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Effects of Radon-222 Exposure on Lung Oxidative Stress and Superoxide Dismutase in Male Albino Rats: Roles of Melatonin as an Antioxidant

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ABSTRACT

This study examines the impacts of radon exposure on male albino rats' lungs, which is a prominent cause of lung cancer, as well as the defensive roles of melatonin as an antioxidant by examining the activities of superoxide dismutase SOD, malondialdehyde MDA, and hydroxyproline. The study is comprised of two experiments. In the first experiment, in a newly built chamber, rats were exposed to radon from (5µCi) of ^{226}Ra source. Rats were divided into seven groups of eight rats. Unlike the control group, the other six groups were exposed to radon, which was assessed using RAD-7 detector, specifically for 30 minutes (D1), 1 hour (D2), 2 hours (D3), 3 hours (D4), 6 hours (D5), and 9 hours (D6). The concentrations were measured to be (600±0.014, 1210±0.028, 2421±0.057, 3600±0.086, 7260±0.172, and 10875±0.259) Bq.m^{-3}, respectively. The second experiment examined the protective effects of melatonin against radon exposure. The rats were given a low (60 mg/kg diet) and a high (120 mg/kg diet) dose of melatonin and then exposed to radon for six hours. Radon exposure did not significantly alter SOD activity. However, the first and third radon doses lowered SOD activity modestly. After exposure, hydroxyproline levels increased significantly compared to the control group. A statistical result found that radon exposure elevated lung MDA. Melatonin enhanced SOD reductions after radon exposure. However, both MEL doses lowered MDA below the positive control. In conclusion, radon inhalation induces lipid peroxidation. Melatonin reduces MDA and increases SOD activity, making it a good antioxidant against radon exposure.

Keywords: Radon Inhalation, Superoxide dismutase, Melatonin, Pulmonary Fibrosis, MDA.

INTRODUCTION

Radon and its offspring provide significant natural radiation exposure. Radon exposure may have caused lung cancer in uranium miners. Alpha rays from radon progeny in the trachea and bronchial region reached the basal cell layer (Ma *et al*., 1996; Othman *et al*., a2023). The earth and atmosphere harbor diverse concentrations of inherent radionuclides, such as uranium-238 (^{238}U) and thorium-228 (^{228}Th) decay sequences (Othman *et al*., b2023; Yaseen *et al*., 2024).

Radon, an inert gas, has a lengthy half-life and can enter the body. Radon is poorly soluble in human fluids; therefore, it is evenly distributed throughout the body. Radon gas and its breakdown products, ^{214}Po and ^{218}Po, can cause lung injury (Al-jomaily *et al*., 2021). Inhaling decay products directs alpha radiation to lung tissues. In particular, items coupled to small aerosols or left unattached might damage sensitive lung cells, disrupt DNA, or create free radicals (Council, 2014; NCRP, 1997), therefore enhancing the likelihood of acquiring pulmonary illness (Field, 2019).

Inhaled radon exposes bronchial and nasal pharynx cells instantly. Also, some radon decay products settle in the lungs. High radon levels raise lung cancer risk since the bronchus and lung are the principal radon-affected organs. High liposolubility causes it to accumulate in fat-rich organs like the endocrine glands and nerve fibers. Radon and its children are quickly absorbed by fat-rich tissues and circulated. Radon exposure is thought to occur in the lungs and blood cells (Keith *et al*., 2012).

It is well known that radon decay produces alpha particles with high linear energy transfer and modest penetration. Alpha particles generate reactive oxygen species (ROS) like hydrogen peroxide (H_2O_2), singlet oxygen (O^2), superoxide anion ($O^{2.-}$), and hydroxyl radicals ($OH^{\cdot}$). (Ramadhani *et al*., 2021). (ROS) are very active and have the ability to damage numerous types of biomolecules, including proteins, DNA, and lipids such as polyunsaturated fatty acids (PUFAs). This occurrence is commonly referred to as "oxidative stress" or "oxidant stress." The PUFA, known as arachidonic acid, undergoes peroxidation, resulting in the formation of malondialdehyde (MDA). The specific interaction between ROS and lipids is commonly referred to as "lipid peroxidation." MDA is a widely recognized indicator and end result of oxidative stress, specifically lipid peroxidation (Tsikas, 2017).

The lung reacts slowly to ionizing radiation. After intense radiation, this radiation-sensitive organ releases cytokines and chemokines. Organs acquire inflammatory cells. Multiple macrophage and neutrophil cytokines cause edema and pneumonitis. ROS and RNS also pile up collagen in the extracellular space, causing lung fibrosis (Sheikholeslami *et al*., 2021). Oxidative stress in the lungs can cause pulmonary fibrosis. As a result, it is critical to prevent and reduce the severity of fibrosis. The determination of hydroxyproline concentration serves as a crucial diagnostic marker for assessing the extent of fibrosis (Qiu *et al*., 2014).

Internal (mitochondrial electron transport system) and external (ionizing radiation) antioxidant systems reduce oxidative damage. We get antioxidants from supplements, carotenoids, and polyphenols. Naturally occurring antioxidants and enzymes control ROS. GSH is the main non-enzymatic antioxidant; SOD, CAT, and GPX are enzymatic (Kuciel-Lewandowska *et al*., 2018).

Superoxide dismutase (SOD) plays a crucial role in protecting against superoxide radicals and serves as the main defense mechanism against oxidative stress. It catalyzes the dismutation of superoxide into hydrogen peroxide, effectively preventing the continued production of free radicals (Ighodaro and Akinloye, 2018). SOD facilitates the conversion of superoxide radicals $O_2^{\cdot-}$ into hydrogen peroxide. SOD activity is frequently employed as an indicator of radical dynamics in animals (Ma *et al*., 1996; Kataoka *et al*., 2017). Many studies have shown a change in SOD activities after radon exposure (Ma *et al*., 1996; Kataoka *et al*., 2017; Kataoka *et al*., 2021; Ramadhani *et al*., 2021; Othman *et al*., 2024).

A neuroendocrine hormone that the pineal gland secretes is melatonin. Melatonin is widely recognized for its potent antioxidant properties, enabling it to effectively scavenge free radicals. It functions as a potent scavenger of ($O_2^{\cdot-}$), (H_2O_2), (OH.), and organic peroxyl radicals (RO_2), singlet oxygen (O_2), effectively neutralizing its effects (Reiter *et al*., 2014). Furthermore, melatonin governs the activity and genetic manifestation of enzymes that counteract oxidative stress as well as those that

promote oxidative stress (Reiter *et al*., 2000). Melatonin, an antioxidant, destroys free radicals and reactive oxygen intermediates. Hydroxyl radicals, peroxynitrite anion, singlet oxygen, and nitric oxide are examples. Melatonin acts as an antioxidant (Bhatia and Manda, 2004). In addition, melatonin has the ability to pass through several barriers in the body, including the blood-brain barrier and the placenta, and it is evenly distributed among cells. These attributes enhance the efficacy of melatonin as an antioxidant hormone (Reiter, 1995).

