Feinendegen 2003; Preston et al. 2004). These doses are able, within hours or days after radiation exposure, to increase oxidative stress, inflammation, generate DNA changes, causing damage to different organs and systems, with severe

morphological changes (Bielefeldt-Ohmann et al. 2012; Chemyavskiy et al. 2017; Imaoka et al. 2007).

In addition to studies on gamma ray radiation, some authors have investigated the effects induced by X-irradiation on rats (Sharma et al. 2017). It was concluded by (Sharma et al. 2019) that low doses of X-rays exposure may afford radiation hormesis.

Sharma et al. (2017) showed that using fractional doses of X-rays for 15 days with approximately 70 rads in Sprague-Dawley female rats were able to significantly alter the neural morphophysiology of these animals.

However, according to some studies, exposure to low radiation doses promotes a protective role for biological systems as they consider that low partitioned doses of radiation increase cellular antioxidant power, activating antitumorigenic mechanisms and tissue repair (Baldwin and Grantham 2015; Feinendegen et al. 2004; Pollycove and Feinendegen 2003; Preston et al. 2004).

The concept of radioactive hormesis is being widely discussed among researchers, who consider that low doses of radiation can promote mechanisms to protect biological systems. A study by Sharma et al. (2019) using acute doses of 20 X-ray rads in female rats found an increase in the levels of antioxidant enzymes, decreased lipid peroxidation in the liver and kidneys. They also saw an increase in leukocyte, lymphocyte, and eosinophil counts, thus showing that low doses of radiation promote an increase in oxidative and immune capacity.

The radiation effects can be influenced by various factors such as age, gender, exposure time and affected tissue. Thus, until today it has not been possible to precisely determine a threshold and linearity of low radiation dose effects, which makes it difficult to establish a safety limit for human health (ICRP 2018); Inano and Onoda 2003; Jacob et al. 1994; NEA-OECD 1979). Some tissues are more sensitive to radiation exposure than others (ICRP 2018). Cells and germ tissues are considered one of the most sensitive tissues to exposure. Their exposure can usually cause damage such as accelerate reproductive age, premature ovarian failure, changes in ovarian follicles, amenorrhea and infertility (Lo Presti et al. 2004; Said et al. 2016; Sapmaz-Metin et al. 2014). At acute doses up to 5 Gy radiation was also able to produce changes in other tissues important for the proper functioning of organisms such as liver and kidney in animal models (Khan et al. 2014; Taylor et al. 2014).

A study by Veiga et al. (2006) showed that Guarapari beaches in the state of Espirito Santo (ES) have the highest incidence of gamma radiation in Brazil. Special

beaches of Guarapari as Areia Preta and Meaípe, have natural radiation values of the same magnitude as those obtained in Kerala, India. In Guarapari, these beaches are mainly formed by monazite sands, responsible for the emission of gamma radiation due to the presence of thorium in their constitution. These radiation values are well above those recommended by international regulatory institutions, which set exposure for the general population at 3 mSv y^{-1} (ICRP 2002; NEA-OECD 1979; UNSCEAR 2013). Same value is adopted by the Brazil's National Nuclear Energy Council (CNEN 2005).

Studies such as Veiga and Koifman (2005) showed an increase in mortality from different types of cancer in regions that naturally emit radiation. Therefore, low doses such as those found in natural environments need further investigation to be better understood, mainly because there is no consensus on its effects. In order to obtain more accurate data regarding the risks associated with exposure to natural radiation to human health, it is necessary to create a model that mimics natural conditions to obtain more reliable results regarding their effects (ICRP 2018; Veiga and Koifman 2005; Weber and Zanzonico 2017). Low radiation doses such as those found in natural environments are difficult to reproduce in laboratory, making it difficult to better evaluate their biological effects (ICRP 2018; Jacob et al. 1994; UNSCEAR 2000; UNSCEAR 2013).

The overall objective of the present study was to reproduce the natural and continuous radiation condition found in the coastal region of Meaípe, using a physical simulator of gamma radiation, to investigate the biological effects of low doses of natural radiation in an open environment, as found in Meaípe. The Meaípe beach ($20^{\circ}44'17'' \text{ S}$, $40^{\circ}32'20'' \text{ W}$) is located in the city Guarapari (estimated population of 110,000 inhabitants), Brazil. The coastal area of Meaípe is illustrated on Figure 1.

It is hoped that the results obtained here may contribute to the indication of low radiation thresholds in different biological systems. The PS may be used in the future as a tool for *in vivo* and *in vitro* researches.

2. Metodology

2.1 Physical simulator of gamma radiation

The physical simulator of gamma radiation (PS) was built to reproduce the physical composition of the monazite sand found on the beach of Meaípe, located in the city of Guarapari, state of Espírito Santo, Brazil (Fig. 1). The sand of Meaípe has dense minerals such as magnetite (Fe_3O_4), ilmenite (FeTiO_3), rutile (TiO_2), zircon (ZrSiO_4) and monazite (Ce, La, Y, Th) PO_4 . Due to the presence of thorium, the monazite sand presents natural levels of gamma radiation (Coelho et al. 2005).

Absorbed dose rate of gamma radiation exhibited a significant radiative level variation in time and space at Meaípe beach (Fig. 2). The radiation measurements were performed, at 10 cm in height over the sand, using a Gamma Scouter detector coupled to an Etrex 10 GPS locator (Garmin). The results showed that Meaípe beach is an open and dynamic system, preventing a unique characterization of the local radiation. Thus, any discussion regarding radiation levels on Meaípe beach has its validity restricted to a certain time and place.

In this work, it was chosen to investigate the biological response of wistar rats to extreme levels of natural radiation ($20 \mu\text{Sv h}^{-1}$ and $3.6 \mu\text{Sv h}^{-1}$) found in Meaípe. Thus, PS was constructed with a sample of 20 kg of sand, collected at (20.739° S ; 40.545° W), on 19 March 2017, representing an extreme dose rate of gamma radiation of about $20 \mu\text{Sv h}^{-1}$, as observed in Fig.2. The intensity of $3.6 \mu\text{Sv h}^{-1}$ was obtained using cages at a vertical distance of 50 cm from the monazitic sand (of $20 \mu\text{Sv h}^{-1}$), as illustrated in Figure 3. These radiation values ensure that human exposure at this site will be, mostly of the time, lower than those investigated in this study.

The collected sand was submitted to a sieve with a 1 mm mesh for the removal of shells and small pieces of organic material. The sand was then washed with hypochlorite and dried for 8 hours in an oven at 120° C , eliminating organic substances and living organisms.

