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To cite this article: Su'adod Osama Al-khateeb *et al* 2021 *J. Phys.: Conf. Ser.* **1963** 012083

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Study of the effect of Ultraviolet UV-induced oxidative stress in male white rats (*Rattus rattus*)

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Abstract The present study was designed to investigate some of the physiological effects in the blood of white male rats exposed to oxidative stress induced by hydrogen peroxide in drinking water at a concentration of 0.5% for a period of 50 days and to compare it with the group exposed to ultraviolet rays at a rate of 6 watts per candle for a period of 50 days

First:- The results showed a significant increase ($P \leq 0.05$) in the group of rats exposed to radiation in the group of ultraviolet rays (18 watts) UV3 and hydrogen peroxide both when compared with the control group. In radical Peroxy nitrite level and in total white blood cell count and for both Ly % and GR%. The results also showed a significant increase ($P \leq 0.05$) in the level of glucose.

Second:- There was a significant decrease at a significant level ($P \leq 0.05$) in the level of glutathione in the group of rats exposed to UV3 radiation and the hydrogen peroxide group alike when compared with the control group, with a significant decrease at a significant level of ($P \leq 0.05$) in the activity of the enzyme glutathione. Glutathione peroxidase and a significant decrease ($P \leq 0.05$) in the volume of packed blood cells, the hemoglobin value, the red blood cell count, and the platelet value, with a significant decrease of MO. %

Third:- The occurrence of signs of blurred vision in the eyes of the ultraviolet rays group with a strength (3 candles) in addition to the darkening of the tail in a clear and striking way and the lack of movement and activity compared to the hydrogen peroxide group and the rays group, the strength of one candle.

Key words: Ultraviolet UV, Oxidative Stress, Blood Parameters, biophysics, H₂O₂

1.Introduction

Ultraviolet (UV) rays are rays whose photons possess high energy sufficient for the transmission of electrons between the orbits of the atoms of the molecules that make up the material that have been exposed to the radiation [1]. Harmful to human connective tissue cells, leading to premature aging, deep wrinkles, and various forms of skin cancer Between (100-400) nm [2].

The types of UV rays differ depending on the amount of their energy. High-energy UV rays are "ionizing" rays that can damage the DNA in cells; Which may lead to cancer. But even the highest types of UV rays do not have sufficient energy to penetrate deeply into the body, which limits their effect mainly on the skin [3].

There are symptoms that may appear upon exposure to radiation, as the severity of symptoms depends on the total radiation dose and the dose rate. He indicated [4] that the risks arising from human exposure to ionizing radiation may be physical risks, genetic risks, intestinal infections, central nervous system injuries. Studies and experiments on laboratory animals have shown that if the dose is increased to a high level, some symptoms appear that indicate some damage to the central nervous system [5].



It may lead to an increase in the occurrence of point mutations (point mutation) in the DNA (deoxyribonucleic acid) and at the chromosomal level and according to the severity of the dose, and these effects are transmitted over generations and cause deformities and genetic diseases and increase the rate of miscarriage and death in the newborns. The chromosomal changes that can be caused by radiation are either numerical or structural changes [6].

Oxidative stress is defined as an unstable state between the production of active oxygen species and the ability of biological systems to resist their harmful effects [7], as the concentration of many compounds and many oxidizing substances in cells increases, including the negative super oxide anion radical ($O^{\cdot-}$), And which is considered one of the most dangerous and toxic types of active oxygen, hydrogen peroxide (H_2O_2) and hydroxyl radical (OH^{\cdot}) [8].

The danger resulting from free radicals lies in the damage caused when they interact with the most important components of the cell, for example, DNA or the cell membrane, which leads to its destruction and its inability to perform its functions as required [9].

As the active oxygen varieties, for example, hydroxyl radicals, superoxide anion radicals, and effective oxygen species that do not have roots, such as hydrogen peroxide (H_2O_2), which are agents that attack unsaturated fatty acids of cell coatings, they are responsible. Peroxidation in living systems and lipid peroxidation is strongly involved with aging and cancers [10], as well as its role in the occurrence of mutations that may lead to the occurrence of carcinomas [11].