Lipid peroxide breaks down in living organisms, releasing aldehydes like MDA that damage cells by interacting with lipids, proteins, and nucleic acids. In addition to scavenging free radicals, melatonin has been shown to lower MDA levels. (Dobsak *et al*., 2003; Reiter *et al*., 2014; Rodriguez *et al*., 2004). Bhatia found that melatonin scavenges free radicals and quenches singlet oxygen in 2004. Melatonin also protects mice from radiation-induced oxidative stress, according to the study. Multiple studies demonstrate the robust antioxidant effects of melatonin (Reiter *et al*., 2016; Nuszkiewicz *et al*., 2020). In 2007, Serin *et al*., examined radiation-induced histological lung abnormalities and melatonin's effects on acute lung damage (Serin *et al*., 2007).

The aims of the present study are to study the effects of different doses of radon exposure on oxidative stress markers (MDA), lung fibrosis markers (hydroxyproline level), and SOD activity; also, to study the effects of low and high doses of melatonin against radon exposure on MDA, hydroxyproline, and SOD activity.

MATERIALS AND METHODS

Radon exposure system

A novel radon inhalation chamber design was created in this investigation. Fig. (1) displays a snapshot of the chamber, while Fig. (2) presents a simplified representation of it in the form of a block diagram.

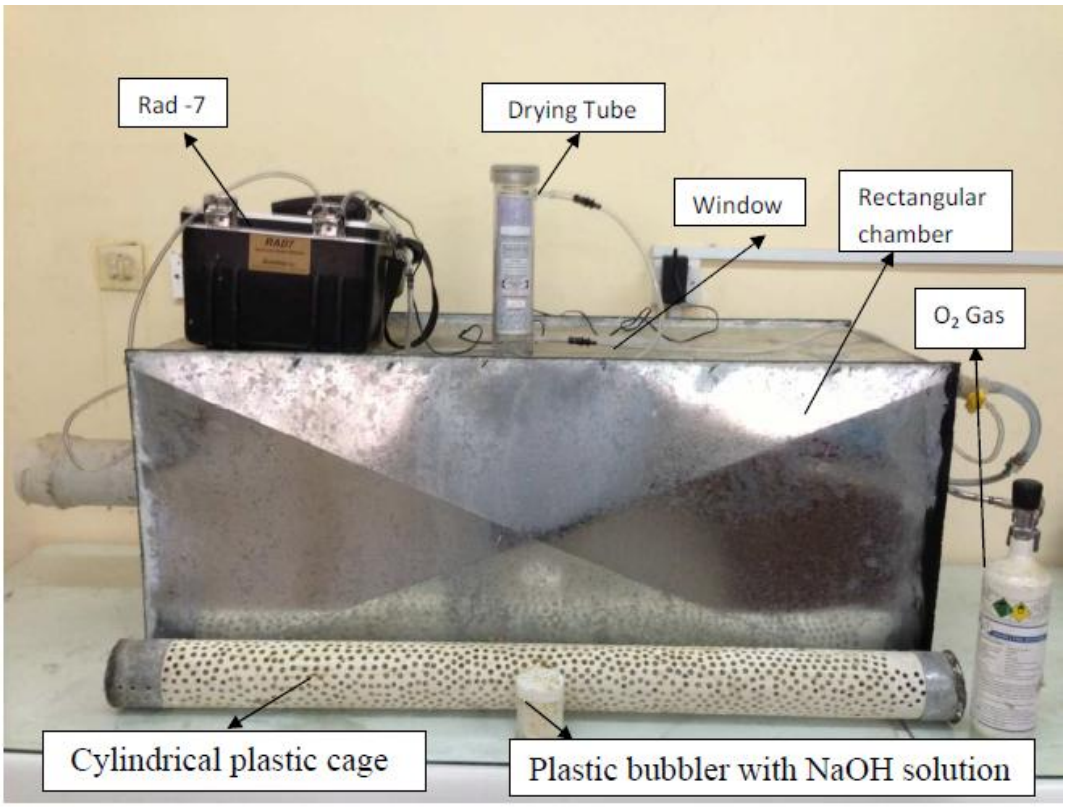

Fig. 1: Photograph of the chamber design.

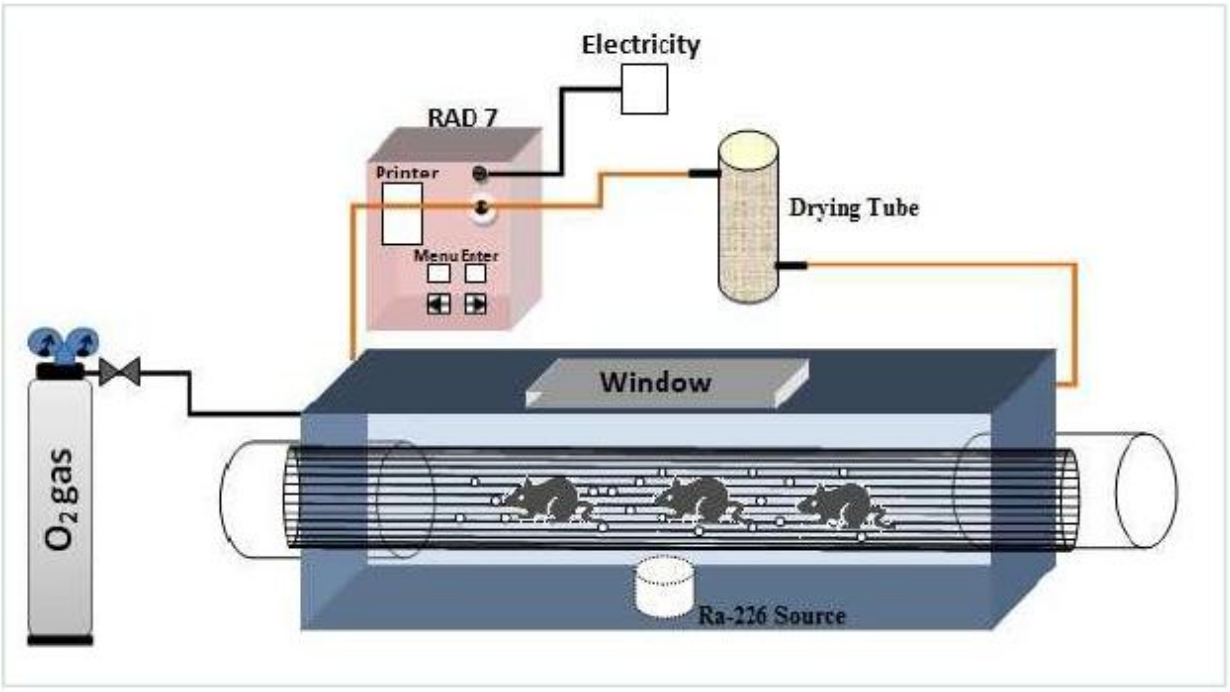

Fig. 2: Block diagram of the chamber design.

