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## Effects of Gamma Ray as Oxidative Stress on Several Blood Parameters of Male Chickens

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### Abstract

The present work aims to detect the effects of gamma ray radiation emitted from Co-60 on several blood parameters (PCV, Hb, WBCs and RBCs) of male chickens. Also, the potential antioxidant impact of grape seeds oil exposed to cold plasma as a protective material to lower the damage caused by gamma ray was tested for a long time and low dose rate. Healthy male chickens were used in this study which had an age range of 2 to 3 months and a weight range of 1.5 to 2 kg. Animals were kept in plastic cages (100 x 400 x 40 cm dimensions) within humidity and temperature preserved at limited values. Four experimental groups were included; the first was the un-exposed control. The second group was administered with a dose of grape seed extract. The third group was exposed to gamma ray radiation only. The fourth group was administered with a dose of grape seed extract and irradiated with gamma waves. Forty male chickens were used in this work as they were parted and administered with the selected doses. The control group was considered as the first group that contained 10 male chicken without being exposed to waves of gamma rays. The next group also contained 10 male chicken irradiated with 5 Gy/h at interval 7 hour/day for 20 days as second group. Another group contained 10 male chicken and was dealt with oil of grape seed (400 mg/kg wt) with orally administered dose for 20 days as third group. The fourth group contained 10 male chicken irradiated with 5 Gy/h at interval of 7 hour/day for 20 days and dealt with orally administered dose (400 mg/kg wt) of grape seed oil for the same time. Overall groups (2-4), male chicken number became 30 used for 40.60 days at 7 hours per day. The schedule of work was achieved at 5 Gy/h of radiation equivalent dose with different exposure time for (20 and 40,60 days) on male chicken and at period 7 hours daily. The grape seed extract was bought from local market in Mosul City and filters from impure, as we found significant decrease in ratio of blood factors RBCs, WBCs, Hb and PCV with increasing of exposure time. This data changes with adding antioxidant materials as (grape seed oil) were the value of blood factors ratio enhancement by increasing the time of dose of these material compared with control group. The blood factors ratio of male chickens change with exposure time, with significant decrease by increasing exposure time, however, the protective material (grape seed oil) reduces the change in ratio % of the some blood factors compared with control group.

**Keywords:** male chicken, hematological parameters, gamma ray, grape seed oil, free radical.

دراسة تأثير اشعة كاما المنبعثة من الكوبلت -60 كاجهاد تاكسدي على بعض معامات الدم لذكور

الدجاج

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### الخلاصة

البحث الحالي يهدف الى ايجاد تأثير اشعة كاما المنبعثة من المصدر كوبلت -60 (Co-60) على بعض معاملات الدم لذكور الدجاج التي وردت من قبل جامعة الموصل. من هذه المعاملات خلايا الدم الحمراء، البيضاء، الهيموكلوبين والصفائح الدموية (RBC,WBC,Hb,PLT) ومعاملة النتائج المتغيرة كما تم اختبار التأثير المضاد للأكسدة المحتمل لزيت بذور العنب المعرضة للبلازما الباردة كمادة وقائية لتقليل الأضرار الناجمة عن أشعة جاما لفترة طويلة وبمعدل جرعة منخفضة. استخدم في هذه الدراسة حيوانات بعدد 40 من ذكور الدجاج وبأوزان 1.5-2 كيلوغرام وبأعمار من 2-3 شهر تم جلبها من كلية الطب البيطري / جامعة الموصل وتم وضعها بأقفاص بلاستيكية ابعادها (10 \* 40 \* 40 سم 3). قسمت الحيوانات الى 4 مجاميع واعطيت العليقة الغذائية 35% وحنطة 34% ذرة و20% فول الصويا و10% بروتين و1% حليب مجفف مع الماء لكل المجاميع. المجموعة الاولى مجموعة السيطرة اعطيت الماء والغذاء فقط بدون تعريض للأشعة النووية. المجموعة الثانية عرضت لجرعة اشعاعية 5 Gy/h لفترة 7 ساعات يوميا لمدة 20 يوما. المجموعة الثالثة اعطيت جرعة فموية من مستخلص بذور العنب ب 400 g/kg.wt لمدة 20 يوما. المجموعة الرابعة عرضت لأشعة كاما بجرعة 5 Gy/h لفترة 7 ساعات يوميا لمدة 20 يوما مع جرعة فموية 400 g/kg من مستخلص بذور العنب لمدة 20 يوما. أعيدت التجربة للمجاميع من 2-4 لزمان تعريض 60 و40 يوما لنفس الظروف. اظهرت النتائج تناقص معنوي ( $P \leq 0.05$ ) في قيم معاملات الدم اعلاه مع الزمن 20 يوما، اذ بينت ان المجموعة الثالثة زيادة معنوية مقارنة مع مجموعة السيطرة بينما المجموعة الثانية هي نقصان معنوي بالمفرنة مع مجموعة السيطرة. الزيادة في زمن التعريض ايضا اظهر نقصان معنوي في معاملات الدم لجميع الحالات لكن تأثير المادة المضافة مستخلص بذور العنب بين تأثيرا في تغيير الحالة الى تحسين او تقليل تأثير الجرعة الاشعاعية في المجموعة الرابعة لكل الحالات.