For the PS assembly, the clean sand was placed in four boxes (60 cm x 40 cm x 10 cm) occupying 1/3 of the volume of each box. Between the box walls and the sand, it was installed a lead sheet with 2.5 mm of thickness to eliminate the radiation emission in all directions except the normal direction to the sand layer surface. Two PVC cages, each one with 4 rats, were then installed on these sand surfaces (referred here as VHR – very high radiation level) and two other PVC cages, also with four rats each, on a shelf immediately above this set, 50 cm high (referred here as HR – high radiation level), as illustrated in Fig. 3. Besides these cages, other two cages with 4 rats each, were mounted without sand (referred here as COM - control group).

The animals of the VHR group were exposed for 24 hours of the day at an average radiation intensity of $20 \mu\text{Sv h}^{-1}$ (or around 175 mSv y^{-1}). The animals in the HR group recorded a value around $3.6 \mu\text{Sv h}^{-1}$ ($\sim 32 \text{ mSv y}^{-1}$).

Since the radioactivity of the Meaípe sand is linked to the content of the thorium chemical element it becomes necessary to quantify this content in the highest dose rate of gamma radiation region observed in 19 March 2017. For this purpose, a 20 kg sand sample was analyzed. The granulometric characterization (Table 1) of the sampled sand indicates that most of the radiation is associated with particles smaller than $200 \mu\text{m}$ (hereafter refer to as sand fraction).

To improve the quantification of the thorium, the magnetic components were withdrawn from the sand fraction by applying a magnetic field of 0.3T. The sand fraction, without magnetic components, was submitted to X-ray fluorescence analysis (Table 2).

It was verified that sand samples with absorbed dose rate of gamma radiation of $20 \mu\text{S h}^{-1}$ were associated with the presence of particles smaller than $200 \mu\text{m}$ and that the sand fraction without the magnetic components had 4.3% of thorium mass.

2.2 Animals

Adult female Wistar rats (10-week-old) were maintained under controlled temperature between 23 and 25° C with a 12:12 h light/dark cycle. Rat chow and filtered tap water were provided ad libitum. All protocols were approved by the Ethics Committee of Animals of the Federal University of Espirito Santo (UFES, N° 05/2018). Additionally, all experiments were performed in accordance with the Biomedical Research Guidelines for the Care and Use of Laboratory Animals available online and followed the recommendations of the American Veterinary Medical Association Guidelines (2007). The female rats were maintained according figure 3 and divided into three groups. Control (CON, n = 8) group rats were not exposed to gamma radiation. VHR (n = 8) group rats were exposed to very high radiation (VHR ~ 20 $\mu\text{Sv h}^{-1}$) and HR (n = 8) group rats to high radiation (HR ~ 3.6 $\mu\text{Sv h}^{-1}$) levels. The rats were maintained in observation and exposition for 3 months. Rat weights were assessed at the beginning of the experiment and weekly until the end of the study.

2.3 Collection and weighing of organs

The animals were weighed weekly. Prior to euthanasia, all rats were anesthetized using ketamine (50 mg kg⁻¹, i.p.) and xylazine (10 mg kg⁻¹, i.p., Fontoveter, Brazil) and their wet organs were weighed after median laparotomy with 2 to 3 cm extension to collect biological material (pituitary, heart, lung, thymus, spleen, kidneys, adrenal, uterus, ovaries, soleus muscle and extensor digitorum longus muscle).

To estimate the organ hypertrophy/atrophy index, the organs were isolated, washed with physiological solution and excess liquid was removed with filter paper. Then, the organs were weighed and the wet tissue weight (mg) corrected for the tibial length (cm), to calculate the wet weight ratio.

2.4 Tissue preparation

The organs (ovaries, liver and kidney) were fixed in paraformaldehyde diluted in phosphate-buffered saline (4% PF-PBS) pH 7.4 for 24–48 h at room temperature. After fixation, the tissues were dehydrated in a graded ethanol series, cleared in xylol, embedded in paraffin at 60°C and for sectioned into 5 μm slices. The sections were

stained with hematoxylin and eosin (H&E), Picrosirius Red and Alcian blue (Coutinho et al. 2016).

2.5 Histomorphometry assessment

For histomorphometry ovaries, a total number of 5 animals per group was used. There were 10 slices per animal with 50 μm thinning, in a total of 50 fields analyzed per group. Follicles were reported as normal, atretic, or cystic according to the morphology. The follicles were classified as preantral when containing two to four layers of granulosa cells with no antral space. Antral follicles were classified when containing three or more layers of granulosa cells and a clearly defined antral space (Lee and Yoon 2005). A degenerated oocyte nucleus, wrinkling of the nuclear membrane, vacuoles in the oocyte, or degenerative changes in the granulosa layers, including cell shrinkage, pyknosis, and karyorrhexis, were all regarded as signs of atresia (degeneration) of follicles. Cystic follicles were defined as follicles that were dilated, filled with cavities of follicular fluid, and lined with one- to four-cell-thick layers of round to flattened granulosa cells (Shi et al. 2009). Primordial and primary follicles were not counted. The 10x and 40x objective utilized for analyzed the follicles.

For kidney histomorphometry, a total number of 5 animals per group was used. It was captured 25 glomeruli per animal with a 40x objective and then analyzed. The superficial collagen deposition in the cortical interstitial area and glomerular tuft was determined using Picrosirius Red (Sigma-Aldrich, St. Louis, MO). The images were measured by Image J software (version 1.33, Public Domain) (Coutinho et al. 2016).

For liver histomorphometry, a total number of 5 animals per group was used. There were 8 slices per animal with 50 μm thinning, in a total of 50 fields analyzed per group. The histomorphometry of the liver was performed according to Bertuloso et al. (2015). To calculate the hepatocyte nucleus area, it was used the Image J program (Vekemans and Braet 2004).

The High-quality images (2048 x 1536 pixels) were obtained with an Olympus microscope (AX70; Olympus, Center Valley, PA), photographed with an AxioCamICc1 camera and exported to Axio-Vision Software (AxioVision Rel. 4.8 software). The images were measured by Image J software (version 1.33, Public Domain).

2.6 Mast cell assessment

Tissue sections were stained with Alcian blue according to a standard protocol (Sigma-Aldrich, St. Louis, MO). Each 5 mm section was used to obtain 20 photomicrographs (40x objective). The number of positively stained cells (i.e., cells containing purple cytoplasmic granules) within the tissue was evaluated. The areas to be analyzed were randomly selected, with the exception that fields containing medium-sized blood vessels were carefully avoided. The number of positively stained cells was then expressed as units per area (mm^2) as described previously (Coutinho et al. 2016).