Ultraviolet (UV) rays are non-ionizing rays whose photons possess high energy sufficient for electrons to move between the orbits of the atoms of the molecules that make up the irradiated material, thus moving these atoms from the original state to the excited state [12]. [13].

As a result of the modern uses of radiation, it has become a very dangerous and continuous challenge in the biological environment, and this is what made it raise the concern of the human being on the globe, so many researchers around the world have conducted many scientific studies and research to find out the effect of radiation pollution to find out the negative effects on the people who are exposed. To different radiation sources. According to these many effects of radiation and for all that, this study was designed, which aims at the following:

Comparison of H_2O_2 induced oxidative stress with ultraviolet ray induced stress in male white rats (*Rattus rattus*). The following tests were performed and parameters measured:

1. To study the effect of induced oxidative stress in Ultraviolet ray and compare it with induced stress with H_2O_2 .
2. Determination of Serum Glutathione (GSH) and Glutathione peroxidase and the level of Malondialdehyde (MDA) by measuring the activity of the enzyme glutathione peroxidase and the concentration of peroxy nitrite in the blood serum.
3. Hematological test such as estimation of (Hb and PCV volumes), Total count of red blood corpuscles, Total count of white blood cells. (Blood Platelets count)

2. Materials and Methods

2.1. Studies Animals

Male white rats (Sprague Dawley) obtained from Baghdad used medical laboratories. The weights of the animals used ranged between (300-200) g and their ages ranged between (2-4) months. She was placed in aluminum cages with dimensions (45x35x25). The cages were kept clean and sterilized with 70% ethanol disinfectant twice a week. And it was left to acclimatize in the small animal house that we created in a simple way for the Department of Life Physics in the College of Applied Sciences/Heet-Anbar University under the appropriate breeding conditions of a temperature of (22 ± 2) m and the duration of lighting was (10) hours of light and (14) hours of darkness) throughout the breeding period. And the study, with its supply of water and feed [10].

2.2. The experience of studying

In this study, 20 white male animals were used. They were randomly divided into four groups. Each group included 5 animals and their weights ranged between 250 gram-300 grams, and their

ages ranged between 2-4 months, and with similar weights, as they were treated with different treatments throughout the 50-year experiment. Days and as follows:

The first group:- (control group) This group was treated with 1 ml of distilled water, and this group was given regular drinking water for 50 days.

The second group:- This group was given regular drinking water containing hydrogen peroxide at a concentration of (0.5%) for 50 days. With the replacement of (0.5%) hydrogen peroxide every 48 hours to be effective.

The third group:- was exposed to ultraviolet rays with a power of three candles, ie, 18 watts. Since each candle was 6 watts, for 50 days, and at an amount of four hours per day.

The fourth group:- I exposed to ultraviolet rays a 6-watt candle for 50 days and an amount of four hours a day.

2.3.Obtaining blood samples:

After 50 days, which represents the end of the experiment, the animals were starved for 24 hours and then anesthetized by placing the rats in a glass container with an airtight cover containing a cotton saturated with chloroform for a few seconds and then drawing the blood with the heart stab of the anesthetic animal with a 5ml plastic syringe. A section of blood in tubes containing the EDTA anticoagulant for hematological tests. As for the other section, it was placed in plastic tubes free of anticoagulants to obtain blood serum from them, and to conduct biochemical, enzymatic and oxidation tests and their antioxidants in the serum.

2.4.Blood tests:-

2.4.1.Determination of Serum Lipid Peroxides: was used. According to this method, the level of Malondialdehyde (MDA), which is one of the main products of the lipid peroxidation process, was measured[14].

2.4.2.Measurement of serum glutathione (GSH) Level: serum (GSH) was determined by the modified method of [15], GSH product the colored which read the absorbance at 412 nm.

2.4.3.Determination of the concentration of peroxy nitrite in serum: The concentration of radical Peroxy nitrite was determined using the modified method [16]. The absorption intensity is measured at 412 nm.

2.4.4.Determination of serum glutathione peroxidase efficacy: the determination was made according to the method [17].