## 1. Introduction

Ionizing radiation is used in many fields of life with animals and efficiency of human. There are certain effects of radiation on live tissues, also passive impacts resulting from irradiation disease within birds as well as mammals when radiation pass through specific levels [1]. The hazards of the higher term impacts can rise by small doses like those used in treating cancer. Bereaved effects of gamma on accretion and survival were noticed as radiation scale and time raise of rainbow trout larvae [2].

The impacts of ionizing radiation on people can lead to variations in chemical equilibrium of tissue [3]. Hazardous waves have a power greater than the bonding strengths of many molecules, which can result in the separation of hemolytic bonds and the release of free electrons. First power sedimentation steps and molecular bond breaks occur at a time scale of 10-13 s [4].

Soft dose radiation (SDR) can yield stimulatory or adaptive responses, the ability to limit effects of subsidiary rise-dose radiation exposures. (incomplete sentence) This firstly change was shown by [5]. but later response was noted in unlike test system containing plant cell [6].

The effectiveness of ionizing wave based on the kind of radiation, its power, its intensity and the time of exposure to that wave. The level of ionizing wave based upon the wave kind, radiation power and intensity of wave as well as exposure time. Two mechanisms of radiation lead to biological effects, directly by isolating molecules based on their methods of ionization and excitation, and indirectly by producing radicals without chains in the form of hydrogen

peroxide in bodily fluids such as water [7]. For hundreds to thousands of meters, gamma wave photons can travel at the speed of light before running out of energy. They have the ability to puncture several materials., including organic components found in humans and animals [8].

### *1.1 Grape seeds oil*

The oil which consists of seed of grape was observed from 11.6 to 19.6 percentage relying on set and pubescent of grapes relative to the total weight involved in the crashing presses [9]. The fatty acid composition of grape seed oils is similarly influenced by puberty. Linoleic acid (66.76–73.61 % acid) and oleic acid are the primary fatty acids found in grape seed oil 17.8 to 6.5 percentage, palmitic acid 6.35 to 7.93 percentage and stearic acid 3.64 to 5.26 percentage, respectively [10]. With high ratios of PUSFA/SFA scaling from 2.80 - 3.11 and increasing ratios from 20.8 to 36.9 percentages, the polyunsaturated fatty acid content of oil was observed at Cabernet Sauvignon and Royal Rouge pomace ranging from 60.9 to 64.4 percentages [11]. The sum of the percentage of fat that isn't saturated numbers for higher than 0.86x10<sup>2</sup> percentage due to liquid, and also contains overall original fatty acids (EFA) [12]. EFA of Dietary was noticed to find neuronal membrane fluidity and directing the brain physiological roles [13]. Defect of EFA over childhood extensions development of brain and longevity speeds the de composition of brain jobs. Increased consumption of monounsaturated fatty acid meals reduced total cholesterol data by 10% and low-density lipoprotein data (LDLD) by 14%, while high-density lipoprotein data (HDL) remained unchanged, according to studies conducted on human subjects. [14]. Survey containing a higher number of sisters' tattletale that the group with minimum linoleic acid intake exhibited the maximum breast cancer incidence [15]. With regard to the grape diversity, increased provision, oil retrieval method, and level of cleaning, the fatty acid and antioxidant structures of red grape seed liquid, as well as other aspects of its nurturing and organic properties, most likely overacted. Most research has focused on the prospect of utilizing a liquid retrieval method based on supercritical CO<sub>2</sub> to identify the optimal red grape seed oil variety [16].

