

## Study of the Effects of Naturally Occurring Radioactive Materials on Blood Indices in Blood's Rats

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**T**O UNDERSTAND the toxicity and the potential role of naturally occurring radioactive materials (NORM) as risk factors on rat's blood characteristics. These materials cover both naturally occurring and man-made or artificial sources. The most common material is Radon, which results from naturally-occurring  $^{232}\text{Th}$  and  $^{238}\text{U}$  and serially passing as isotopes through a number of decays till a stable isotope evolves. The aim of this study was to investigate the potential effects and the oxidative damage caused by gamma radiation emitted from naturally occurring radioactive materials with lowdose levels on the blood of male rats. Groups of adult male rats were classified and exposed to different doses result from NORM. The expected changes the changes in the concentration of total Protein, Albumin and Globulin according to the exposure were measured, in addition to the change in some hematological parameters. The results indicated that all irradiated groups showed significant decreasing in the concentration of Total Protein, Total Albumin, Total Globulin, Albumin / Globulin Ratio, the Platelets numbers (PLTs) and platelets Volume (MPV) Globulin and Albumin / Globulin Ratio and a significant decreasing in the Platelets numbers and volumes (PLTs), (MPV) At the same time, the results showed increasing in the PLTs/Lymphocytes ratio compared with control group. The results established that the exposure to TENORM dose has some deleterious effects as it observed in Albumin and Lymphocytes elevation. In conclusion , the natural occurring radioactive materials have a significant effect on the blood system in the male rats.

**Keyword** : Naturally Occurring Radioactive materials, Blood Indices, Protein, Albumin, Globulin

### Introduction

The radiobiological information which is related to the effects of ionizing radiation on the biological systems was limited with the experimental studies on some types of animals and radio-humanly accidents. On the other hand, these results of the effect of ionizing radiation were based on many factors. These factors are radiation types, radiation dose and radio-sensitivity of exposed tissues<sup>[1]</sup>. However, the exposure to the ionizing radiation can lead to a disturbance in the sequence of components in the biological systems<sup>[2]</sup>. The excessive in free radicals and/or low antioxidant defense due to the exposure to the ionizing radiation are the reasons of oxidative stress in biological system. The oxidative stress can produce chemical alterations of biomolecules causing structure and functional modifications. Reactive nitrogen species (RNS) and reactive oxygen species (ROS) are the products of normal cellular metabolism<sup>[3]</sup>. The harmful cellular agent for cell is the overproduction of ROS. Effect on the lipid's concentration, protein

and DNA sequence are associated also with the excessive production ROS. This harmful process is based on the interaction between radiation and water in cell<sup>[4]</sup>.

More researches are interested with the production of cell abnormal activities due to the effect of ROS on cells. The ionizing radiation might be naturally or artificially. Nearly, soils, rocks and water include small amounts of naturally radioactive materials (e.g. Uranium, Radium, Thorium and their decay products). When a naturally radioactive material in its natural state becomes purposefully concentrated either in waste by products or in a product, it becomes technologically enhanced naturally occurring radioactive material (TENORM). According to this concept, we can be defined the TENORM as any naturally occurring materials whose radionuclide concentration or potential for human exposure has been increased above levels encountered in the natural state as a result of human activities<sup>[5]</sup>. The aim of this work is to investigate the potential

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radioprotective effects of TENORM asoxidative damage caused by gamma-irradiation on normal blood's rat.

### **Materials and Methods**

#### *Animals preparation:*

The animals were prepared to be irradiated under as the following conditions:

#### *Animals selection and feeding*

24 Male albino rats with average weight  $220 \pm 10$  g, the animals were feed with normal rat food with balanced meal. Each meal was contained about 21%  $10 \pm$  protein.

#### *Animals housing*

The animals were housed in plastic cages and preserved at room temperature and pressure. The room light was controlled (12-h day/night cycle). The cages were cleaned daily at fixed time. The animals housing and feeding were done for this study at the biophysics lab, faculty of science, Al-Azhar university.