2.4.5.Compacted Cell Volume (PCV) measurement: Measuring the volume of compacted cells using capillary tubes and a blood centrifuge at a speed (12,000 rpm) for five minutes and the results were determined by the device's ruler [18]

2.4.6.Estimate the amount of hemoglobin: by dividing the volume of compressed blood cells by 3.3 by describing that hemoglobin represents 1/3 the volume of the red blood cell [19]. According to the following law:- $Hb = \frac{PCV(\text{value})}{3.3} = g/l$. This is for the healthy control group. As for the other groups

subjected to oxidative stress and for the aqueous extract, the Sahli method was used because they were exposed to oxidative stress. It is better not to calculate the above-mentioned equation for an increase in accuracy.

2.4.7.Calculating the total number of white blood cells: According to the total number of white blood cells / cubic millimeter of blood, using the Haemocytometer slide and diluting the blood with Turk's solution. [20]

2.4.8.Total red blood cells count: The number of red blood cells (red blood cells) in healthy and infected animals was calculated by using a counting slide or the so-called Hemocytometer, to deduce the number of blood cells depending on the dilution factor and the area and volume of the chamber inside which the cells were counted[21]

2.4.9.Measurement of the platelet rate: The platelet rate was measured using a platelet solution, and the appropriate counting slide was used according to the method [22]

2.4.10.Serum glucose concentration was determined: enzymatically using the British Plasmatec Kit [23].

2.5. Statistical analysis

The results were analyzed statistically by the SPSS statistical program. The data were statistically analyzed according to the analysis of variance (ANOVA) test using the statistical program. The arithmetic averages were compared using the Duncan Multiple Range test [24] and under the level of significance ($P \leq 0.05$)

3. Results and Discussion

3.1. Determination of Serum Lipid Peroxides

The effect of different treatments on the level of malondialdehyde (MDA) in the blood of male white rats

Fig.1 shows a significant increase ($P \leq 0.05$) in the group of rats exposed to both UV3 and hydrogen peroxide when compared with the control group.

The reason for this is that oxidative stress results in the generation of free radicals that attack the unsaturated fats present in the structure of cellular membranes, thus causing the occurrence of lipid peroxidation represented by the (MDA), which works to break down the cell membrane [11]

The high concentration of (MDA) leads to rapid consumption of defense antioxidative systems, which leads to tissue damage [25].

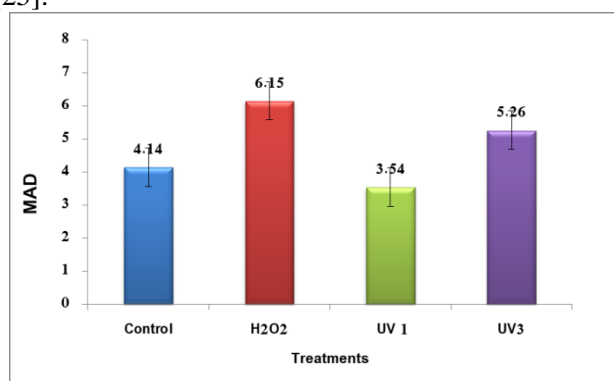


Figure 1 The effect of treatment with ultraviolet rays (18 watts) and (6 watts) for a period of (50 days) the level of concentration of malondialdehyde (MDA) in the blood serum of male rats

- The number of animals is six for each group.
- Values are expressed as the mean \pm the standard deviation.
- The different letters mean that there are significant differences at the level of significance ($P \leq 0.05$)

3.2. Determination of Serum Glutathione (GSH)

The results in Fig. 2 showed that there was a significant decrease at a significant level ($P \leq 0.05$) in the level of glutathione in the group of rats exposed to UV3 radiation and the hydrogen peroxide group alike when compared with the control group.

The reason may be attributed to the conversion of glutathione from the active form to the inactive form of disulfur, and the sulfur group in the synthesis of glutathione is a good reducing factor that blows the hydrogen atom easily, due to the weak bond between sulfur and hydrogen (SH) and the strength of the bond between carbon and hydrogen (CH) in free radicals. By protecting cell membranes from the risk of free radicals [26]

In addition, there is a shortage of the raw materials necessary to build it during oxidative stress, including NADPH resulting from the five-phosphate pathway, which is the catalyst for the action of the enzyme glutathione reductase, which works to restore glutathione from the inactive form of bisulfur to its active form [27].