2.7 Oxidative stress

The levels of lipid peroxidation were determined using the thiobarbituric-acid reactive substances (TBARS) spectrophotometric assay based on the reaction between malondialdehyde and triobarbituric acid as previous described by Zovico et al. (2016). Briefly, the samples were homogenized with trichloroacetic acid and butylated hydroxytoluene. After being vortexed, the samples were placed in a dry bath and then centrifuged. The upper phase was diluted with thiobarbituric acid and placed in dry bath for 30 min, and the upper phase was read at 532 nm using a spectrophotometer. Additionally, the advanced oxidation protein product (AOPP) assay protocol was used to evaluate the oxidation of proteins as previous described (Zovico et al. 2016). Briefly, samples were diluted with phosphate buffer solution, potassium iodide (KI) and acetic acid, vortexed for 6 min and then measured at 340 nm. Chloramine-T absorbance at 340 nm was used as standard curve (0 to 100 μM). Total protein content was determined by the Bradford method.

2.8 Blood pressure (BP) measurements

Systolic BP (SBP) was evaluated in conscious rats after 60 days of the radiation exposition and was determined by an indirect tail-cuff method (IITC Life Science, Inc., USA). Animals were restrained for 5–10 min and conditioned to the procedure with cuff inflation-deflation cycles. The results of three stable measurements of SBP were

averaged. The pressure was automatically controlled, and systolic pulses were detected by a pulse transducer. A suitable cuff size was selected for each animal (Claudio et al. 2017).

2.9 Statistical analysis

The data are expressed as the mean $\pm$ standard error of the mean (SEM), and the statistical analysis was performed using GraphPadPrism 6 (GraphPad Software, La Jolla, CA–USA). Differences between groups were assessed by one-way analysis of variance (ANOVA), followed by Tukey post hoc test. Statistical significance was set at $P < 0.05$.

3. Results

The results did not show significant anatomical alterations nor presence of tumor mass in the female rats submitted to radiation. There were no changes in initial and final weight among groups. No changes were also observed in the wet weight of the organs and tissues analyzed among the groups (Table 3).

Measurement of systolic blood pressure by caudal plethysmography after 60 days of radiation exposure showed no change in systolic blood pressure between groups, with CON (113.00 ± 4.76) mmHg; HR (113.71 ± 3.53) mmHg and VHR (114.85 ± 3.51) mmHg. In addition, there was no hypertrophy in the heart of animals exposed to radiation (Table 3).

The evaluation of the ovarian structure showed that the cystic follicles of the VHR group presented larger volumes than those found in the other groups (Fig 4). No differences were found in the quantification of inflammatory cells by Alcian blue staining (CON 2.15 ± 0.16 ; HR 1.94 ± 0.16 ; VHR 2.06 ± 0.19 , mast cell/mm², Fig. 4J), and no differences were found between groups in the analysis of oxidative stress by AOPP (CON 39.78 ± 1.71 ; HR 38.60 ± 2.03 ; VHR 42.99 ± 4.08 , μ M cloramina-T/mg protein, Fig. 4K) and TBARS methods (CON 2.39 ± 0.39 ; HR 2.42 ± 0.60 ; VHR 1.59 ± 0.53 , nMol/mg protein, Fig. 4L).

Figure 5 shows the results of counting ovarian follicles. There was not difference in the quantification of cystic follicles of the groups (HR 2.90 ± 0.48 and VHR 2.87 ± 0.10 follicles mm⁻²) compared to the CON (2.75 ± 0.17 follicles mm⁻²), as displayed in

Fig 5A, and there was no difference in the quantification of atresic follicles among the groups (CON 1.68 ± 0.10 ; HR 1.59 ± 0.18 ; VHR 1.34 ± 0.13 ; follicles/mm², Fig 5B).

In our study, we found no difference between the preantral follicle count in the HR and VHR groups compared to the CON group (HR 3.16 ± 0.50 ; VHR 3.18 ± 0.48 vs. CON 4.06 ± 0.08 ; follicles/mm², Fig. 5C), and also in the antral follicles (HR 4.79 ± 0.76 ; VHR 3.23 ± 0.30 vs. CON 5.64 ± 1.06 ; follicles/mm², Fig. 5D).

Regarding the histomorphometric aspects, qualitative analyses showed no damage to renal tissue, such as basolateral vacuolization, brush edge attenuation and pyknotic nuclei, with the cortex and spinal cord presenting normal morphological aspects in H&E staining (Fig. 6). There were no changes in the quantification of inflammatory cells by Alcian blue placement (CON 3.09 ± 0.21 mm²; HR 3.27 ± 0.27 mm²; VHR 3.33 ± 0.22 mm²; Fig. 6J) or by Picrosirius red. Furthermore, in collagen deposition, no differences were identified (CON 3.98 ± 0.16 mm²; HR 4.18 ± 0.21 mm²; VHR 4.27 ± 0.21 mm²; Glomerular collagen fibers area/ glomerular tuft area (%); Fig. 6K).

Histological analyses performed on the liver (Fig. 7) showed small infiltrates of inflammatory cells in the space near the central lobular veins and small granulomas in the hepatic parenchyma in both the HR and VHR groups by H&E staining in relation to the CON group. Despite the observed granulomas, there were no changes in the mast cell quantification of mast cells (CON 3.07 ± 0.17 mm²; HR 3.23 ± 0.23 mm²; VHR 3.15 ± 0.24 mm²; Fig. 7K). Significant quantitative changes were also observed in the size (increase) of the nucleus areas of the hepatocyte cells of the groups subjected to radiation (HR 50.22 ± 1.69 μm², VHR 51.03 ± 2.27 μm²; $p < 0.05$) in relation to the control group (38.77 ± 1.25 μm², Fig. 7J).

4. Discussion

In this study it was created a physical simulator of gamma radiation (PS) capable of emitting gamma radiation found in natural environments from Meaípe beach monazitic sands, of Guarapari (Brazil), a place known for its natural beauty and intense tourism. In this place the radiation emission is not constant nor homogeny, changing according to climatic variations, because it is an open system. Despite that, throughout the experiment it was used an average of $20 \mu\text{Sv h}^{-1}$ (or around 175 mSv y^{-1}) for the VHR group and $3.6 \mu\text{Sv h}^{-1}$ ($\sim 32 \text{ mSv y}^{-1}$) for the HR group. This is the first study done

directly with Guarapari sand in the laboratory showing the direct effects of exposure on normal rats. The experiment allowed constant monitoring of animals and evaluation of radiation (Gamma Scout apparatus) for 90 days

The values used are well above the annual average for 2017, 1.5 ± 1.2 (mSv y^{-1}), and those recommended by the Organization for Economic Cooperation and Development (NEA-OECD 1979), International Council for Radiation Protection (ICRP, 2002) and the United Nations Committee on the Effects of Atomic Radiation (UNSCEAR 2000). It is also above the recommended doses by BEIR VII (2005).

