Egypt. J. Biophys. Biomed. Eng., **Vol., 24** No. 1 pp.63- 71 (2023)

Brain Imaging and hematological evaluation of the risk of chronic exposure to naturally occurring radioactive materials from petroleum oil fields in rats

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T HE GROWING activities of the oil and gas industries produce a huge product value of underground materials including naturally occurring radioactive materials as a form of products or waste. The present research aims to assess the possible effects of exposure to a chronic dose of TE-NORM on the rat's brain by analyzing the magnetic resonance imaging (MRI) of pre- and post-irradiation, in addition to studying the changes in body weight and some hematological indices such as the WBC count, percentage of Lymphocyte, Mean Platelet Volume, and Platelet count. The examined rats were exposed for 60 days to TE-NORM with a total dose of 16 mSv. Subsequently, the rats were scanned with magnetic resonance imaging for brain anatomical examination for an MRI study. The results indicated that there are no significant changes between MRIs of pre- and post-irradiation. In the remaining tests, there were significant changes observed in the hematological indices tested and there is a noteworthy weight loss after the period of exposure. Detection of the neurological and physiological effects of TE-NORM exposure needs more efforts in the future with different methods and at different exposure intervals.

Keywords: TE-NORM, Magnetic resonance imaging (MRI), rat's brain, blood indices.

Introduction

The historical experimental studies of the effect of ionizing radiation aimed to understand the radiation effects on biological systems not only by accident reports but also by animal experiments [1-3]. Based on the radiobiological results, the effect of radiation on the biological system is not fixed and is not the same for all experimental cases [4]. However, the difference depends on some factors, including types, quality, the quantity of radiation, and the level of the radiosensitivity of the affected cells as well [5]. Particularly, the type of radiation meant the type of radioactive material that has the ability to emit types of radiation even waves or particles. Of course, not only radioactive materials are the main sources of ionizing radiation but also there are other sources such as x-ray tubes, accelerators, and nuclear reactors.

Humans are exposed to ionizing radiation encountered in the environment such as cosmic rays, radioactive elements in the earth's crust, radon and its decay products, besides the radioactivity naturally present in the body. In addition, man-made sources as in radio-therapy and radio-diagnosis, research laboratories, and nuclear accidents have increased the risk of exposure to ionizing radiation.

However, this work was focused on radioactive materials, particularly Naturally Occurring Radioactive Materials (NORM) which contain radioactive sources (Uranium, Radium, Thorium, and their decay products). These materials were created naturally, and almost have the same age as the earth's age [6]. These materials can be found in some types of soils, rocks, and sources of water. If the NORM becomes in its natural state and hence purposefully concentrated either in waste by-products or in a product, it becomes technologically enhanced naturally occurring radioactive material (TE-NORM). Based on this concept, the TE-NORM can be defined as any naturally occurring material whose radionuclide

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Recieved 10/10/2023 ; accepted 13/11/2023
DOI :10.21608/EJBBE.2023.241800.1066

concentration or potential for human exposure has been increased above levels encountered in the natural state as a result of human activities [7]. The main probability of the effect of ionizing radiation could be represented in the shape of the disturbance of the sequence of the molecules in the living system [8]. Exposure to low levels of a chronic dose of TE-NORM may lead to some stochastic effects that result in delaying the development of some forms of cancer such as stomach, bone, leukemia, esophagus, and cancer of lungs [9]. Recent literature still scopes the radiobiological effects of TE-NORM [10]. Chronic radiation exposure has some deleterious effects as it appeared in EPR (Electron Para-Magnetic Resonance) and lipid peroxides elevation [11], Furthermore, another research explored that, the in-vivo chronic low-level natural radiation provides an increase in the level of DNA damage and this damage can be balanced by the internal defense system especially an increase in the antioxidant enzymes [12]. In this work, the effect of the TE-NORM on the rat's brain was studied by evaluating the changes that might appear in the anatomical structure of the rat's brain using the images of MRI of the groups of rats with almost the same weight and ages. The evaluation was performed using image analysis software used for MRI analysis to compare two images. On the other hand, the periodic weighting follow-up of examined rats was investigated. Also, some hematological analyses were performed to assess the harmful effect of TE-NORM. Investigating the effects of post-treatment brain damage using MRI exists in the literature [13-16], but the approach of investigating the effect of chronic exposure to TE-NORM using MRI is limited in the studies, in addition to almost the classical evaluation was dependent on the ability of human eyes for image differentiation [17,18]. Brain damage discovery via MRI using codes of MATLAB or machine learning procedures is considerable in recent research [19-21], Hence, the aim of this work is to evaluate the effects of TE-NORM on rat's brain using a code of the MATLAB image analysis program, besides changes in some hematological indices and body weight.