#### *Animals grouping and irradiating*

The animals were randomly selected and classified into four groups (G), each G consist of 6 male rate. Each group was exposed to certain dose from TENROM and Each group was exposed to certain dose from TENORM and the effect of every dose level on rat's blood was studied. TENORM doses were measured using a calibrated *Digilert-100*<sup>®</sup> nuclear radiation monitor, manufactured by S. E. International Co, Inc. USA. G1 was conserved as a control for all examinations. G2 & 3 were classified according to the exposure for TENORM doses. For G2, the animals of this group were exposed to TENORM with dose rate 10mGy/month for 30 days. The animals of G 3 were irradiated to the same dose rate for 60 days. For last group (G4), the animals were exposed to another radioactive source to compare between the effect of exposure to different type of radioactive material with different dose rate. This radioactive source was <sup>137</sup>Ci with dose 0.695 Gy/minute. The delivered dose to this group was 2 Gy is a single fraction. The irradiation process for all groups was done at the National Center for Radiation Research and technologies, Egyptian Atomic Energy Authority, EA EA.

#### *Blood collection and Hematological parameters:*

After irradiation, all groups were prepared to anesthesia and blood collection. Diethyl Ether solution was used to anaesthetizing process. Investigated blood samples were accumulated

by rat's eye puncture. Collected samples were prevented using EDTA (ethylene di-amine tetra-acetic acid). 12  $\mu$ l of whole blood samples were evaluated via the (*Diff3*) *Mek6410/Mek-6420* hematology Analyzer system. Mean Platelet Volume (MPV) was assessed. Lymphocyte counts were obtained for the spleen. Also, Platelet counts (PLTs), percentage of Lymphocytes were assessed. The applied method for hematological parameters account was *Wintrobe* protocol.<sup>[6]</sup>

#### *Determination of Total Protein*

According to Biuret method performed by Doumas (1975), the serum of total protein was prepared and determined. In an alkaline solution, the total protein formed a violet to blue color with copper ions. The absorbance for this color was 546 nm (Spectrophotometer (*UVT9000*®China)). The instrument was adjusted at zero, and the mixture was incubated for 5 min at room temperature before the measurement to draw up the environmental condition.<sup>[7]</sup>

#### *Determination of Albumin*

Albumin was determined in serum according to the method described by Doumas et al., (1971). Albumin reacts with Bromo cresol green to yield green color which is can be measured at 628 nm. The instrument was adjusted at zero. Then, the composition mixed well and incubated for 10 min at room temperature. Finally, the absorbance of samples and standard against the blank was measured.<sup>[8]</sup>

The Total Globulin fraction is generally determined by subtracting the albumin from the total protein.

#### *Computational and statistical analysis*

The analysis of results was done using the computer program of Statistical Packages for Social Science (SPSS 23) for analysis and data comparison. The control group based as a standard value to calculate the difference percentage using excel program.

### **Results**

#### *Effects of radiation on PLTs count and volume*

Figures 1(A & B) show the effect of radiation on the mean values of PLTs counts and PLTs volume for the investigated groups. As shown in these figures, there is a significant decreasing in the mean values of PLTs related to the increase in radiation as compared with the control group.