The animal chamber was constructed using rectangular chingo plates with a thickness of 1 cm. The length of the chamber is 1.13 m, while the width and height are both 0.48 m. Consequently, the total volume of the chamber amounts to 0.2603 m^3. Located above the chamber is a window with an area of 0.6 m^2, allowing for observation of the rats inside the chamber. A cylindrical plastic cage, measuring 3 inches in diameter and 1 meter in height (with a volume of 0.01823 m^3), was placed in the inner chamber. The cage was specifically built to facilitate the passage of radon through small holes during the exposure period. The chamber contained CO_2 gas, which was captured using a plastic bubbler containing a sodium hydroxide (NaOH) solution. Sodium hydroxide is a white, corrosive, solid compound that absorbs carbon dioxide from the chamber. This process is done to prevent CO_2 retention and carbon dioxide poisoning in rats by removing carbon dioxide from their breathing gases. For each experiment, the rate of oxygen (O_2) was regulated by introducing it into the chamber every 30 minutes. This was done to ensure that the rats did not experience hypoxia while inhaling radon in the chamber.

Radon concentration measurement

The rats were subjected to radon exposure in a recently constructed chamber, using a 5 microcurie ^{226}Ra source as the radon source, as shown in Fig. (2). The ^{226}Ra source is placed inside the chamber for a period of at least one month in order to achieve a state of secular equilibrium between radon and its decay products. Radon-226, formed by the radioactive decay of ^{226}Ra, has a half-life of 1600 years and is of interest for secular equilibrium because it decays to ^{222}Rn, which has a half-life of 3.8235 days (Rao, 2001). The RAD-7 detector was utilized for quantifying chamber air radon. US-based DURRIDGE manufactured the RAD7 electronic radon monitor. The RAD7's solid-state semiconductor detector detects alpha particles. It measures in an internal hemisphere chamber. This gadget can generate an alpha-energy spectrum for ^{220}Rn and ^{222}Rn progeny alpha particles. The spectrum is 0–10 MeV with 0.05 MeV precision. This detector measures ^{222}Rn and ^{220}Rn concentrations autonomously and quickly. The configuration includes a vinyl tube with desiccant ($CaSO_4$), a RAD7 professional electronic radon detector, and chamber. Connection to the closed loop triggered both valves (DURRDGE, 2012).

A RAD7 radon monitor is linked to the chamber to assess radon levels during the exposure period. In the closed loop, gas circulates. Radon will travel from the chamber's air to the desiccant, then to the RAD7 intake filter. RAD7 expels air. The RAD7 device detects alpha particles generated

by polonium isotopes when its air decays. The RAD7 device then quickly converts the alpha particle into an electrical signal to assess radon levels. This detector can distinguish electrical pulses from ^{218}Po and ^{214}Po, which have energies of 6 and 7.69 MeV, respectively. After analysis, the RAD7 data shows the radon concentration in $Bq.m^{-3}$ or Bq. L^{-1} units. RAD7 has a detection limit (MDA) of 3.7 $Bq.m^{-3}$. The specific activity of ^{222}Rn is obtained from a calibration factor determined from radon chambers run by the US EPA and the DURRIDGE Co. were equal to 0.479 CPM/(pCi/L) for normal mode and 0.232 CPM/(pCi/L) for sniff mode with uncertainty (2%) (Durridge, 2015). Furthermore, this company has calibrated RAD7 to specifically detect alpha radon. The efficiency of the RAD7 detector for measuring radioactivity is (0.0086 counts per minute/Bq. L^{-1}) (Abdullah *et al*., 2023). The nominal sensitivity of RAD 7 for monitor and sniffer mode were equal to 0.5 counts/min/pCi/L and 0.25 counts/min/pCi/L, respectively. Also, the range were equal to 0.1 to 20,000 pCi/L (Azeez, 2010; DURRDGE, 2012). The experiment is conducted in a dry environment with humidity below 8% (by purging RAD7 10 minutes before testing). One day of tests with 30-48 minute cycles. A succinct report takes 30 minutes, and then the same thing happens again after 30 minutes with an absolute accuracy 5% (Najam *et al*., 2017; Diab, 2019; Ershaidat *et al*., 2015).

RAD 7 generates a brief report one day after the program begins. This report comprises the mean radon measurement from 48 cycles, a bar chart, and a cumulative spectrum. The radon concentration in the chamber was measured to be 29055±691 $Bq.m^{-3}$. Also, the RAD-7 detector was used to measure the change in radon levels in the chamber during every experiment. The radon levels have been measured at various time intervals and denoted as doses (D) due to different exposure times, specifically 30 minutes (D1), 1 hour (D2), 2 hours (D3), 3 hours (D4), 6 hours (D5), and 9 hours (D6). The radon concentrations were measured to be (600±0.014, 1210±0.028, 2421±0.057, 3600±0.086, 7260±0.172, and 10875±0.259) $Bq.m^{-3}$, respectively (Azeez, 2010; Diab, 2019).

Animals and sample preparation

The current investigation was carried out on a sample of 72 mature male albino rats (Rattus norvegicus). The animal research ethics committee, which is a part of Salahaddin University's College of Science, gave its approval to the ethical and legal worries about the samples used in this investigation. It was determined that the permission number (19/10/20234/90) was appropriate for the approval. The rats exhibited apparent good health, with weights ranging from 200 to 330 grams, and were around 12 weeks old at the commencement of the trial. The animals were selectively bred and kept in plastic enclosures measuring (56 x 39 x 19 cm). The enclosures were filled with wooden chips and contained eight rats per enclosure. The animals were kept in a room with a regulated temperature of 24±3°C, located in the animal facility of the Biology Department within the College of Science at Salahaddin University in Erbil, Kurdistan Region, Iraq. The animals were subjected to a 12-hour light/dark schedule throughout the duration of the experimental investigation. The animals were provided with a regular rat diet and unrestricted access to tap water ad libitum.

Experimental design

The study comprised two distinct studies, with each experiment meticulously structured as outlined below:

Experiment I

The objective of this experiment was to explore the impact of inhaling six different doses of radon at various exposure times on tissue MDA levels, tissue SOD levels, and lung fibrosis in male albino rats. The experiment involved the categorization of rats into seven groups, with each group including eight rats housed together in a cage. The rats in this cohort were given standard rat food and unlimited access to tap freshwater.

1. The control group, includes 8 rats, was conducted under conditions where there was no exposure to radon. The animals were maintained under identical conditions, ensuring that they were not exposed to radon inhalation within the chamber.
2. The first group includes 8 rats labelled D1; they were exposed to radon inhalation for about 30 minutes, and the radon concentration during this period was measured to be 600±0.014 $Bq.m^{-3}$.

3. The second group includes 8 rats labelled D2; they were exposed to radon inhalation for about one hour, and the radon concentration during this period was measured to be 1210±0.028 $Bq.m^{-3}$.
4. The third group includes 8 rats labelled D3; they were exposed to radon inhalation for about two hours, and the radon concentration during this period was measured to be 2421±0.057 $Bq.m^{-3}$.
5. The fourth group includes 8 rats labelled D4; they were exposed to radon inhalation for about three hours, and the radon concentration during this period was measured to be 3600±0.086 $Bq.m^{-3}$.
6. The fifth group includes 8 rats labelled D5; they were exposed to radon inhalation for about six hours, and the radon concentration during this period was measured to be 7260±0.172 $Bq.m^{-3}$.
7. The sixth group includes 8 rats labelled D6; they were exposed to radon inhalation for about nine hours, and the radon concentration during this period was measured to be 10875±0.259 $Bq.m^{-3}$.