Materials and Methods

TE-NORM preparation

A sample of TE-NORM was collected from a petroleum oil field in the Western Desert of Egypt and prepared and packed in a plastic bottle. This sample was about 400g. The sample was sealed and kept in storage for one month to create secular equilibrium between the daughter and parent atoms. A High purity Germanium gamma-spectrometer with 40% efficiency and 2.0 Kev resolution at 1.33 Mev photons of Co-60, shielded by 4"Pb 1 mm Cd and 1 mm Cu linked up to a multichannel analyzer was used for gamma measurement. The radioactivity spectrum of the sample indicated that the sample includes Uranium (^{238}U), Thorium (^{232}Th), and Potassium (^{40}K). The activity concentrations of ^{238}U, ^{232}Th, and ^{40}K, are given in terms of 150.336, 125.571, and 8.99 K Bq Kg^{-1}

Animals' preparation

In this work, 12 male rats weighing (150 ± 10 gm) were used for experiments. The animals were treated following the recommendations of the National Institute of Health for human treatment of animals. The animals were housed and fed in a suitable cage. Also, the animals were kept for one week for adaptation before the experiments. The rats were weighed before starting the experiment (zero time) and after the exposure. To check the effect of TE-NORM on the mean weight of animals' bodies. The daily nutrition of animals was 25g, containing 21% protein, 5% fibers, 3.5% fats, and 6.5% ash, however, the daily feeding waste was cleaned every new day of feeding. The environmental condition of the animals was controlled at room temperature and a normal level of humidity. Meanwhile, male rats were chosen for this study to avoid hormonal interference in females during the different stages of the estrous cycle. The daily conditions of animals were observed. After the adaptation period, the animals were divided into two groups, control group and irradiated group.

Animal's irradiation

The control group was kept in a separate cage in the animal room to avoid the TE-NORM source. The second group was in another cage in a different room with the same condition as the adaptive environment. The volume of the TE-NORM plastic bottle was 1570 cc which got from one of the fields of the western Desert of Egypt. The TE-NORM sample was inserted in the middle of the cage of the second group. The base of this bottle was stuck on the base of the cage to be more fixed and more resistant to the animal's movement during the exposure period. As mentioned, the time of exposure was 60 days after the adaptation time. The emitted dose rate of the TE-NORM was 11μSv/h. This dose rate was measured using a calibrated survey. This monitor was *Digilert 100* nuclear radiation monitor, manufactured by S. E. International, Inc. USA. The total absorbed dose received during the exposure days was calculated based on the guideline of ICRP 60 [22], The total chronic absorbed dose received was 16 mGy.

MRI rat preparation

The tested group was investigated for MRI pre- and post-irradiation to check the anatomical changes in the rat's brain. The animals were scanned as three rats by three rats. Before scanning, the Animals were anesthetized using ketamine hydrochloride (50mg/kg of animal) by application of intraperitoneal injection.

MRI of rat's brain

MRI scanning was performed using a 3T MRI scanner (Philips MRI machine) of 90-mm equipped with a shielded gradient capable of producing a 300 mT/m as a maximum gradient amplitude with a rise time equal to 80 μs. The inner diameter of the RF (Radio Frequency) coil was 38 mm. Animals were flatted to be head-first setup Fig.1 to avoid image artifacts. In this work, all the stuff used for the rats' positioning was devoid of parts that may cause image deprivation. The internal laser pointers were used to place the head of the rat relative to the magnetic field. A fast spin-echo sequence with 3000/80 ms was used for axial T2-weighted imaging throughout the whole brain. The slice-thickness was 2.5 mm, and the selected distance factor was 0. Also, the field of view was 150 mm, the pixel matrix was 224×202, and 15 RF excitations for T2- weighed images were applied. The scanning acquisition time was 08:34 min for T2-weighted images [23]. This imaging protocol and imaging procedure were applied pre- and post-animal irradiation.

Pre-and post-MRI analysis

In-house software designed by a code of MATLAB version R2018a was used for MRI analysis [24], this software is free and available for research, this code is similar to that used by Yousif et al and Chow et al [24, 25]. The software can convert the image pixel to the numerical value of pre- and post-irradiation images. The pre-irradiation images were considered as the reference value for comparison. Once images were converted, any compare program can be used for comparison, the used software in this study is the office spreadsheet compare software, each pixel in the post-irradiation image was subtracted from the corresponding pixel in the pre-irradiation image to calculate the pixel-by-pixel difference. Any significant difference that may appear between the two images will be recorded. This difference may indicate a change in the anatomical structure of the rat's brain, which in turn may be a tumor cell growth as a foreign mass.