Fig.2 illustrates the difference percentage for

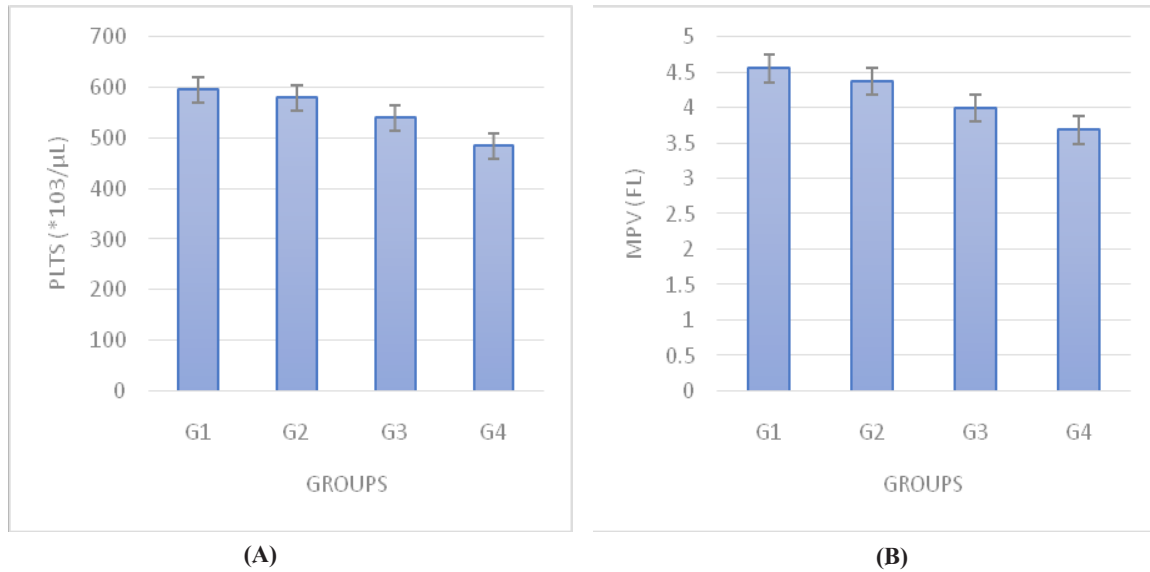


Fig. 1. Mean values of PLTs (A) count and (B) volume for all groups "G".

G 2, 3 and 4 to G1 for PLTs counts and PLTs volume. As indicated in this figure, there is a deeply decreasing in both PLTs count and volume as the time of exposure increasing and/or exposing for gamma ray radiation from a radioactive source. The difference percentage in PLTs count in G 2, 3 & 4 to G1 were -2.68, -9.40 and -18.62 %, while they were -4.06, -12.28 and -19.13 % for PLTs volume respectively.

#### Effects of radiation on percentage of Lymphocytes

Figure 3 shows the effect of radiation of TENORM and Gamma rays on the percentage of lymphocytes in the blood's rats. The percentage of lymphocytes in the control group was (80.10 %) as a normal value. After irradiating other groups, the percentage values of Lymphocytes showed decreasing with the increasing for TENORM or gamma radiation. The values were (80.1, 72.78, 66.48, 59) for G 1, 2, 3 and 4 respectively.

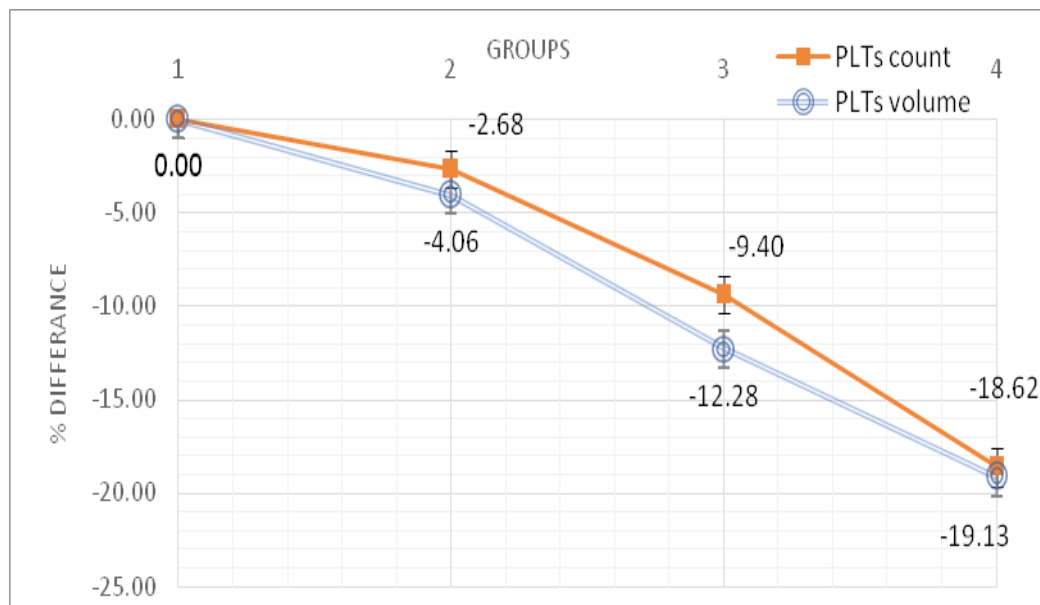
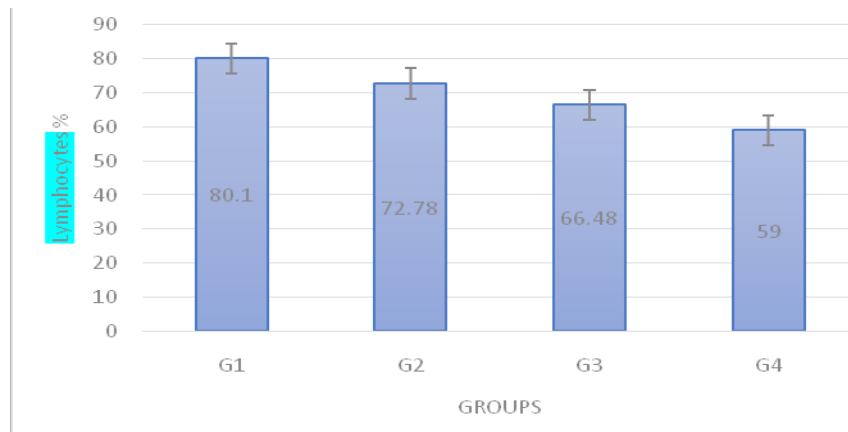