Experiment II

The aim of this experiment was to investigate the impact of two different dosages of melatonin on the levels of tissue MDA, tissue SOD, and lung fibrosis in male albino rats exposed to radon.
1. Positive control group: The rats in group five (D5) from the initial experiment were designated as the positive control group for radon exposure, with a duration of six hours and a radon concentration of 7260±0.172 $Bq.m^{-3}$.
2. The low-dose melatonin (60 mg/kg diet) group consists of 8 rats: The rats in this group were randomly allocated to receive treatment with a low dose of melatonin (60 mg/kg diet), along with unrestricted access to drinking water, for a duration of one week prior to being exposed to radon. Subsequently, they were placed in a chamber for a six-hour period of radon exposure.
3. The high-dose melatonin (120 mg/kg diet) group consists of 8 rats: The rats in this group were randomly allocated to receive treatment with a high dose of melatonin (120 mg/kg diet), along with unrestricted access to drinking water, for a duration of one week prior to being exposed to radon. Subsequently, they were placed in a chamber for a six-hour period of radon exposure.

Tissue sample collection

Following each trial, which lasted one week following exposure to radon, the rats were administered ketamine hydrochloride (50 mg/kg) to induce anesthesia (Khudhur and Maulood, 2023, Maulood *et al*., 2015). The animals underwent dissection to acquire lung tissue in all experimental groups. Subsequently, the lung tissue was rinsed with a normal saline solution. The sera were preserved at a temperature of -80°C using a Sanyo Ultra-Low Temperature freezer from Japan until they were analyzed.

Biochemical analysis:

Tissue halogenation

Each rat's lung was flushed with a cold normal saline solution. The lungs were then broken up in 20 mM phosphate buffer (pH = 7.4; tissue/buffer ratio, 1/5 w/v) using a hand-held glass homogenizer device (Chowdhury *et al*., 2013). The homogenates were subjected to centrifugation at a force of 2000 times the acceleration due to gravity (2000g) at a temperature of 4°C for a duration of 10 minutes using a Beckman J2-21 centrifuge. The liquid portion of the mixture was gathered and preserved at a temperature of -80 degrees Celsius until it could be analyzed.

Determination of MDA in lung tissue

The (MDA) concentration was determined using the Kartha and Krishnamurthy methods (Kartha and Krishnamurthy, 1978). A 20% trichloroacetic acid solution was combined with 1 ml of tissue homogenate. The mixture was centrifuged at 6,000 RPM for 10 minutes. After centrifugation, one milliliter of liquid above the sediment was transferred to a test tube. Each test tube received one milliliter of a 0.7% thiobarbituric acid solution. Subsequently, the test tubes were immersed in a water bath that was heated to its boiling point for a duration of 20 minutes. If needed, spin the centrifuge at 3000 RPM. The measurement of the progress of the pink color was conducted at a wavelength of 535 nm. The MDA concentration was determined using the following equation:

$$\text{MDA (µmol/g of tissue)} = \frac{\text{Absorbance at 532nm}\times D}{L\times E_0} \quad \ldots\ldots\ldots\ldots \qquad (1)$$

Where L: light path (1cm), E_o: Extermination constant $1.56\times105\ M^{-1}.\ Cm^{-1}$, D: Dilution factor. The MDA concentration in the homogenized tissue was determined by multiplying the optical density (O.D.) by 420. To obtain the concentration in µmol/g of tissue.

Determination of sod activity in lung tissue

The lung tissue's SOD concentrations were evaluated using a microplate enzyme immunoassay, namely the TSH accu-bind enzyme-linked immunosorbent assay microwells.

Test principle

This ELISA kit uses competitive ELISA. This package includes a SOD1-coated microtiter plate. The rat SOD1 present in the sample or standard competes with a fixed quantity on the solid phase support for the binding sites on the biotinylated rat SOD1 detection antibody. After taking out any sample or standard that is still not bound, avidin coupled with horseradish peroxidase (HRP) is put into each well of the microplate and left to sit there for a while. The TMB substrate solution is then added to each well. To assess the enzyme-substrate reaction, a sulfuric acid solution is added, and the color change is detected using spectrophotometry at 450 nm±2 nm. Comparing sample optical density (OD) to the standard curve quantifies Rat SOD1 in samples.

Procedure

Additional 50µL of a standard or sample was added to each well. To each well, 50µL of biotinylated detection antibody was quickly added. The samples were incubated at 37°C for 45 minutes. The specimens were collected and washed three times. Add 100 µL of HRP conjugate to each well and incubate at 37°C for 30 minutes. Next, samples were suctioned and rinsed five times. Subsequently, a volume of 90 µL of substrate reagent was introduced to each sample and subjected to incubation at a temperature of 37°C for a duration of 15 minutes. After adding 50µL of stop solution, the samples were examined at 450 nm. Fig. (3) shows the computed results.

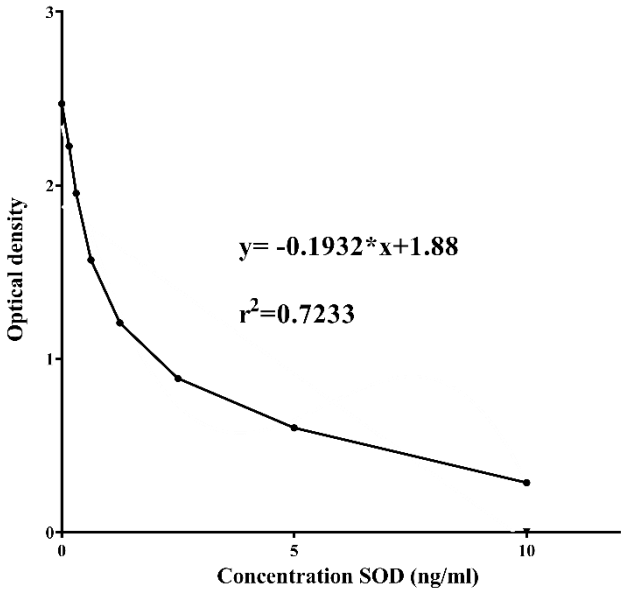

Fig. 3: SOD standard curve.

Determination of hydroxyproline in lung tissue

The hydroxyproline concentrations in the lung tissue were measured using a microplate enzyme immunoassay (TSH accu-bind ELISA microwells).

Test principle

This kit uses Sandwich-ELISA. This kit's micro-ELISA plate has HYP-targeting antibodypre-coating. The right antibody is introduced to micro-LISA plate wells with standards or samples. A biotin-modified Hyp and-specific detection antibody follows. Avidin-Horseradish peroxidase (HRP) conjugate is successively added and incubated in each microplate well. Water conveys unpaid laborers. The substrate solution is introduced into every well. The blue wells consist of Hyp, biotinylated detection antibody, and Avidin-HRP conjugate. The enzyme-substrate reaction turns yellow with sulfuric acid. Optical density (OD) is measured at 450 nm±2 nm wavelength using a spectrophotometer. Hyp concentration influences OD. The comparison of samples' optical density (OD) to the standard curve determines Hyp concentration.