Hematological analysis

To assess the changes that may occur in some immune systems in the blood due to the exposure to the radiation of TE-NORM, some tests that evaluate the change in blood immunity indices have been measured. The investigated indices are white blood cell (WBC) counted, percentage of Lymphocyte (P-LYM), Mean Platelet Volume (MPV), and Platelet count (PLT). After the 60 days of TE-NORM exposure, considered as chronic exposure, the two groups of animals were

Fig. 1. Rats' preparation and MRI scanning.

prepared for anesthesia and blood assortment. A solution of ketamine hydrochloride was used to anesthetize animals before surgery processing. At the end of the experimental time, blood samples were collected by rat's heart puncture. Accumulated samples were preserved using EDTA (ethylene di-amine tetra-acetic acid). 10 µl of whole blood samples were assessed via the (Diff3) Mek6410/Mek6420 Hematology Analyzer System. After that, the WBC, P-LYM, PLT, and MPV were measured. The applied method for the hematological parameters account was the *Wintrobe* protocol [26,27].

Results and Discussion

The body weight assessment

The results obtained in the current study revealed a decrease in the body weight of animals that started to be noticeable and significant after 35 days of TE-NORM exposure of the irradiated group compared to the control group as shown in Table 1. It was also noted that the activity of the irradiated animals is much weaker than that of the control group, which may indicate a loss in the metabolic rate and a decrease of energy in addition to the weakness of self-defense behavior or resistance to others Figures 2(a & b).

TABLE 1.The change in body weight at different intervals after TE-NORM exposure.

No. of days	Weight (gm) Control group (n=6)	Weight (gm) Irradiated group (n=6)	Percentage of change
0	150±10.00	149±10	0%
7	200±7.00	200± 11	0%
14	212.5±9.4	207.5±9.4	-2%
21	230±7.18	215±9.35	-8%
28	244.5±7.45	220±7.12	-10%
35	261.5±7.61	230.5±7.18	-12% *
42	275.5±7.18	240.5±7.18	-13%*
49	302±7.76	250±7.12	-17%*
56	310±7.2	260±7.12	-16%*
63	315.5±7.18	270±7.12	-15%*
67	325.5±7.18	280±7.12	-14%*

Each value represents mean ± standard deviation (n=6), *: the p-value ≤ 0.05, and the percentage of change is the percentage change from the corresponding control value.

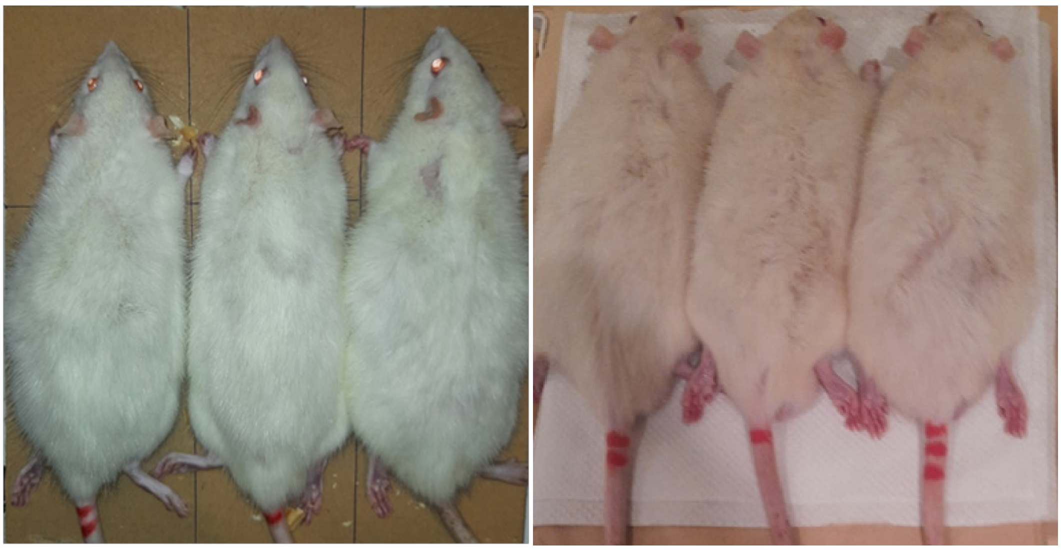

Fig. 2. The overview of the rats' bodies for (a) controlled group and (b) the irradiating group.