Fig. 2. Difference percentage in irradiated groups to G1, for PLTs count and PLTs volume.



**Fig. 3. The percentage of Lymphocytes in all groups.**

*Effect of radiation on the PLTs to lymphocyte (PLTs/ Lymphocytes) ratio*

Figure 4 describes the effect of radiation on the PLTs to lymphocyte ratio. As indicated in this figure, there was an increasing with (7%) in the PLTs to lymphocyte ratio between the values of G1 as a control group and G2 which was exposed to 30 days TENORM, also there a slightly increasing (9%) for G3 & (10%) for G4 whose were exposed to 60 days TENORM and 2Gy of gamma ray respectively compared with G1.

*Effect of radiation on the blood protein, Albumin and Globulin concentrations*

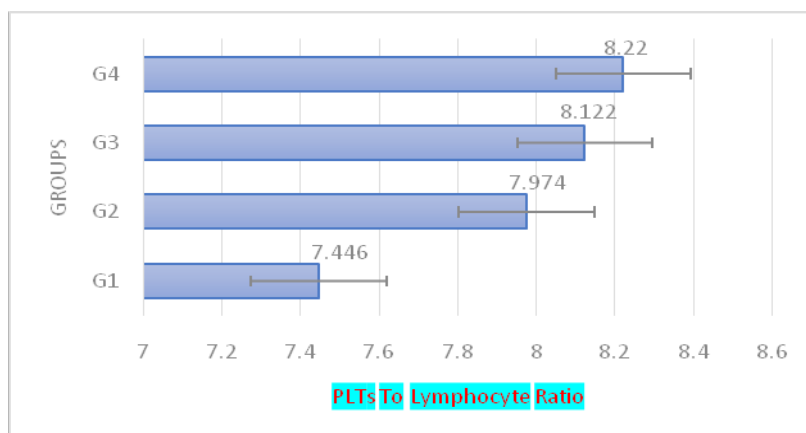
Figure 5 shows the effect of radiation on Protein, Albumin and Globulin of the investigated groups.

As shown in this figure, the Protein concentration for examined groups compared with the control decreased gradually. The concentration difference percentage for G2, 3 and 4 compared with G1 were (8%, 24% and 36%) respectively. Also,

this figure indicates that there was a significant drop in the concentration of blood Albumin. The difference percentage between G 2 to G1 was (13%), while it was (33%) and (47%) difference between G3 to G1 and G4 to G1 respectively. The effect of radiation on the Globulin concentration of G2, 3 and 4 showed slightly differences compared with the concentration of Protein and Albumin as shown in figure 5. The statistical differences in the concentration of Globulin indicated that there were 2.1 % difference between G2 to G1, 8.8 % between G3 to G1 and 18 % between G4 to G1.

*Effect of radiation on blood Albumin to Globulin (A/G) ratio*

Figure 6 explains the effect of radiation on the Albumin to Globulin ratio. As indicated in this figure, there is a significant decreasing with (11%) in the Albumin to Globulin ratio between the values of G1 as a control group and G2 while there is a fall with (28 %) for G3 & (35 %) for G4 respectively compared with G1.