Procedure

Each well received 100 µL of a standard or sample. After 90 minutes at 37°C, the liquid was withdrawn and 100 µL of biotinylated detection antibody was added. After 1 hour of incubation, samples were aspirated and cleaned three times. We added 100 µL of HRP conjugate. The specimens were incubated at 37°C for 30 minutes. After that, the samples were suctioned and rinsed five times. After that, 90 µL of substrate reagent was added. For 15 minutes, the specimen was kept at 37 degrees Celsius. Next, 50 microliters of stop solution were added. Measure the absorbance at 450 nm immediately and calculate the results as shown in Fig. (4).

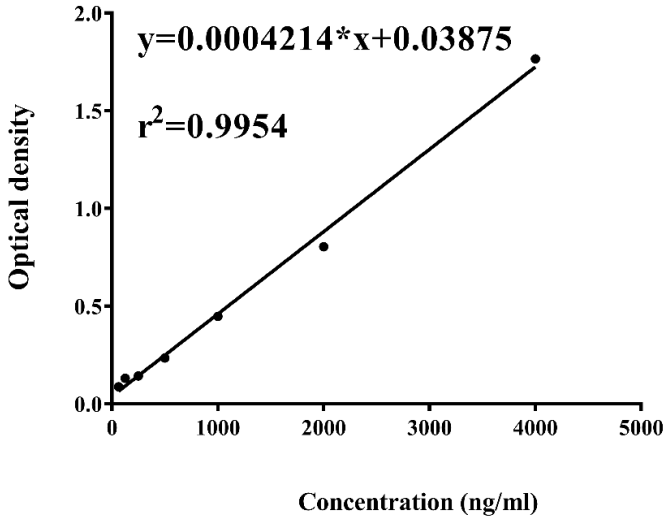

Fig. 4: Hydroxyproline standard curve.

Statistical analysis

The data provided displays the average ± standard error of the average (SEM), and statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS, version 28). Based on the results of the Shapiro-Wilk and Kolmogorov-Smirnov tests, it can be concluded that the current data exhibits a normal distribution. All the gathered data was subjected to a parametric test. The data was analyzed using one-way analysis of variance (ANOVA). The group comparisons were conducted using the post-hoc Duncan test. The study revealed a statistically significant difference when the p-value was below 0.05. The existence of distinct letters on bars implies statistical significance, while the presence of identical letters on bars signifies no statistically significant difference.

RESULTS AND DISCUSSION

This study investigates the impact of various levels of radon exposure on oxidative stress and indicators of lung fibrosis in rats. Additionally, it investigates the potential of melatonin as an antioxidant to lessen radiation-induced lipid peroxidation. The statistical analysis revealed a strong correlation between radon exposure and the concentration of MDA in lung tissue, specifically in D4 and D5 (82.005± 14.18 and 80.79±7.693 µmol/g of tissue), respectively. The concentration in D6 showed a substantial rise (94.02±7.283 µmol/g of tissue) ($p<0.05$) compared to the control group (21.33± 4.522). Also, when radon was present, the levels of MDA went up significantly in groups D1, D2, and D3 (45.41± 4.694, 53.56± 6.711, and 65.78±11.55µmol/g of tissue), compared to the control group (21.33± 4.522µmol/g of tissue), as shown in (Table 1) and Fig. (5).

Table 1: Effects of different doses of radon exposure on oxidative stress and lung fibrosis markers

Parameters	Lung MDA* (µmol/g tissue)	Lung SOD* (ng/ml)	Lung hydroxyproline* (ng/ml)
Control	21.33± 4.522^{d}	1.166±.0787^{a}	59.56± 2.363^{bc}
D1 (1/2hr exposure)	45.41±4.694^{cd}	1.047±.1515^{ab}	56.25± 7.439^{bc}
D2 (1 hr exposure)	53.56± 6.711^{c}	1.179±.0525^{a}	53.20± 1.105^{c}
D3 (2 hr exposure)	65.78±11.55^{bc}	1.022±.04641^{ab}	62.46± 4.505^{abc}
D4 (3 hr exposure)	82.005±14.18^{ab}	.8939±.0428^{b}	64.064±3.945^{abc}
D5 (6 hr exposure)	80.79± 7.693^{ab}	.8717±.04101^{b}	67.85± 4.431^{ab}
D6 (9 hr exposure)	94.02± 7.283^{a}	.8920±.08527^{b}	74.52± 3.858^{a}

The different letters on bars mean significant and the same letters on bars mean no significant difference. The data represented mean ± SEM *P<0.05 considered a significant difference according to ANOVA followed by post hoc, Duncan test.

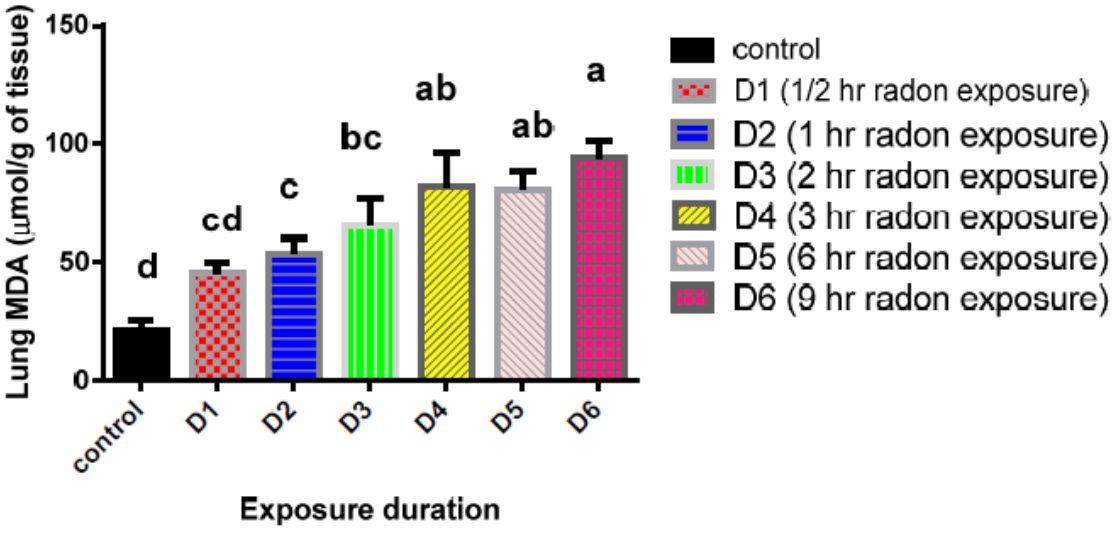

Fig. 5: Effects of different doses of radon inhalation on lung MDA level.

It is established that, following radiation exposure, the levels of MDA exhibited a substantial increase (Bhatia and Manda, 2004). MDA is widely recognized as the primary product of lipid peroxidation, and its concentration is typically used to represent the overall level of lipid peroxidation products (Drewa *et al*., 2002). MDA, a byproduct of lipid peroxidation, has the ability to generate ozone. This ozone then quickly reacts with cellular structures, resulting in the production of hydrogen peroxide and other reactive oxygen species (ROS). This process ultimately leads to the peroxidation and denaturation of membranes (Ajamieh *et al*., 2004; Scassellati *et al*., 2020).