As shown in Table 1, the weight rate for both groups increased but without similarity and there is a noticeable decrease in an irradiated group when compared to the control. As we indicated, the irradiated dose was a chronic whole-body dose with 16 mGy; In a recent closed study by Takahashi et al when they investigated the effect of radiation on body weight in the spontaneously hypertensive rats' model and Wister Kyoto rats, they found that: the body weight gain decreased after exposure to radiation. [28]

MRI analysis

Using the method of MRI imaging in rats' models is considered one of the core tools that may be used for organ investigation [29,30]. Most of these studies are focused on investigating the mass change progression in the rats' models using different protocols of MRI [29,31]. The use of MRI images in rats has a strong impact on the result verification. In addition, using other modalities of tumor investigations such as Computed Tomography (CT) and combined images of (CT) & Positron Emission Tomography (PET-CT) in rats are also still used in research [32,33]. Here, estimating brain damage (as strange mass, inflammation, even tumor tissue) of rats due to exposure to TE-NORM is our interest in this research which may be limited in the literature. Figures 3(a & b) shows an example of an MRI cut image for pre- and post-irradiation for experimental animals. The total scanning cuts were applied in the MATLAB code for image analysis. The examined rostro caudally cuts of the serial of MRI images encompassing the whole rat brain, and it started from the caudal part of the olfactory lobe to the most caudal part of the inferior colliculus.

The results indicated that the similarity between the two study-sets images was 100%, which means that there was no percentage difference between the value of pixels of pre-and post-irradiation MRIs for the six rats, which in turn means that the MRI images of rats exposed to TE-NORM for two months cannot be an investigating tool to indicate if there are any existed foreign masses.

a

b

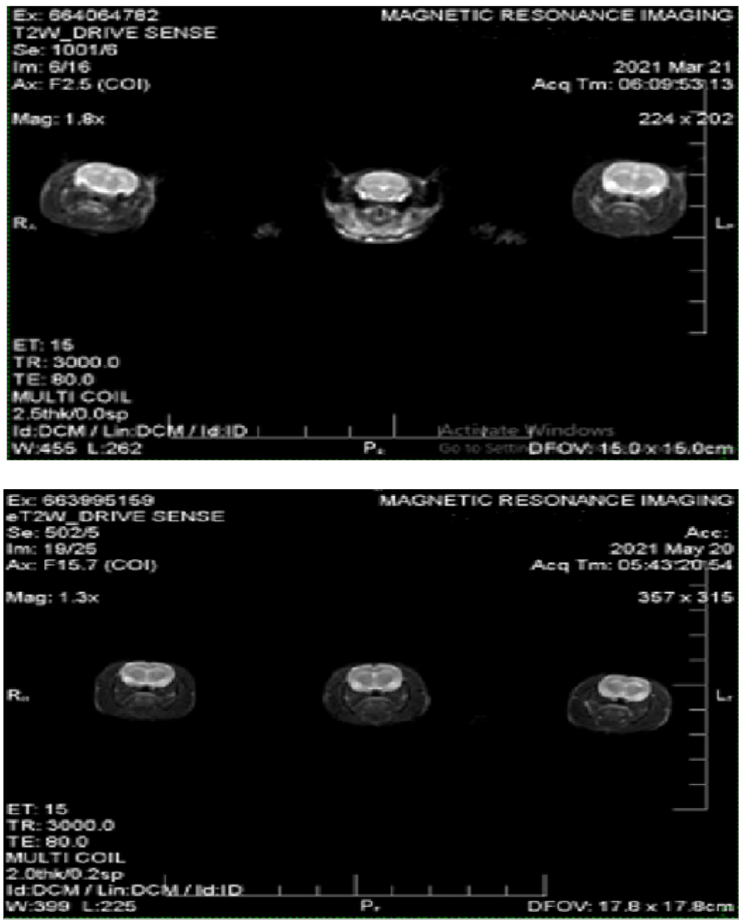

Fig. 3. Scanning MRI cuts of rat's brain for (a) pre- and (b) post-TE-NORM irradiation.

Hematological Parameters

Table 2 shows the effect of ionizing radiation emitted from the TE-NORM on the examined indices of blood. As mentioned above the WBC, P-LYM, MPV, and PLT were examined for control and irradiated animals.

Hematopoietic cells are highly sensitive to radiation damage even at relatively low levels of exposure [34] and chronic exposure of mammals to ionizing radiation affects proliferating cell systems [35]. Suppression of blood cell counts can lead to many adverse side effects. Low RBCs cause anemia, low WBCs increased susceptibility to infection and low platelets are responsible for excessive bleeding [36].