## **2. Materials and Methods**

The grape seed oil was extracted from the Mosul City local market and purified to remove contaminants. Using a crushing mechanism within an ice bath, 50 grams of grape seed with a volume of 250 cm<sup>3</sup> of ethanol was crushed to extract the oil. The concentration data for the seed was 95%. An electric field generator was used to agitate the mixture for an hour at room temperature. The filtration was performed using many layers of filter paper. The centrifuge of the filterable was obtained with a speed of 1000 revolutions per minute for (15 minutes) and then the top layers of the seed oil were selected. Since the atmospheric pressure plasma jet effect strongly on the fatty acid content in the food oil as was obtained by Soro et al [17] the refined oil of grape seed was exposed to the plume of the argon plasma jet. The atmospheric pressure plasma jet was generated by applying a high frequency 28 kHz high voltage 8Kv on the Argon gas flow in the dielectric tube. As a result of that a cold plasma plume was obtained with a length of few centimeters. This plume was directed to grapes seed oil for 6 min [17]. The animals as male chickens were used in this study which ranged between (2 to 3 months) of age and (1.5 to 2 kg) of weight. Healthy chickens were given from the Veterinary Department at Mosul University, where they were housed in plastic cages with metal covers. (100\*400\*400 m<sup>3</sup>). Applications were received to guarantee proper hygiene. In all groups, the control team , oil of grape seed, the gamma waves exposure, and oil of grape seed with Irradiation by gamma waves within humidity and temperature during the experiment were preserved at a limited value and replacing the mulch every week.

A mixture of nutrition includes corn, soybean, protein, dried milk, and wheat with percentages of 34,20,10,1,35 respectively. Also, water was administered during the experimentation time.

### 2.1 Cobalt-60 Properties:

A cobalt -60 (Co-60) has specific activity 1 MCi as original activity and the radiation energy of gamma ray 2.5 MeV [18] and for beta particles 0.097 MeV with exposure constant with 1.3 R. m<sup>2</sup>/Ci . h. The half-life of Co-60 equals to 5.27 year.

Dose rate (R/h) =  $\Gamma \cdot A / d^2$ . Where:  $\Gamma$ , gamma constant in R. m<sup>2</sup>/Ci. h

Specific activity in Ci/kg and d as distance per square meter m<sup>2</sup>.

After 20 years about the original specific activity then, the activity computed as following:

$$A = A_0 e^{-\lambda t} \quad \dots\dots\dots (1)$$

$$A_0 = 1 \text{ MCi, decay constant } \lambda = 0.693/t_{1/2} \quad \dots\dots (2)$$

After 20 years the specific activity can be computed as,

$$A = 1 \text{ MCi} \times \{e - (0.182) \text{ year}^{-1} \times 20 \text{ year}\}$$