SOD activity goes up when cells are exposed to radon. SOD removes superoxide radicals, and this effect has been seen in several organs of rats and rabbits as well (Kataoka *et al*., 2012; Kataoka *et al*., 2021; Ma *et al*., 1996). Furthermore, the alterations in SOD activity following exposure to radon are presented in Fig. (6) and (Table 1). The SOD activities in lung tissues increased non significantly with the increase of radon exposure in D2 group (1.1792±.05259ng/ml) as compared to the control group (1.1669± .0787 ng/ml). However, SOD activity in lung tissue was slightly reduced in the first and third dose of radon exposure, and its activity significantly decreased ($p<0.05$) in D4, D5 and D6 groups (1.047±.1515, 1.022±.0464, .8939±.0428, .8717±.0410 and .892±.0852 ng/ml), respectively, as compared to the control group (1.1669± .0787 ng/ml). Another study found that radon's effects are outweighed by its SOD inhibition after 16 hours of inhalation at 1000 $kBq.m^{-3}$. On the other hand, our data demonstrated that SOD activity in lung organs significantly increased after two hours of radon exposure because of the short period time of exposure is consistent with (Ma *et al*., 1996). In the current study, it is important to note that SOD activity in the lung tissues of rats exposed to radon was enhanced in the second group, while SOD activity transiently regained a near-normal level in the D1 and D3 groups and was subsequently reduced again in the D4, D5, and D6 groups. These findings suggest that prolonged exposure to radon inhalation leads to the activation of two distinct kinds of SOD. This discovery is similar to the documented impacts of ionizing radiation exposure in rats (Yamaoka *et al*., 1998), these outcomes may be related to homeostasis (Kataoka *et al*., 2011) which is consistent with our findings in lung tissues. The lung is the primary organ susceptible to radon exposure (Maier *et al*., 2020).

The findings of our study confirmed the activation of (SOD) activity in the lung (Maier *et al*., 2020). However, another study showed that there were no significant changes in the (SOD) activities in the lungs, even when exposed to high doses of ionizing radiation. The changes in (SOD) activity after radon exposure are because lung tissues are very sensitive because they are always exposed to oxygen. This suggests that the lung may be resistant to oxidative damage from oxygen and radiation (Kataoka *et al*., 2011; Sasaok, 2018). There is a potential danger that the adipose cell membrane has a higher propensity to absorb radon compared to other cellular components. As a result, radon and its decay products may emit more alpha radiation, which could expose the cell membrane to more of it. The cell membrane can be induced to transmit signals to other cellular compartments in order to produce SOD. If the duration of exposure is significantly extended, the cell membrane may potentially lose its responsiveness to stimulation, resulting in a decrease in SOD activity (Ma *et al*., 1996). After a 1-hour exposure, the activities of SOD showed a slight increase, then a slight drop after 0.5 and 2 hours of exposure. Subsequently, there was a slight decrease in SOD activity after 3, 6, and 9 hours of exposure, as depicted in Fig. (6).

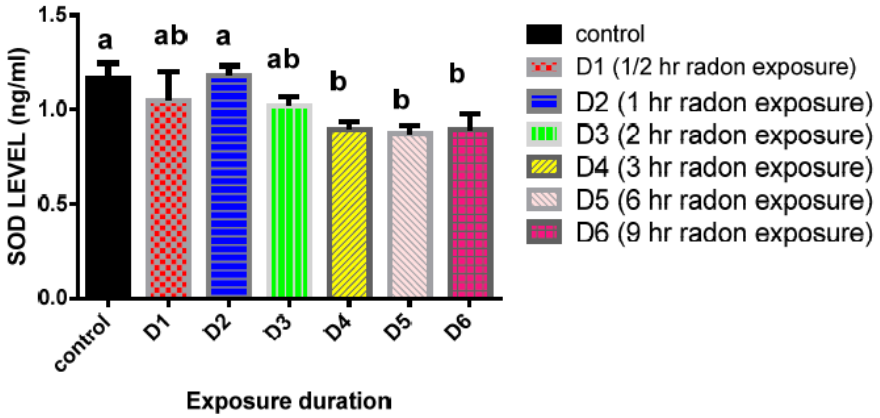

Fig. 6: Effects of different doses of radon inhalation on SOD activity.

Two plausible mechanisms could be taken into account. The first effect is the inverse relationship between low dose rates and alpha irradiation, as observed in experimental experiments. The second mechanism involves the dominance of SOD formation control due to prolonged exposure, surpassing the stimulating effects. The production process of SOD may be stimulated shortly after exposure begins, but this stimulation may not persist for extended periods of time, such as 6 and 9 hours (Kataoka *et al*., 2017; Kataoka *et al*., 2011). In our present investigation, we observed a notable reduction in the activities of superoxide dismutase (SOD) in lung tissues, leading to an overall increase in the concentration of the superoxide free radical. This decline in SOD activities may have contributed to the buildup of O_2^{-}. Protein oxidation by free radicals has been detected (Güney *et al*., 2004), As a result, it is believed that the increased attack from free radicals is to blame for the decline in SOD activity. The current findings, which point to a higher level of MDA, support this hypothesis. These results align with those previously reported by (Güney *et al*., 2004; Xiao *et al*., 2022; Xin *et al*., 2022).

As shown in Fig. (7) and (Table 1), the level of hydroxyproline in the sixth group showed a significant rose ($p<0.05$) in hydroxyproline concentration in the rat's lung tissue (74.52 ±3.858 ng/ml) after radon exposure as compared to the control group (59.56± 2.363 ng/ml). Moreover, there was a non-significant difference between the hydroxyproline levels in the third, fourth and fifth groups (62.46±4.505, 64.06±3.945 and 67.8±4.431 ng/ml), respectively, while the concentration of hydroxyproline in lungs of first and second groups had a substantial reduction in hydroxyproline content (56.25±7.439 and 53.2±1.105 ng/ml) as compared to the control group (59.5± 2.363 ng/ml). The current findings have shown that exposure to radon leads to lung damage and fibrosis, which aligns with the findings of (Chen *et al*., 2020) They discovered that inhalation of radon led to lung damage and fibrosis in mice, a condition that worsened with higher levels of exposure. This suggests that oxidative stress, which results from the release of ionizing radiation during radon exposure, has the potential to harm lung cells (Giuranno *et al*., 2019).

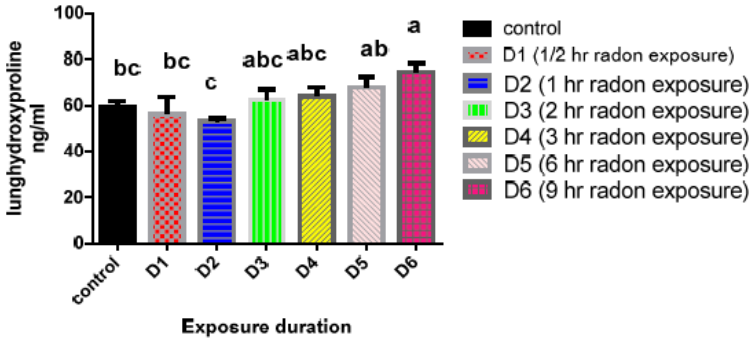

Fig. 7: Effects of different doses of radon exposure on hydroxyl proline concentration in lung tissues.