**Fig. 4. PLTs to Lymphocyte ratio.**

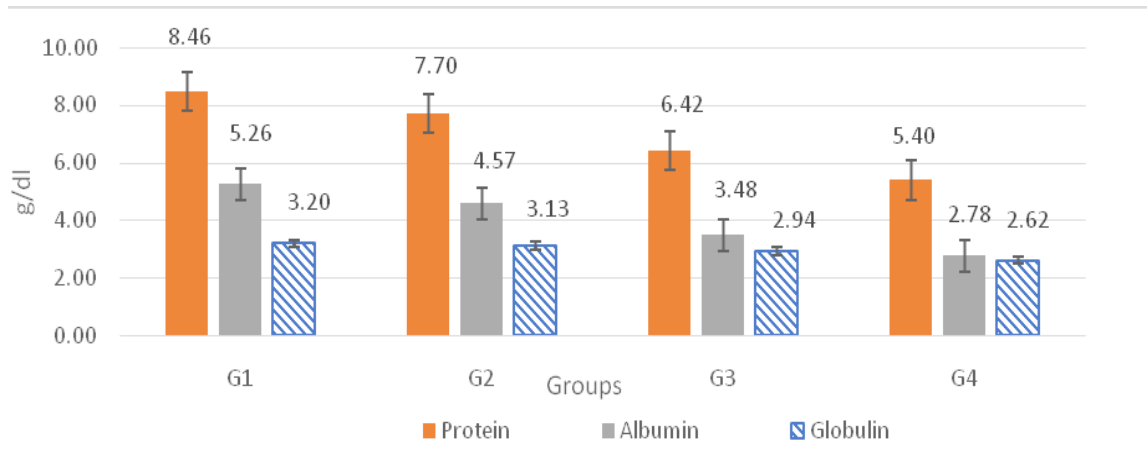


Fig. 5. Concentration of Protein, Albumin and globulin for all groups.

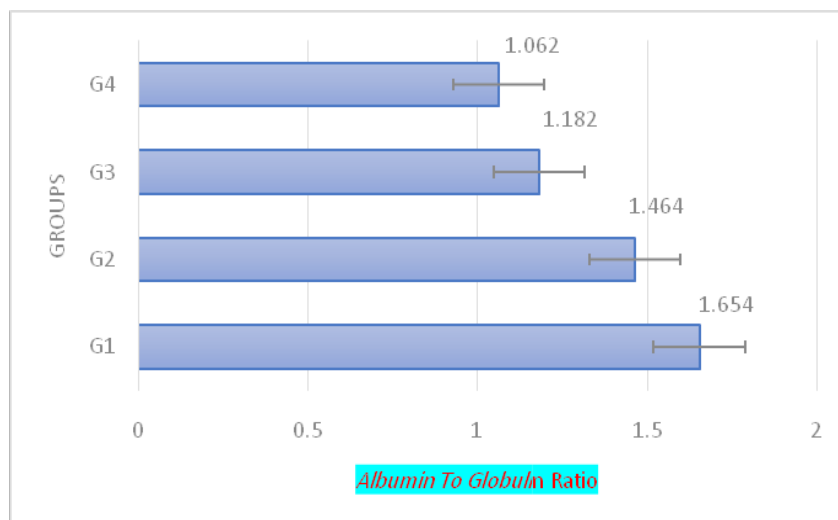


Fig.6. Effect of radiation on the Albumin to Globulin ratio.

## Discussion

The results showed that there were changes in the normality of rat's blood due to the exposure to radiation. The decreasing in PLTs count and MPV is accompanied with decreasing in erythrocyte count. The harmful effects of radiation on RBCs count may be associated with the cessation of erythrocytes' production in the bone marrow additional to the loss of cells from the circulation blood system by hemorrhage or leakage through the capillary walls, and the direct destruction of mature circulating cells. Based on that, the decreasing in the normal values of blood indices following radiation exposure may be assigned to directly damage caused by a fatal dose of ionizing radiation [9]. (Moroni et al., 2011) demonstrated when they examined the effect of radiation on the mini pigs, they found that the neutrophil-to-