The effects of low radiation doses are more complex to be predictive and analysed. Adaptive mechanisms may promote cellular protection, but there is no well-defined threshold of their effects. Therefore, there is no definitive conclusion about exposure to low radiation doses (Bielefeldt-Ohmann et al., 2012; Daino et al. 2018; Ezz et al. 2018; Inano et al. 1999; Inano and Onoda 2003; Sharma et al., 2019). Thus, international commissions have set conservative exposure limits below 3 mSv y^{-1} for the general population, considering the lower probability of an individual developing cancer (NEA-OECD 1979; UNSCEAR 2000; ICRP 2002).

However, according to BEIR VII (2005), doses below 100 mSv y^{-1} pose no risk to human health. This conclusion was drawn from analysis of the results of population and experimental studies such as the Radiation Effects Research Foundation (RERF). The RERF revealed that more than 60% of survivors of radiation emitted by bombs in Japan received a dose of less than 100 mSv y^{-1} . This study also investigated approximately 30,000,000 children of atomic bomb exposed survivors and found a lack of significant adverse genetic effects at doses below 100 mSv y^{-1} . Therefore, there is no consensus on the minimum possible radiation limit to cause biological damage. Research using reactors does not reproduce the variables of a natural environment or its dose range to arrive at more accurate data at radiation below 1 mSv y^{-1} .

Here, it was chosen to leave the animals exposed for 3 months to overcome possible adaptive mechanisms that could generate false positive results in a shorter exposure time. In this work, the animals radiated all over their bodies and were constantly in the radioactive material, being removed only for a short time during the evaluation and weekly weighing.

Pollycove (2007) showed in his review study that acute doses up to 300 mSv y^{-1} stimulated cellular antioxidant and antimutagenic systems, strengthening enzyme and immune repair promoting protection for biological systems. However, these effects

were lost when these doses exceeded 1 Sv y^{-1} . Unger (1980) postulated that low doses in chronic exposures are not associated with reduced cell damage but with the role of early tissue regeneration. He also concluded in his studies that acute doses are more damaging to biological systems compared to smaller and fractional doses. These results corroborate our choice to expose the animals for 3 months, due to the chronological time of the animals, mimicking the radiation received by a local resident who constantly lives under the influence of this radiation.

It was chosen to evaluate germ tissues, because according to the literature they are extremely sensitive to radiation, and doses close to 1 Sv y^{-1} are responsible for impaired reproductive function in humans and lower doses of $40\text{-}60 \text{ mSv y}^{-1}$ may be detrimental to reproductive organs. animals such as rats and mice (Kimler et al. 2018; Meirow and Nugent 2001; Mishra et al. 2018; Lo Presti et al. 2004; Sapmaz-Metin et al. 2014).

In this study, we found no differences in the ovary morphology of the female rats analyzed compared to the control. There was no difference in the quantification of atretic follicles, cystic follicles, and antral/preantral follicles between groups. These results corroborate the idea that germ tissues are not more sensitive to exposure to ionizing radiation, however we cannot confirm a change in the reproductive function of the animals, as it is necessary to carry out further tests, such as hormonal dosage, the reproductive cycle, the count number of pups and number of pregnancies of the animals exposed. This is one limitation of this study.

However, some signs that could indicate a possible failure in the ovarian mechanisms were not observed in the present study. One of these indicators would be the increase of weight and deposition of fat in the animals, since the estrogen released by the granulosa cells modulate the fat distribution and the energy metabolism in the animals. The literature clearly describes increased fat deposition by women in menopause and an increase in adipose tissue in animal models that have suffered some depletion in estrogen production (Danilovich et al., 2000; Heine et al., 2000; Naaz et al 2002). In our study, there were no changes in the animals' weight and in any anthropometric value, indicating that cell function was preserved in exposed animals.

Another finding that supports the preservation of histological function was that there were no differences in the quantification of inflammatory cells by Alcian blue staining in the ovarian follicle, and there were also no differences between groups in the analysis of oxidative stress by the TBARS and AOPP method.

These changes in the quantification of follicles without the presence of oxidative stress and inflammation may be linked to a direct mechanism on the ovarian follicles. According to previous studies, ovarian follicles in the early stages of development are more sensitive to radiation than follicles at more advanced levels, causing damage to cell functions, impairing their development, but preserving their function (Adrianens, Smits, Jacquet, 2009) .

Studies with doses much higher than those used, such as that performed by Lee and Yoon (2005) with high acute doses of 7.2 Sv were able to cause damage to the ovarian tissue, with total deletion of the follicles after 2 days of irradiation. The work carried out by Kimler et al. (2018) showed that acute doses of 1 Sv caused loss of ovarian follicles two days after exposure, and within 6 days there was total deletion. However, acute doses of 0.1 Sv were not able to cause structural damage to the ovaries. This last result corroborates what was seen in our work.

The biological effects evaluated by other researchers on reproductive parameters showed that doses close to and below 0.1 Sv were not able to generate structural damage to the ovaries and doses above 3 Sv caused total depletion of ovarian follicles impacting reproductive function. These values are close to those received by women undergoing radiotherapy. Human studies also indicate ovarian failure, accelerated reproductive age, amenorrhea and infertility with acute doses of ionizing radiation (Lo Presti et al., 2004; Suit et al., 2007). No work found in the literature has reproduced the same radiation dose to our work, so we need to elucidate better with other parameters to infer the impact on the reproductive function of humans who are exposed to monazitic radiation.

There is no work evaluating cardiovascular events at low radiation doses. Results obtained by plethysmography did not identify changes in systolic blood pressure. Given the regulatory role of the kidney in the renin-angiotensin-aldosterone system and due to the exposure time of the rats, if the renal tissue was impaired the animals would show signs of cardiovascular changes, as animal models such as 2K1C are used to generate hypertension secondary to renal damage (Dias et al. 2014). There was no change in the renal structure of animals exposed to radiation in our study, nor a difference in the concentration of inflammatory cells, indicating that the kidney maintained its structure and function preserved. Preserving the kidney ensures in part the control of blood pressure and preservation of the cardiovascular function of the animals.

No studies have been found in the literature evaluating liver and kidney at doses similar to ours for comparison. Most studies use much higher doses to evaluate the effects of radiation on these tissues (Taylor et al. 2014). The work of Khan and colleagues (2014) found liver tissue damage (inflammatory cell infiltration and liver enzyme abnormalities) after 6 hours of acute gamma-ray exposure of 5 Sv y^{-1} , well above the dose offered by our simulator (maximum of 0.175 Sv y^{-1}).

In our work, histological analyses performed on the liver did not show any severe structural changes. Small granulomas in the liver parenchyma were seen in H&E staining in all groups. Despite the observed granulomas, we did not identify structural changes that could indicate a possible organ failure such as vacuolization and degeneration of hepatocytes, spacing of the hepatic space, hepatocellular hypertrophy, the presence of steatosis and or hepatic necrosis (Asaoka et al., 2016; Komolkriengkrai et al., 2019; Khan et al., 2014).