The lung tissues of rats that received low doses of MEL (60 mg/kg diet) and high doses of MEL (120 mg/kg diet) showed a significant decrease in the lipid peroxidation marker MDA ($p<0.05$). MDA levels in lung tissues were 30.34±1.955 µmol/g of tissue in the low dose group and 20.89±2.202 µmol/g of tissue in the high dose group. In the positive control group, they were 80.79±7.693 µmol/g of tissue. The decrease in lung MDA levels resulted in their convergence with the levels found in the control group, as depicted in (Table 2) and Fig. (8).

Table 2: Effects of low and high doses of melatonin against radon exposure on oxidative stress and lung fibrosis markers

Parameters	Lung MDA* (µmol/g tissue)	Lung SOD* (ng/ml)	Lung hydroxyproline*(ng/ml)
Control	21.33± 4.522[d]	1.166±.0787[a]	59.56± 2.363[a]
Positive Control (D5) (6 hr exposure)	80.79±7.69[a]	.8717±.04101[c]	67.85 ±4.431[a]
Melatonin (Low dose) (60 mg/kg diet)	30.34±1.95[b]	1.214±.1052[ab]	55.80 ±4.995[a]
Melatonin (High Dose) (120mg/kg diet)	20.89± 2.202[b]	1.196±.0798[ab]	67.25±5.028[a]

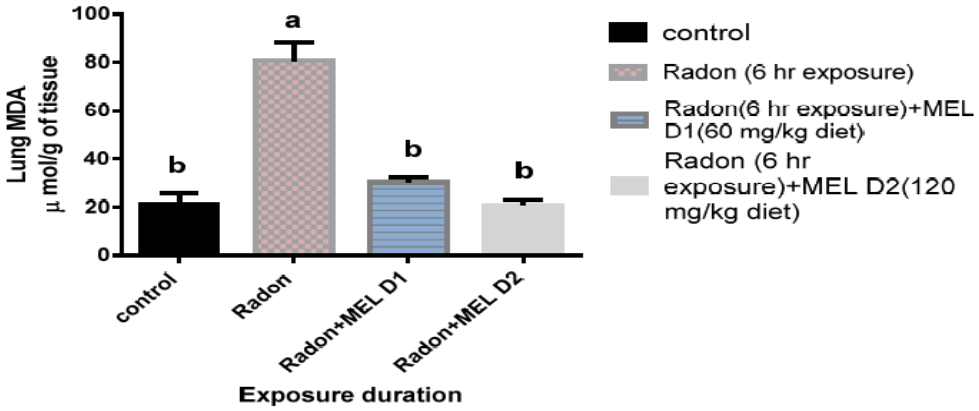

Fig. 8: The effect of melatonin on lung MDA level.

The main reason why MDA levels go down after taking MEL supplements is that they may boost SOD and glutathione reductase (GSH-Rd), two enzymes that are known for being powerful antioxidants (Hacışevki and Baba, 2018; Goc *et al*., 2017). In addition, MEL has the ability to counteract hydrogen peroxide, singlet oxygen, NO, and peroxyl nitrite anions. These substances are free radicals that trigger oxidative stress in biological membranes and lead to lipid peroxidation in cells (Sener *et al*., 2002). Administration of MEL also led to a decrease in MDA levels *(Tahamtan et al*., 2015). Several studies prove the potent antioxidant impacts of melatonin against oxidative stress (Nuszkiewicz *et al*., 2020; Reiter *et al*., 2017; Motallebzadeh *et al*., 2020; Wu *et al*., 2021). Melatonin has solubility in both aqueous and lipid locations, leading it to serve as antioxidant across the interior watery environment, blood stream fluids, plasma membrane, and cellular components (Asghari *et al*., 2017). Research on the protective impacts of melatonin has verified that this hormone and its byproducts fight several ROS, and RNS which are in accordance with our results *(*Vishnoi *et al*., 2016).

The statistical analysis revealed a notable rise in SOD activity in the lungs of rats in the positive group D5 ($p<0.05$) following treatment with low and high doses of MEL (60 mg/kg diet and 120 mg/kg diet) (1.214±.10525 and 1.196±.07988), respectively, compared to the positive control D5 (.8717±.041). This increase in SOD activity surpassed that of the control group, as shown in (Table 2) and Fig. (9). The findings indicate that melatonin has the potential to decrease oxidative stress by enhancing the activity of superoxide dismutase (SOD) in a manner that is dependent on the dosage. This effect was observed in rats used as positive controls. Conversely, the levels of MDA were dramatically decreased in a dose-dependent manner in positive control rats compared to the control group, which aligns with (Jang *et al*., 2013). They indicated that the melatonin group showed a considerable increase in SOD activity compared to the group that just received irradiation. This increase may have contributed to the significant reduction in lipid peroxidation, which confirmed the present result.

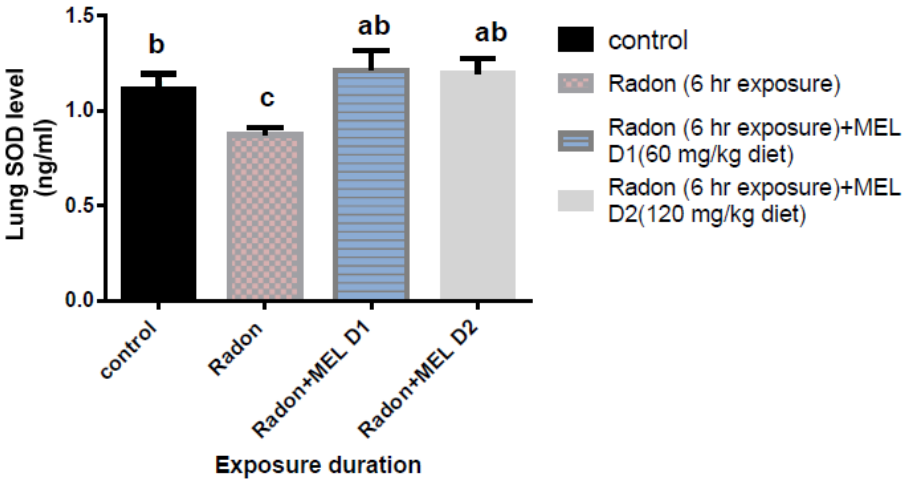

Fig. 9: The effect of melatonin on SOD activity.