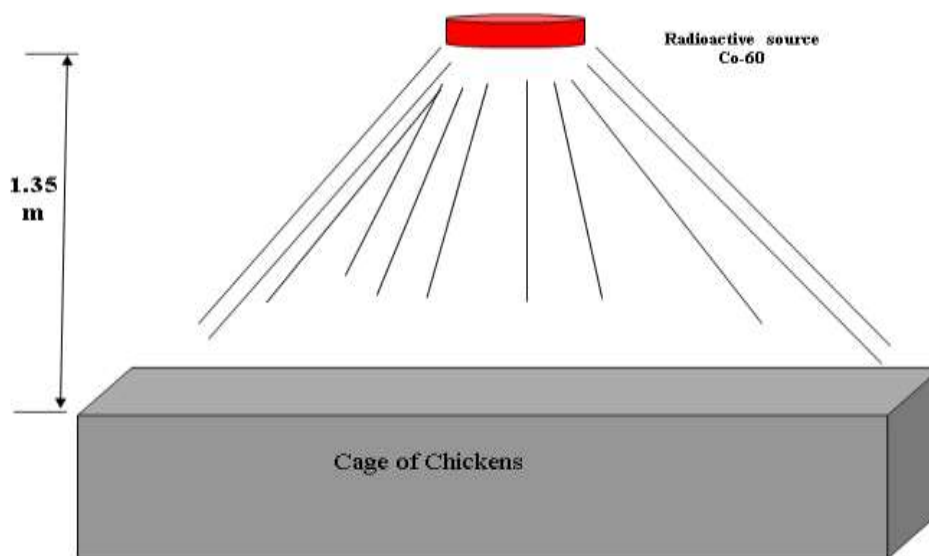
A = 0.072 MCi. The radioactive source put at 1.35 m from cages of chickens the exposure

$$\text{dose rate compute as following: } D(\text{R/h}) = \Gamma \times A / d^2 \quad \dots\dots\dots (3)$$

Where  $\Gamma$ . represents gamma constant = 1.3 h, Ci/ m, A = 0.072 Ci and d= 1.35 m  
 $D(\text{R/h}) = 1.3 \times 0.072 \times 10^{-6} / (1.35 \times 1.35)^2$  then  $D(\text{R/h}) = 0.0514 \times 10^{-6} \text{ R/h}$ , then Gray = 100 R, then, D as Dose rate in (Gy/ h) =  $0.0514 \times 10^{-6} \times 100$ . Then Dose rate = 5.1 micro-Gy/h = 5.0 micro-Gy/h.

### 2.2 Working System

The design includes Co-60 as radioactive material yielding gamma radiation on the chickens with (5 micro Gy) put on the top about 1.35 m from cages as plastic cages like the same method of [19] with the same case in this work to evolve distribution gamma-ray radiation with soft dose rate on all cages to limit and delete the attenuation dose of plastic cages from all measurements containing the chickens. The irradiation time by gamma was 7h/day for a period of 20-60 days. The used radiation dose is very low, and it is close to the environmental pollution dose. Therefore, it takes a long time to show the cumulative effect of the low dose, which is close to the dose used in similar research to this research in the field of environmental pollution. The irradiation of gamma achieved with seven hours' exposure daily for 20, 40 and 60 days. The rate dose was practically determined using the portable Geiger counter with R per hour and concealed on micro-Gy by dividing per 100 equals to 5 micro-Gy. Other applications for the evaluator include the X-ray wave dosage. This source is distinguished in addition to giving it the gamma rays, also it emits a beta craze during dissolution Beta released from the other environment as radiation kinds of addition to gamma ray. Figure 1 represents set up of work. The selection of this radioactive is due to medical and industrial uses. The selection of Co-60 as radioactive source returns to emit gamma rays with soft dose 5 micro-Gy/h and many uses in researchers are considered. It is essential for animal health in small quantities as a component of vitamin B12. Cobalt deficiency, which is very rare, may also be fatal, it leads to anemia [20-21].



**Figure 1:** Set up of working system

### 2.3 Design of Experiments:

In the experiment, forty male chickens were used, and they were divided as follows:

1. Ten male chickens in the primary group (control) have not been subjected to the gamma wave. Ten male chickens in the following group, referred to as the "second group," received an absorbing dose of 5 micro-Gy/lh over the course of 20 days, with a duration of seven hours per day.
2. The third group, called the Third group, consists of ten male hens and is fed 400 mg/kg wt of grape seed oil orally for 20 days.
3. The fourth group consists of ten male hens that were given an oral dose of 400 mg/kg wt of grape seed for 20 days and exposed to 5 micro-Gy/h for seven hours each day.
4. The male chicken numbers in groups (2-4) consist of thirty workers who work seven hours a day for 40 and 60 days. The desired dose of 5 micro-Gy per hour of absorbed radiation was the goal of the job design.

### 3. Statistical Analysis

ANOVA was used to expose all values as mean  $\pm$  S.D. In this project, one direction analysis after variance was used. Duncan helped to obtain the variables. At the 0.05 level, the involve significant state was computed [22].