There were no changes in the quantification of mast cells in liver tissue indicating that the tissue was not suffering from inflammation. Only a quantitative change in the size of the cell nucleus areas of the HR and VHR hepatocytes was observed in relation to the control. These results may be related to other factors and need to be further investigated, with an increase in the area of the hepatocyte nucleus, which may be related to senescence (Aravinthan et al., 2013) or also to an increase in gene expression activity (Gonzalez-Reimers et al., 1988). However, the tissue morphology was preserved, indicating that it did not suffer damage with chronic exposure to low radiation doses.

5. Conclusion

The effects of ionizing radiation on health have been studied and debated for several decades. However, there are still issues about radiation with no conclusive answers, particularly related to the low-dose rate of ionizing radiation in areas of high natural background radiation. However, as far as we know, there is no work in the literature simulating the conditions of continuous and natural radiation similar to that investigated here using a physical simulator. Most research has used nuclear reactors or medical equipment to expose animals to acute or fractional doses of radiation. Therefore, this work was pioneer in the creation of a physical simulator (PS) of gamma radiation to investigate the effects of low dose ionizing radiation in areas of high natural background radiation. The PS was capable of generating continuous ionizing radiation

similar to that observed in natural open environments. It was used the monazite sands of the beach of Meaípe (city of Guarapari), place known for its natural beauties and intense tourism.

Adult female Wistar rats (9-weeks-old) were exposed 24 hours uninterrupted to an average radiation intensity of $20 \mu\text{Sv h}^{-1}$ (or around 175 mSv y^{-1}), referred as very high radiation level (VHR) and around $3.6 \mu\text{Sv h}^{-1}$ ($\sim 32 \text{ mSv y}^{-1}$), named here as high radiation level (HR). The experiment lasted 3 months and the rats were withdrawn from the radiation environment only for weekly weighing. A control group (CON) of the rats without exposure to gamma radiation was also analyzed during the three months of experiment. In complementary studies, a group of male rats will be evaluated and then the findings will be compared.

The results showed that the gamma radiation, from chronic exposure to monazitic sands, did not alter the anthropometric parameters evaluated, did not cause oxidative stress or severe structural damage to the organs analyzed (kidney and ovary) when subjected to radiation rate. However, the liver tissue was slightly altered when exposed gamma radiation of 3.6 and $20 \mu\text{Sv h}^{-1}$. Subsequent studies will be carried out to verify that the exposure does not pose a risk to human health.

6. Additional information

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Disclosure statement

The authors declare that there are no conflicts of interest.

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Table 1: Distribution of sand particle size and associated dose. Sand sample collected on 19 March 2017, with total radiation dose of $\sim 20 \mu\text{Sv h}^{-1}$.

Particle average size (μm)	Mass percentage (%)	Dose ($\mu\text{Sv h}^{-1}$)
< 500	16.67	0.12
< 425	17.26	1.13
< 355	20.13	0.75
< 300	18.21	0.09
< 200	22.48	3.58
< 100	5.25	14.32

Table 2: Chemical composition of the sand with granulometry smaller than 200 μm and without magnetic components.

Element	Mass (%)	Std. dev.
Ce	28.89	0.06
Nd	25.16	0.05
Zr	19.46	0.02
Si	9.6	0.1
Ti	7.96	0.02
Th	4.26	0.01
Ba	2.04	0.09
Ca	0.82	0.03
Y	0.73	0.01
Hf	0.67	0.02
S	< 0.5	
Se	<0.5	

Table 3: Body, organ and tissue weight assessment.

Body weight, organ and tissue	CON	HR	VHR
Initial body weight (g)	160.75 ± 7.95	172.00 ± 6.69	164.75 ± 5.27
Final body weight (g)	277.50 ± 8.32	279.25 ± 4.59	277.57 ± 7.90
Pituitary gland (mg)	0.38 ± 0.02	0.34 ± 0.04	0.39 ± 0.03
Thymus (g)	0.13 ± 0.01	0.09 ± 0.01	0.11 ± 0.01
Heart (g)	0.26 ± 0.01	0.27 ± 0.01	0.26 ± 0.01
Lung (g)	0.42 ± 0.01	0.41 ± 0.03	0.42 ± 0.02
Liver (g)	2.46 ± 0.08	2.47 ± 0.11	2.50 ± 0.13
Spleen (g)	0.16 ± 0.01	0.15 ± 0.09	0.15 ± 0.09
Kidneys (g)	0.51 ± 0.01	0.48 ± 0.01	0.51 ± 0.02
Adrenal gland (mg)	17.98 ± 0.11	20.94 ± 0.11	19.68 ± 0.09
Ovaries (mg)	30.06 ± 0.13	26.30 ± 0.14	27.15 ± 0.34
Uterus (g)	0.19 ± 0.01	0.17 ± 0.01	0.22 ± 0.03
Soleus (mg)	38.93 ± 0.28	40.06 ± 0.41	37.59 ± 0.17
EDL (mg)	33.48 ± 0.16	35.44 ± 0.35	37.27 ± 0.17

CON, HR and VHR represent, respectively, control, high radiation and very high radiation level group (n=8 for each group). EDL stands for Long Digital Extensor muscle. Values are expressed as the (mean ± SEM).

Figures



Figure 1: Spatial configuration of Meaípe beach.