When the rat was given low and high doses of MEL (60 mg/kg diet and 120 mg/kg diet) (55.80 ± 4.995 and 67.25 ± 5.028 ng/ml), there was no significant change in the level of hydroxyproline in its lung tissue compared to the positive control D5 (67.85 ± 4.431 ng/ml). Additionally, there was no significant change compared to the control group ($p>0.05$), as indicated in Fig. (10). Although, there is evidence that MEL reduces pulmonary fibrosis (Hosseinzadeh *et al*., 2018; Zhao *et al*., 2018; Arslan *et al*., 2002), The duration of one week of MEL supplementation in our study may not be sufficient to decrease hydroxyproline levels. Additional research using extended MEL treatment may lead to a notable reduction in the lung fibrosis marker, hydroxyl proline (Serin *et al*., 2007).

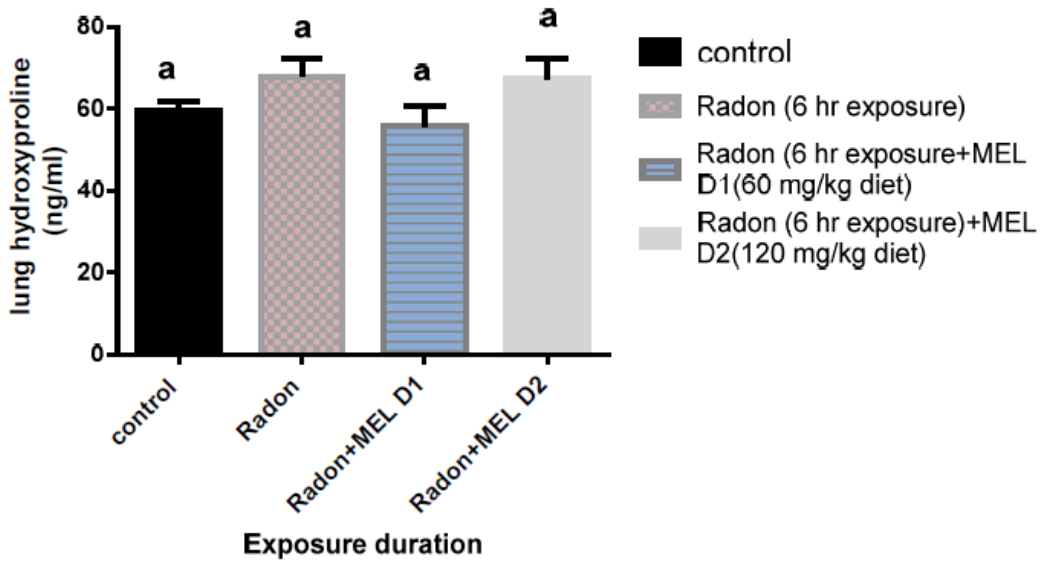

Fig. 10: The effect of melatonin on the hydroxyproline concentration.

CONCLUSIONS

In this study, an investigation was done to examine the impact of radon exposure on the lungs of male albino rats, as well as the protective roles of melatonin as an antioxidant. Our study provided evidence that ionizing radiation, such as that emitted from inhaling radon, can cause cellular damage and lipid peroxidation by generating (ROS) and oxidative stress, and the injury could be repaired with melatonin consumption by increasing SOD activity and lowering MDA levels. Inhaling radon leads to oxidative stress by increasing the concentration of tissue MDA. Inhalation of radon resulted in lipid peroxidation and alterations in the lung, which exhibited a dose-dependent relationship. However, it greatly reduced the activity of lung SOD. More MDA accumulating in tissues may be to blame for the decline in SOD levels. This, in turn, causes a big rise in hydroxyproline content in the lungs, which is a sign of lung fibrosis. By lowering the amount of MDA and preventing lipid peroxidation through an increase in SOD activity, rats given both low and high doses of MEL showed a noticeable decrease in oxidative stress. These findings suggest that it's crucial to think of melatonin as an antioxidant that can stop radiation-induced lipid peroxidation because it can lower MDA levels and increase SOD activity. The significance of this work lies in its exploration of various dosages of radon and melatonin in lung tissue. There is a scarcity of articles that investigate the protective effects of melatonin on the lungs when exposed to radon. This study fills that gap and is therefore valuable for both academics and practitioners. Nevertheless, the precise mechanism by which melatonin provides protection has yet to be fully understood. In the future, our focus will be on studying the defense mechanism of melatonin against lung harm produced by long-term exposure to radon. In addition, the lung tissue will undergo a pathological inspection, while other markers of oxidative stress will be assessed and the expression of relevant genes will be measured.

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آثار التعرض للرادون- 222 على الإجهاد التأكسدي في الرئة وديسموتاز الفائق أكسيد في ذكور الجرذان البيضاء : أدوار الميلاتونين كمضاد للأكسدة

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الملخص

تتناول هذه الدراسة تأثيرات التعرض للرادون على رئتي ذكور الجرذان البيضاء، وهو سبب بارز لسرطان الرئة، بالإضافة إلى الأدوار الدفاعية للميلاتونين كمضاد للأكسدة من خلال فحص أنشطة ديسموتاز الفائق أكسيد SOD، و المالونديالدهيد MDA، و الهيدروكسي برولين. تتكون الدراسة من تجربتين. في التجربة الأولى، في الغرفة المبنية حديثًا، تم تعريض الفئران لغاز الرادون (5μCi) من مصدرراديوم (^{226}Ra) تم تقسيم الفئران إلى سبع مجموعات، كل منها ثمانية فئران. وبخلاف المجموعة السيطرة، تم تعريض المجموعات الست الأخرى لغاز الرادون، والذي تم تقييمه باستخدام كاشفRAD-7، وتحديدًا لمدة 30 دقيقة (D1)، وساعة واحدة (D2)، وساعتين (D3)، 3 ساعات (D4) ، 6 ساعات (D5)، و9 ساعات (D6). تم قياس التراكيز لتكون (600 ± 0.014، 1210 ± 0.028، 2421 ± 0.057، 3600 ± 0.086، 7260 ± 0.172، و 10875 ± 0.259) $Bq.m^{-3}$ ، على التوالي. فحصت التجربة الثانية التأثيرات الوقائية للميلاتونين ضد التعرض للرادون. أعطيت الفئران جرعة منخفضة (60 ملغم/ كغم من النظام الغذائي) وجرعة عالية (120 ملغم/ كغم من النظام الغذائي) من الميلاتونين ثم تم تعريضها للرادون لمدة ست ساعات. التعرض للرادون لم يغير بشكل ملحوظ نشاط SOD . ومع ذلك، فإن جرعتي الرادون الأولى والثالثة خفضت نشاط SOD بشكل متواضع. بعد التعرض، زادت مستويات الهيدروكسي برولين بشكل ملحوظ مقارنة بالمجموعة السيطرة. وجدت نتيجة إحصائية أن التعرض للرادون يرفع مستوى MDA في الرئة. عزز الميلاتونين تخفيضات SOD بعد التعرض للرادون. ومع ذلك، كل من جرعات MEL خفضتMDA إلى ما دون السيطرة الإيجابية. في الختام، استنشاق غاز الرادون يدفع بيروكسيد الدهون. يقلل الميلاتونين MDA ويزيد من نشاط SOD، مما يجعله مضادًا جيدًا للأكسدة ضد التعرض للرادون.

الكلمات الدالة: استنشاق الرادون، سوبر أكسيد ديسموتاز(SOD)، الميلاتونين، التليف الرئوي، (MDA).