#### 3.1 Hematological parameters analysis

Hematological studies were examined using several techniques. Red and white blood cells were discovered based on the results of the Neubauer hemocytometer system, which tested the hemoglobin ratio (Hb) with gm/100 ml in relation to the cyanmethemoglobin test. A micro-centrifuge apparatus was used to attain the packed cell volume [23]. In the final stages of the experiment, a capillary tube tip-pierced hole in the topical venous plexus was used to select blood samples from each group. Additionally, a vial containing a two percent ethylenediamine tetra acetic acid (EDTA) anticoagulant was used to select the blood.

### 4. Results

The present work results reported in Tables (1),(2) and (3) include the impact of gamma wave and deal with grape seed oil for three period 20 ,40 and 60 days on hematological factors in adult male chickens.

**Table 1:** Impact of gamma wave and grape seed oil for 20 days on hematological factors in adult male chickens

Factors of blood	primary Group 1 * mean $\pm$ S.D	Grape Seed oil only with 400 g/kg. wt Group 2 mean $\pm$ S.D	Irradiation with Gamma wave at 5 Gy per hour for 7 hour daily Group *mean $\pm$ S.D	Grape Seed oil with 400 g/kg.wt Irradiation with Gamma wave at 5 Gy per hour for 7 hour daily Group 4 *mean $\pm$ S.D	Ratio Percentage Group2 due to group 1	Ratio Percentage group3 due to group 1	Ratio Percentage Group4 due to group 1
RBC (count $\times 10^3$ /ml)	6117.8 **b $\pm$ 0.33	6517.39 **a $\pm$ 0.36	1096.29 **d $\pm$ 0.25	5011.75**c $\pm$ 0.29	106.53	17.91	81.92
WBC (count/ml)	*6126.8 b $\pm$ 1.47	*6452.29 a $\pm$ 1.21	*1077.91d $\pm$ 0.47	*4097.62c $\pm$ 0.44	105.31	17.59	66.86
Hb (g/100 cm <sup>3</sup> )	13.258 b $\pm$ 0.3 0	14.6 0 a $\pm$ 0.3 1	9.4 2d $\pm$ 0.33	12.69 c $\pm$ 0.45	107.54	70.94	95.71
PCV(%)	40.6625b $\pm$ 1.81	42.88 a $\pm$ 1.74	8.76 d $\pm$ 1.84	35.75 c $\pm$ 1.92	105.46	21.54	87.91

**Table 2:** Impact of gamma wave and grape seed oil only for forty days on hematological factors in adult male chickens

factors of blood	Primary Group 1 mean $\pm$ S.D	Grape seed oil only with 400 g/kg. wt Group 2 mean $\pm$ S.D	Irradiation with Gamma wave at 5 Gy per hour for 7 hour daily Group 3 mean $\pm$ S.D	Grape seed oil with 400 g/kg.wt + Irradiation with Gamma wave at 5 Gy per hour for 7 hour daily Group 4	Ratio percentage Group2 due to group 1	Ratio percentage group3 due to group 1	Ratio percentage Group4 due to group 1
RBC (count $\times 10^3$ /ml)	6117.8 a $\pm$ 0.33	5617.42b $\pm$ 0.26	727.76 d $\pm$ 0,22	4144.86 c $\pm$ 0,23	91.82	11,89	87.76
WBC (count/ml)	6126.8 a $\pm$ 1.47	6087 b $\pm$ 1.52	1007.76d $\pm$ 0.57	4123.16c $\pm$ 0.44	99.36	16.43	67.29
Hb (g/100 cm <sup>3</sup> )	13.258 a $\pm$ 0.35	13.4 2b $\pm$ 0.3 7	6.4 21d $\pm$ 0.31	10.49 c $\pm$ 0,49	101.22	48.43	79.12
PCV(%)	40.6625a $\pm$ 1.85	34.88b $\pm$ 0.79	16.54d $\pm$ 2.33	32.32 c $\pm$ 4.18	85.78	40.67	79.48