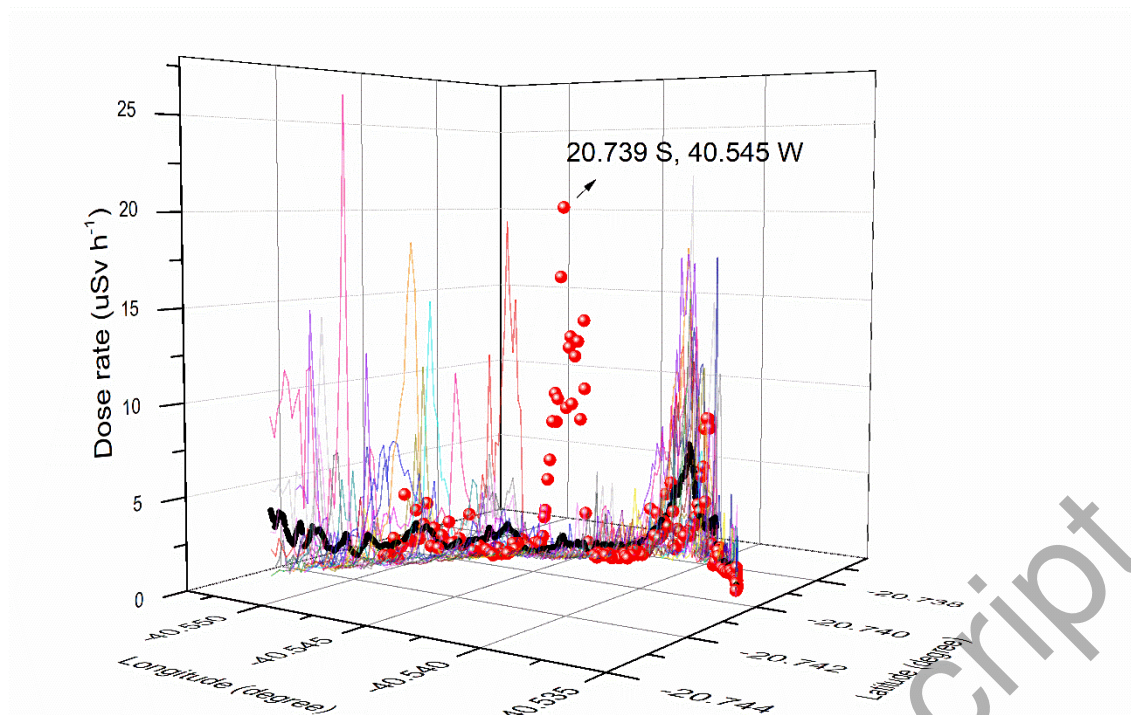


Figure 2: Dose rate of gamma radiation measured along the beach of Meaípe. Values observed from 19 March to 30 December (every two weeks) 2017. The sand used to build the PS was collected on 19 March (red balls) at intensity of $20 \mu\text{Sv h}^{-1}$ at (20.739° S ; 40.545° W). The spatial annual average values are represented in thick black line.

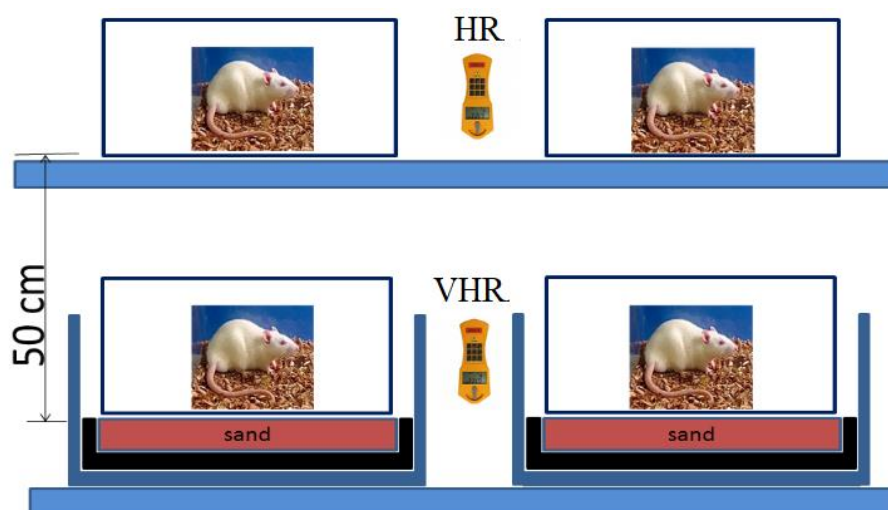


Figure 3: Schematic configuration of the physical simulator of gamma radiation.