lymphocyte and platelet-to-lymphocyte ratios are particularly very important prognostic indicators of irradiation in mini animals [10]. On the other hand, the decreasing in total protein may be attributed to a damage in vital processes or due to the change in the liver functionality or kidney permeability or immune deficiency and other tissues resulting in leakage of protein via the kidney [11]. The changes in the values of hematological parameters like PLTs count, MPV, percentage of Lymphocytes, and the ratio of PLTs/ Lymphocytes, may be the first indicator for an abnormal biological system. Our results in agree with Abbady et al., 2000 who reported in their publication that there was a decline in the level of serum in animals exposed to whole body irradiation with gamma ray at different times of exposure and different doses [12]. A deeply decreasing was also noticed in serum of Albumin concentration in Karalenka et al., 1993,

with agreement with our results<sup>[13]</sup>. Based on our results, it's worth mention that the decreasing in total protein, Albumin and Globulin may be attributed to an impairment in the production of protein in liver due to hepato-cells damage<sup>[14-15]</sup>. In another studies, Keren et al., 1994, and Lessard et al., 1985, stated that the excessive loss through injury to kidneys may be the reason of the decreasing of Albumin concentration<sup>[16-17]</sup>. Choldhari and Chakrabati, 1983 mentioned that the decreasing in Albumin and Globulin could be related with hepatic disfunction<sup>[18]</sup>. These defects in the biological process might be the partially responsible for protein decreasing after radio-exposing.

### **Conclusion**

This study aimed to investigate the effect of TENORM exposure for one month and two months, also the effect of 2 Gy from gamma rays source on the blood indices and concentration of Protein, Globulin and Albumin in rat's blood. The results indicated that there were significant changes in the biological process in these parameters. Further investigations studies are needed on the effects of chronic ionizing radiation irradiation with different doses and periods. Based on our results that there are harmful effects on blood indices and concentration of Protein, Globulin and Albumin elevation in rat's blood according to the exposure for TENORM dose. TE-NORM exposure for one and two months resulted in highly production of free radicals with subsequent elevation in total proteins and deleterious effects on red blood cells.

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### ”دراسة آثار المواد المشعة التي تحدث بشكل طبيعي على مؤشرات الدم في دم الجرذان“

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لفهم السمية والدور المحتمل للمواد المشعة (NORM) والتي توجد بشكل طبيعي على الحالة الطبيعية لدم الجرذان كأداة خطر. تغطي هذه المواد كل من المصادر التي تحدث بشكل طبيعي أو من صنع الإنسان. المادة ذات الإهتمام الواسع هي الرادون، والتي تنبعث من اليورانيوم 232 و 238 والتي توجد بشكل طبيعي

الهدف من هذه الدراسة هو  
التحقق من الآثار المحتملة والضرر التأكسدي الناجم عن إشعاع غاما المنبعث من المواد المشعة التي تحدث بشكل طبيعي مع انخفاض مستويات الجرعات على دم ذكور الجرذان

**المواد والطرق**  
تم تصنيف مجموعات من ذكور الجرذان البالغين وتم تعريضهم لهذه المواد بجرعات مختلفة. تم قياس التغيير في تركيز البروتين الكلي والألبومين والجلوبولين وفقاً للتعرض ، بالإضافة إلى التغيير في بعض المعلمات الدموية.

**النتائج والمناقشة**  
أظهرت جميع المجموعات المشعة انخفاضاً ملحوظاً في تركيز البروتين الكلي ، الألبومين ، الجلوبولين ونسبة الألبومين / جلوبيولين بالإضافة الى انخفاض ملحوظ في أعداد وأحجام الصفائح الدموية (PLTs) و (MPV). في الوقت نفسه ، أظهرت النتائج زيادة في نسبة (PLT / LC) مقارنة (PLTs / Lymphocytes) مقارنة بالمجموعة الضابطة. أثبتت نتائج هذه الدراسة أن التعرض لجرعة TENORM له بعض التأثيرات الضارة كما لوحظ في ارتفاع مستوى الألبومين والخلايا اللمفاوية.

**استنتاج**  
المواد المشعة الطبيعية لها تأثير كبير على نظام الدم في ذكور الجرذان.