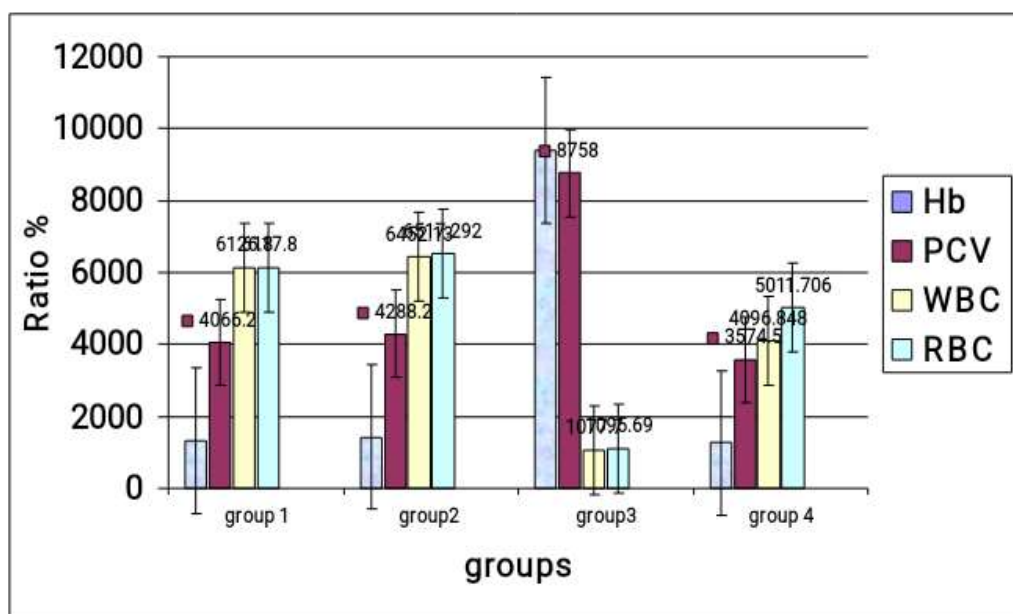
**Table 3:** Impact of gamma wave and grape seed oil for sixty days on hematological factors in adult male chickens

factors of blood	Primary Group 1 mean ± S.D	Grape seed oil only with 400 g/kg.wt Group 2 mean ± S.D	Irradiation with Gamma wave at 5 Gy per hour for 7 hour daily Group 3 mean ± S.D	Grape seed oil with 400 g/kg.wt + Irradiation with Gamma wave at 5 Gy per hour for 7 hour daily Group 4	Ratio percentage Group2 due to group 1	Ratio percentage group3 due to group 1	Ratio percentage Group4 due to group 1
RBC (count x10 <sup>3</sup> /ml)	6117.8 b±0.33	6517.39 a±0.36	1096.29 d± 0.31	5011.75c ±0.30	106.53	17.91	81.92
WBC (count/ml)	6126.8 b± 1.46	6311.29 a ±1.33	1011.91d±0.47	3901.51 2c ±0.44	103.02	16.51	63.67
Hb (g/100 cm <sup>3</sup> )	13.258 b ± 0.34	13.92a ±0.35	9.98 2d±0.37	11.96 c ±0.49	104.99	75.27	90.20
PCV(%)	40.6625b ±1.76	41.34 a ±1.75	7..89 d±1.84	34.94 c ±1.63	101.67	19.40	85.93