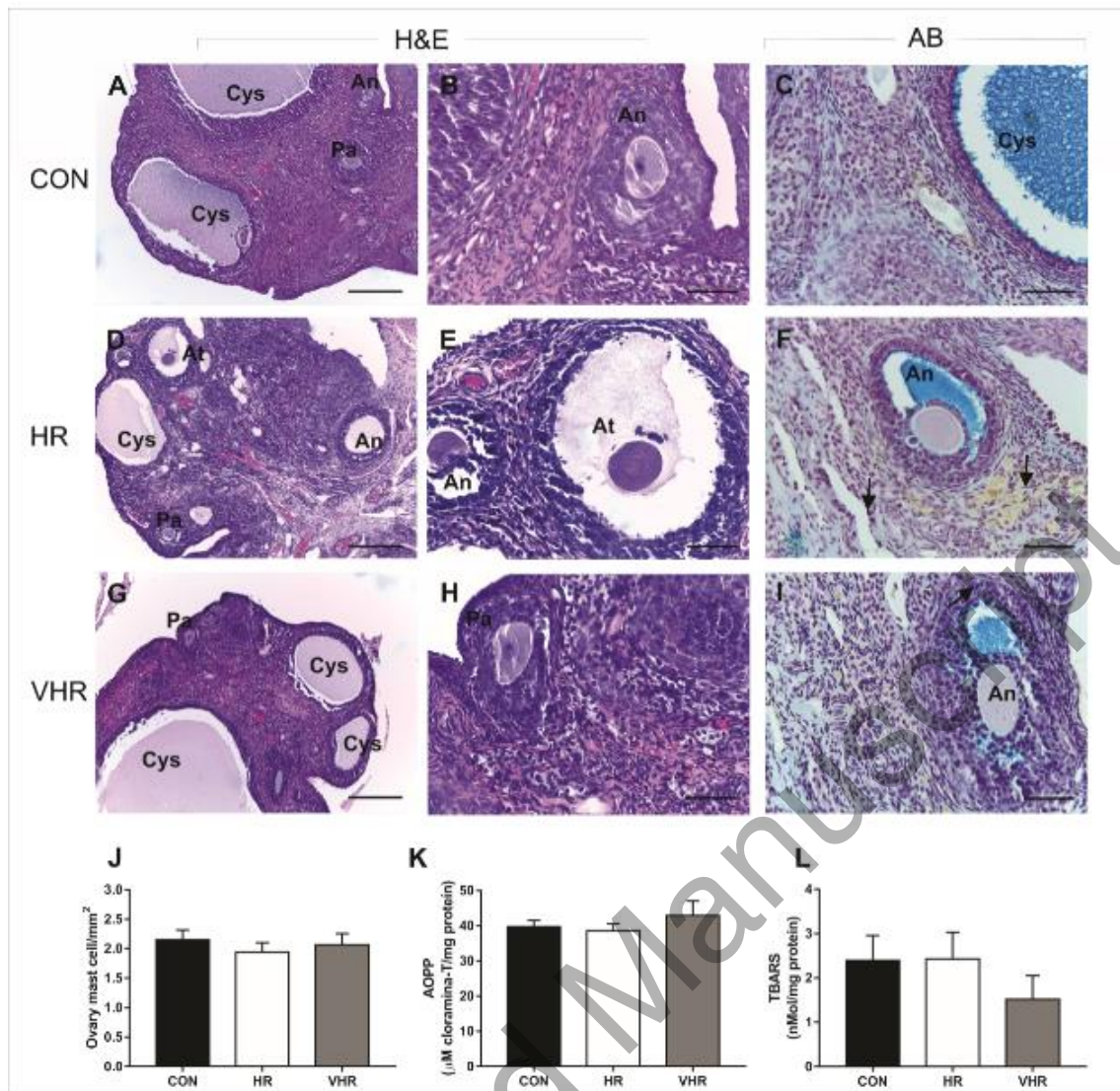


Figure 4: Histological analysis of ovary from the female rats were stained with H&E and Alcian blue. Representative sections from (A, B, C) CON and (D, E, F) HR and (G, H, I) VHR. No significant difference in the mast cells was observed in the ovaries of the groups (J), had no significantly difference in the AOPP: Advanced oxidation protein products (K) and TBARS: Thiobarbituric acid reactive substances (L). Bar=250 μm (A, D, G) and Bar= 50 μm (B, C, E, F, H, I). Pa: pre-antral follicle; An: antral follicle; At: atresic follicle; Cys: cystic follicle. Arrow represents mast cells. Values are expressed as the mean ± SEM (n=5 for each group) (one-way ANOVA followed by Tukey's test).

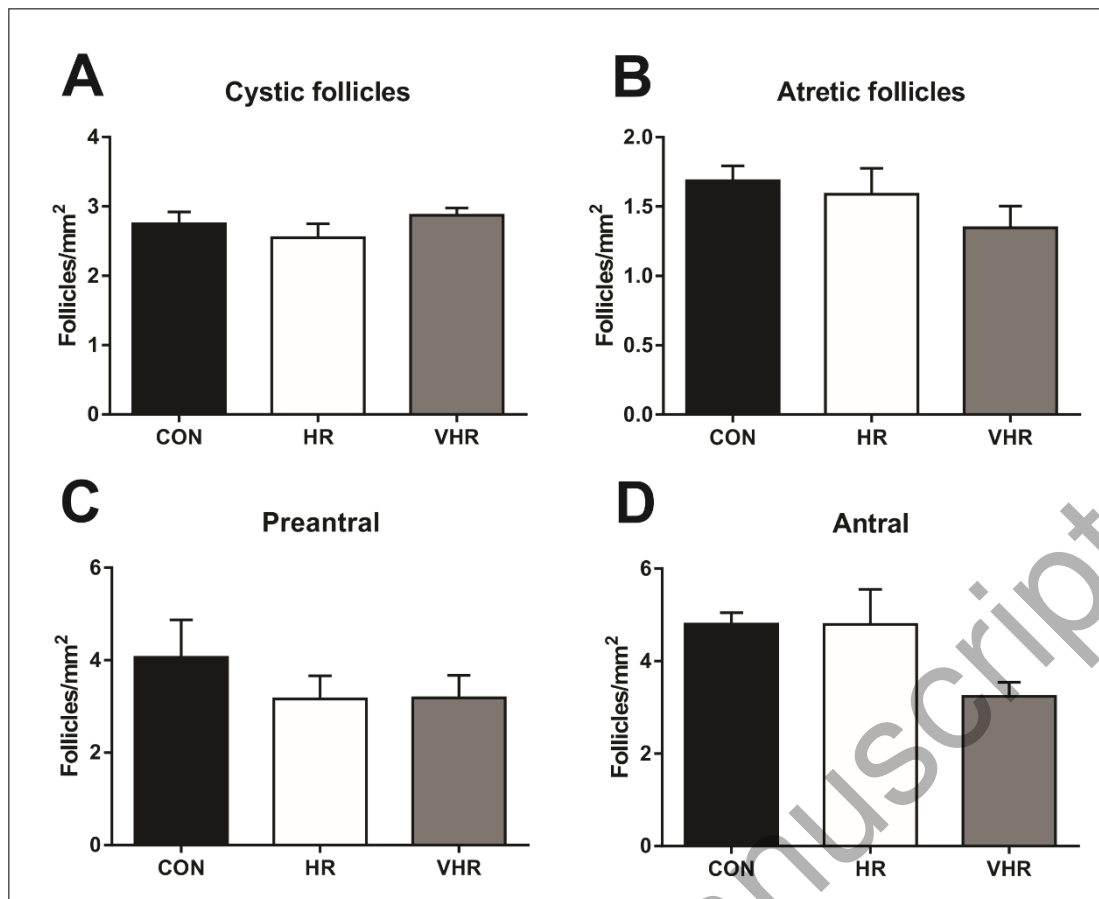


Figure 5: The morphology of the ovary from the female rats (n=5 for each group) were stained with H&E, which indicates (A) cystic follicles, (B) atretic follicles, (C) preantral follicles and (D) antral follicles. Values are expressed as the mean $\pm$ SEM. (one-way ANOVA followed by Tukey's test).