In the current study, as shown in table (2), there is a noticeable decrease in WBC, PLT, and MPV at the end of the time of exposure to the emitted radiation from the radioactive material of the sample of TE-NORM compared with the control group, while there are no detected changes in the P-LYM between the irradiated and control groups. The result corroborates that WBCs, and platelets showed a decrease after cumulative radiation doses [37]. The decrease of WBC was more pronounced which is agreement that WBCs are the most sensitive [35]. Platelets are a rich source of cytokines and growth factors, such as platelet derived growth factor (PDGF), and they play an essential role in blood clotting and wound healing. In the current study, the results corroborate that the platelet count declines following exposure to a mild or moderate IR dose [38].

A reduction in WBC was combined with the drop in PLT count and MPV. The damaging effects of radiation on WBC count may be related to the termination of white blood corpuscle creation in the immune system, in addition to the loss of cells from the circulating blood system by loss or outflow through the capillary walls, and the direct obliteration of developed circulating cells. Accordingly, the drop in the common values of blood indices following irradiation may be assigned to them radiosensitivity [39]. Similar results were proposed by Sanzari et al. They found that there was a significant decrease in the WBC and P-LYM which was dose-dependent and not affected by the type of radiation or dose rate [35]. It is worth mentioning that, exposure to other different types of gamma sources such as Tc-99 and fluorine with low chronic doses has a noticeable effect on the red blood cell account (RBCs) and WBC as well depending on the hours of exposure [40]. The variations of hematological parameters PLT, MPV, and percentage of LYM due to whole-body exposure to gamma rays, maybe the first indicator of an abnormality in the function of the biological system [41]. The effect of exposure to ionizing radiation on the blood indices as an indicator of the radio-harmful effects is not close to the smaller models but it can appear in large animals and humans as well [42].

Conclusion

This study aimed to investigate the effect of TE-NORM exposure for 60 days on changes in brain structure using MRI investigation, body

TABLE 2. The effect of chronic exposure of TE-NORM on the blood indices (WBC, P-LYM, PLT, and MPV).

Parameters	Control group (n=6)	Irradiated group (n=6)	Percentage of change
WBC (10^9/L)	6.4 ± 0.67	4.9 ± 0.45*	-23.44 % *
P-LYM (%)	94.4 ± 2	94 ± 1	- 0.42 %
MPV (fL)	6.6 ± 0.2	5.7 ± 0.19*	- 13.64 % *
PLT (10^9/L)	546 ± 3.5	515 ± 2.7*	- 5.68 %*

Each value represents mean ± standard deviation (n=6), *: the p-value ≤ 0.05, and the percentage of change from the corresponding control value.

weighing and blood indices . The results indicated weight loss in the mean rat's weight, and there were significant changes in the hematological analysis. And no substantial changes in the brain structure of MRI images were observed. Further investigation studies are needed on the effects of exposure to chronic ionizing radiation with different doses and periods.

Acknowledgments

The authors would like to thank professor: Omar S. Desouky professor of radiation biophysics at Egyptian Atomic Energy Authority, for his academic support, also, the authors would like to acknowledge Dr. Ahmed E. Yousif, the assistant lecturer of medical physics, radiation oncology department, faculty of medicine Ain shams university for his assistance and programming support.

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"تصوير المخ وتقييم الدم لخطر التعرض المزمن للمواد المشعة الطبيعية من الحقول البترولية علي الجرزان"

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تنتج الأنشطة المتنامية لصناعات النفط والغاز قيمة ضخمة للمنتجات من المواد الجوفية بما في ذلك المواد المشعة التي تحدث بشكل طبيعي كشكل من أشكال المنتجات أو النفايات.
يركز البحث الحالي على تقييم تأثير التعرض المزمن لمنتجاتTE-NORM على دماغ الفئران.
تم إختيار مجموعتين من ذكور الجرذان البالغين وتعريضهم لهذه للمواد المشعة الطبيعية TE-NORM بجرعه مزمنه منخفضة حوالي ٠,١٦ جراي لمدة شهرين وتم التحكم في العملية باستخدام تقنية التصوير بالرنين المغناطيسيMRI لما قبل وبعد التشعيع بجانب دراسة التغيرات في وزن الجسم وبعض المؤشرات الدموية.
أشارت النتائج إلى أن التعرض ل TE-NORM لمدة ٦٠ يومًا لم يظهر أي تغييرات على صور التصوير بالرنين المغناطيسي قبل التشعيع وبعده بينما كانت هناك تغييرات كبيرة في التحليل الدموي وفقدان الوزن في متوسط وزن جسم الفئران بعد فترة التعرض.
المواد المشعة الطبيعية لها تأثير كبير على نظام الدم ومتوسط الوزن في ذكور الجرذان.