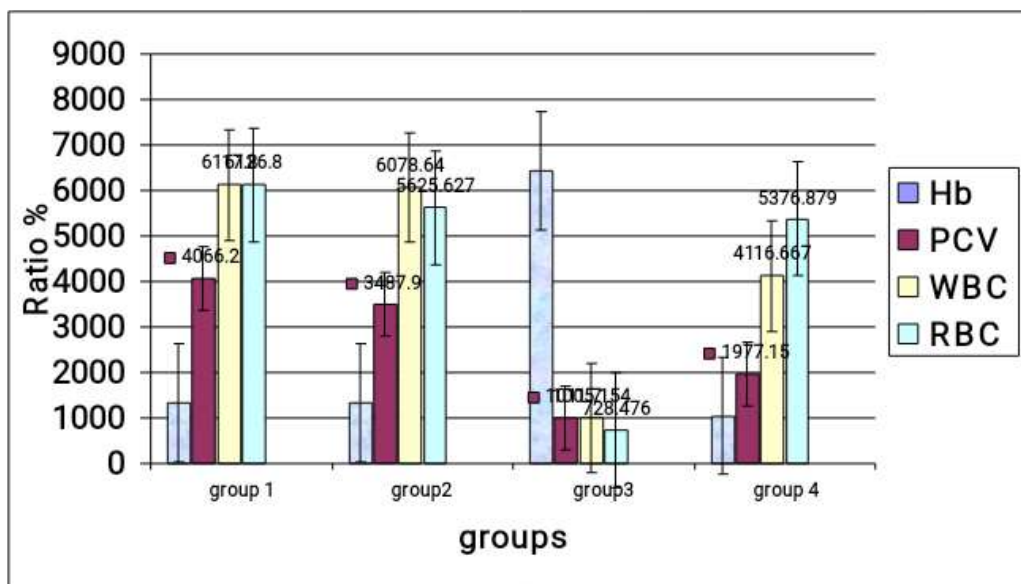
\*Mean±S.D \*\* P=0.05 \*\*\* n=10 \*\*\*\* T= 40

\*Mean and standard deviation reported in tables (1) and (2) and (3);\*\*Level of significance (P≤0.05) as a,b,c and d between groups; \*\*\*Number of animals per group;\*\*\*\*Total number of animals used.

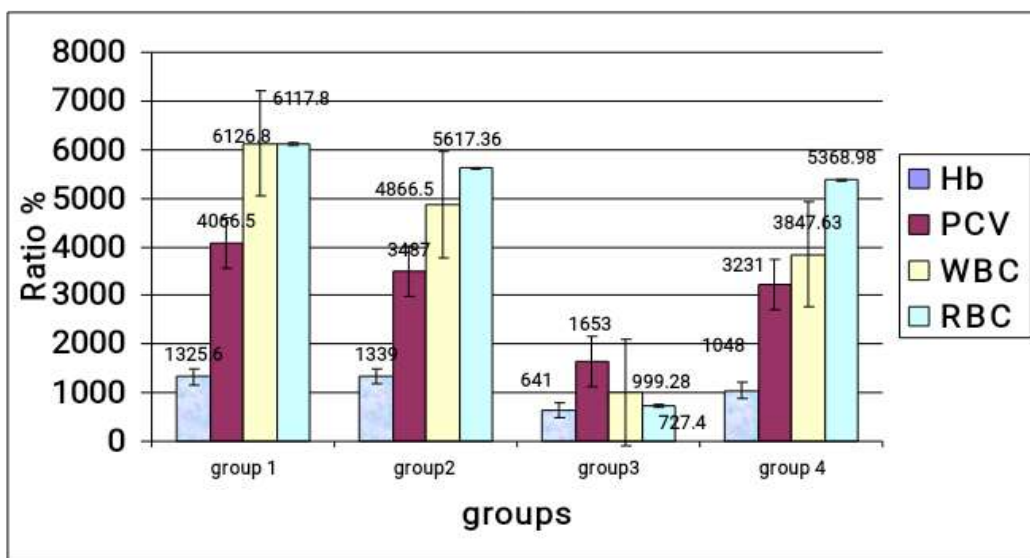
The present work results reported in Figures (2) ,(3) and (4) include the impact of gamma wave irradiation and deal with grape seed oil for 20 ,40 and 60 days on hematological factors in adult male chickens.



**Figure 2:** Ratio % of Hb,PCV,WBC andRBC for Groups(2-4) due to group 1 for irradiation time at 20 days on male chickens



**Figure 3:** Ratio % of Hb,PCV,WBC andRBC for Groups(2-4) due to group 1 for irradiation time 40 days on male chickens.



**Figure 4:** Ratio % of Hb,PCV,WBC andRBC for Groups(2-4) due to group 1 for irradiation time 60 days on male chickens.

**5. Discussion:**

The radioactive sources have many applications in medicine and industry. It was employed to found radiometric calibration of x-rat packed film practically and agreement by theoretical outcomes [24].