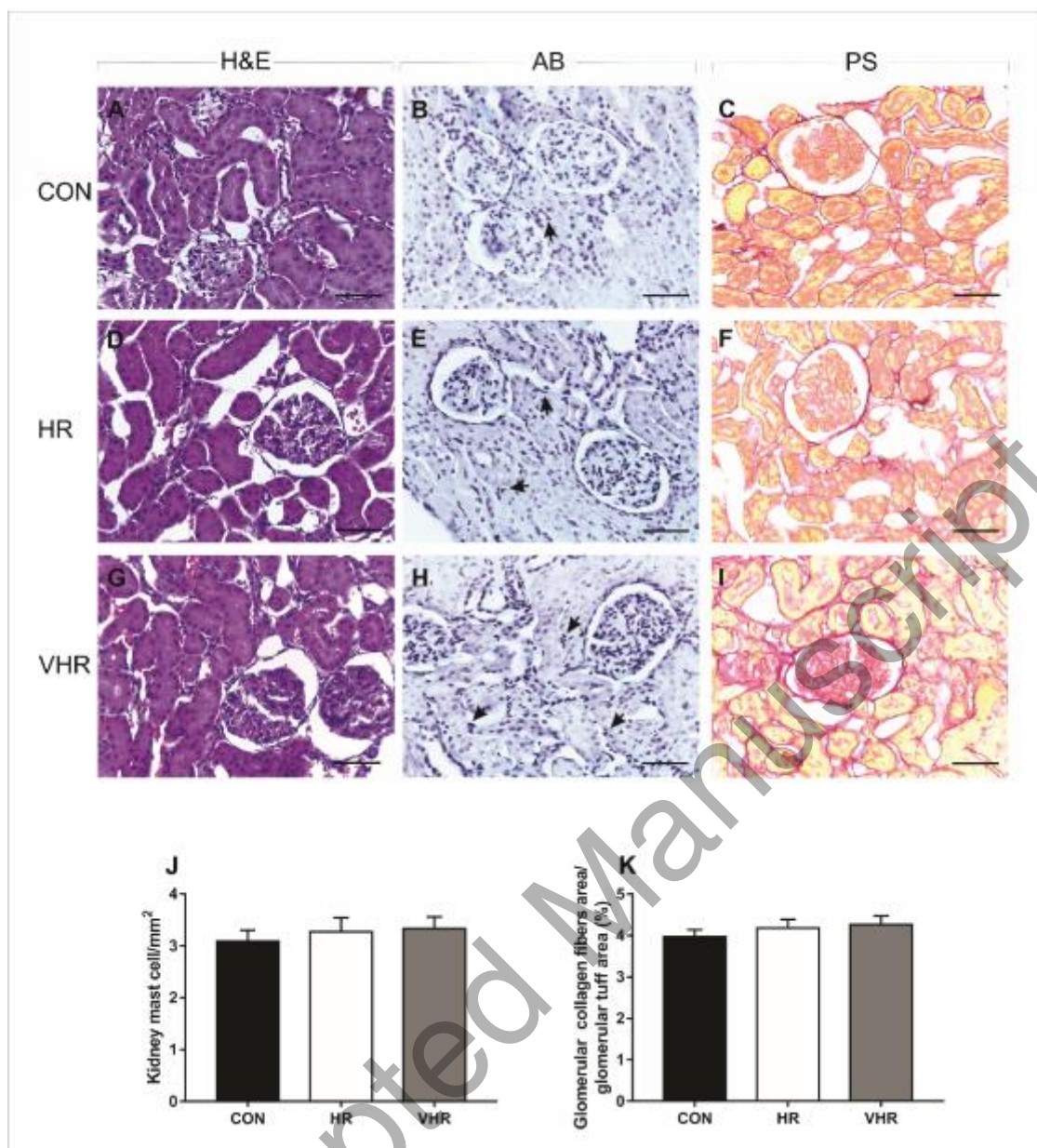


Figure 6: The morphology of the kidney from female rats were stained with H&E, Alcian blue (Ab) and Picrosirius (PS). Representative sections from (A, B, C) CON and (D, E, F) HR and (G, H, I) VHR. There was no difference in the quantification of inflammatory cells by Alcian blue placement between groups (J), there was no difference in quantification of collagen deposition in the glomerular region (K). Bar= 50 μ m. Values are expressed as the mean $\pm$ SEM (n=5 for each group) (one-way ANOVA followed by Tukey's test).

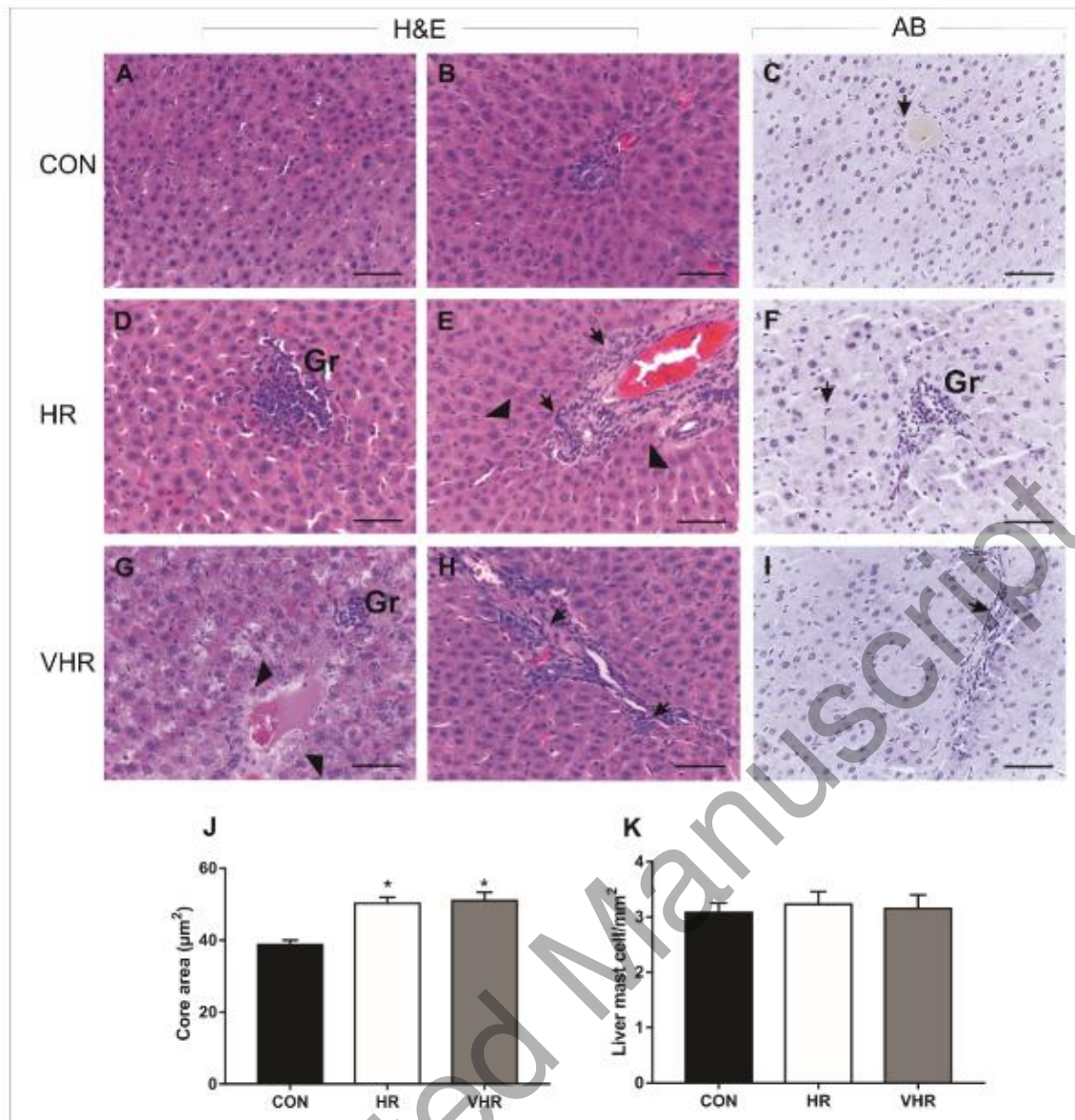


Figure 7: The morphology of the liver from the female rats were stained with H&E, Alcian blue. Representative sections from (A, B, C) CON and (D, E, F) HR and (G, H, I) VHR of the liver showing cellular infiltrations (arrow), granulomas (Gr) and changes in cell nuclei (arrowhead). There was an increase in the cell nucleus area (J). Despite the inflammation of liver tissue seen on H&E staining, there was no difference in mast cell quantification between the groups analyzed (K), Bar= 50 μm. Values are expressed as the mean ± SEM (n=5 for each group). *p ≤ 0.05 vs CON (one-way ANOVA followed by Tukey's test).