The reason of using Co-60 as radioactive source is due to the decays with soft gamma ray, hole body exposure to Co-60 can rise the risks of cancer. Usually, cobalt that is ingested is clips in feces; however, a soft dose is absorbed with the kidneys, liver, and bones. Increasing exposure to radiation and increasing absorption of it for along time may lead to the occurrence of cancer because of increased internal sedimentation of energy in the body parts, especially sensitive ones. This case of absorption can lead to cancer from inside exposure to gamma ray. Large measure respect to Co-60 as industrial source causes outside irradiation large sufficient to lead to skin burns, great radiation. In other hand, using Co-60 as diagnostic and therapy in nuclear medicine limit the selected in this work cages. In this work, the same method of [19,25]



was selected with the same case of the distance between cages respect to radioactive source to evolve distribution gamma ray radiation with soft dose rate on all cages to limit and delete the attenuation dose of plastic cages from all measurements containing the chickens.

The reason for chaining males instead of females in this study is that females suffer from the menstrual cycle, as well as the male role in the greater impact on changing the economic situation in case it is affected by radiation.

From Tables (1-3) the effect of the gamma radiation on different groups can be found, where significant increase in group 2 is noticed, but also significant decrease can be noticed in groups 3 and 4 compared with group 1 due to the hematological parameters Hb, PCV, WBC and RBC.

From group 4 it can be found that there is, for all periods of time exposure time a significant decreasing in hematological parameters Hb, PCV, WBC and RBC compared with control group, rather than we notice the effect of grape seed oil on the group 2 where significant increases can be found in the value of some hematological parameters compared with groups 3 and 4 for all time exposure (20-60 days) but obtained significant decreases with the same time of exposure compared with the control group (1).

From figures (1-3) it is found that the ratio fixed on the forms indicate the percentage ratio between some blood factors express from the idea of work in all tables.

These results agree with [26] we noticed that the Ratio% of the hematological parameters WBC and RBC have significant decrease with increase in period of exposure time until to 42 days rather than decrease ratio between heterophil to lymphocyte (H/L) and ratio between heterophil to lymphocyte (H/L). Ionizing radiation leads quickly to the generation of products that dissolve in water, which includes many free radicals to be subsequently released into the radioactive cells from some radioactive sources. Through the ionizing irradiative effects that depend on the use of hematopoietic cells, they do not directly affect bone marrow generation by decreasing it, in addition to the planned cell demise, which, depending on where in the body a cell is located, is one of its properties. [27]. Moreover, administering an oral dose of 400 mg/kg.wt of grape seed oil for the same duration of 20, 40, and 60 days after irradiating with gamma waves for seven hours a day at a dose of five micro Gy thereafter. It was found that there was a significant change in the decrease in blood parameters section in team 2, 3 and 4 compared with the control team 1. Moreover, in the three cases of exposure to radiation 20, 40 and 60 days the data in team 3 and 4 were lower than the data in the team 2 in the case of the percentage.

Finally the researcher also agree with our study were noticed decrease the hematological parameters in groups of adult rats without treatment by black seed oil compare with rats administrated black seed oil [28].

## 6. Conclusions:

The study's stated results indicate that grape seed oil, an antioxidant material with elevated vitamin C concentration, has a substantial effect in reducing the size of free radical formation. With increased protection for prolonged exposure, this material can be used as a repair solution for the average of toxic data created as a result of thin-dose rate. It could be able to reduce the ratio of oxidative stress data acquired from thin-dose gamma radiation exposure. These investigations suggest that oral grape seed oil may reduce oxidative stress.

Because of the effects of radiation at thin dose gamma waves with long-term dose scale for 20–60 days on several hematological factors, including the PCV, Hb, WBCs, and RBCs of cock chicken, the building housing poultry must be located far from the area of nuclear power laboratories or keep radioactive materials. As a result, poultry should not be kept in buildings

that are close to these locations. to avoid destroying the double strand of DNA that results in genomic mutation, generating free radicals like hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), and more.

## 7. Acknowledgements

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## 8. Disclosure and conflict of interest

There are no conflict of interest

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