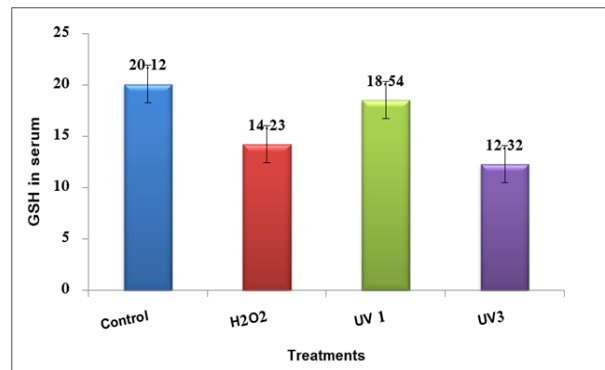


Figure 2 Effect of UV rays (18 Watt) and (6 Watt) for (50 days) of Serum Glutathione (GSH) Blood of Male Rats

- The number of animals is six for each group.
- Values are expressed as the mean \pm the standard deviation.
- The different letters mean that there are significant differences at the level of significance ($P \leq 0.05$)

3.3. Determination of Serum Peroxy nitrite radical

The effect of different treatments on ONOO⁻ concentration in blood serum

The results in Fig. 3 showed a significant increase ($P \leq 0.05$) in the level of radical Peroxy nitrite in the group of rats exposed to radiation, both UV3 and the hydrogen peroxide group when compared with the control group.

The reason for the high concentration of nitrite peroxide, ONOO⁻, is significantly increased due to the oxidative stress resulting from it. The free oxygen radical is generated, which remains active and searches for a single electron to bind with it and settle down, leading to the association of the single oxygen root with nitric oxide forming the peroxide nitrite root ONOO⁻ in the blood plasma and its percentage increases. In skeletal muscles subject to oxidative damage [28]

Also, the decrease in the concentrations of enzymatic antioxidants in the blood plasma, especially catalase and superoxide dismutase (SOD) enzymes in exposure to oxidative stress leads to an increase in the rates of root formation (ONOO) [10]. As the increased concentration of ONOO and other types of free radicals in the case of exposure to H₂O₂ causes inflammatory states that lead to an increase in the release of cytokines, and the latter works to inhibit the activity of insulin in cells and may lead to the development of diabetes [29].

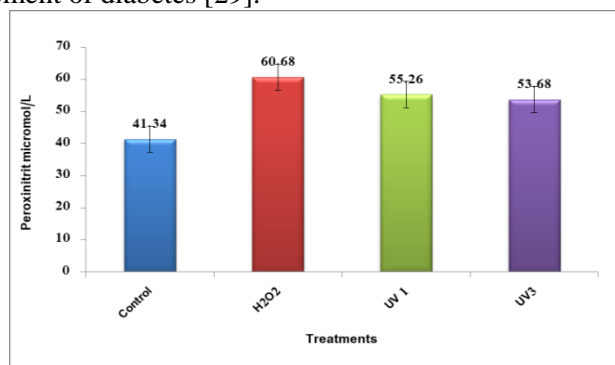


Figure 3 Effect of treatment with ultraviolet rays (18 watts) and (6 watts) for a period of (50 days) on radical Peroxy nitrite blood of male rats

- The number of animals is six for each group.
- Values are expressed as the mean \pm the standard deviation.
- The different letters mean that there are significant differences at the level of significance ($P \leq 0.05$)

3.4. Determination of Serum Glutathione peroxidase

The effect of different parameters on the level of serum glutathione peroxidase concentration

The results in Fig.4 showed a significant decrease at ($P \leq 0.05$) level in the activity of Glutathione peroxidase enzyme in the blood serum of the group of rats exposed to radiation, UV3 and the hydrogen peroxide group with H_2O_2 both when compared with the control group.

Many researchers have attributed the reason to an increase in oxidative stress, which leads to a decrease in the effectiveness of the anti-oxidant enzymes, or it may be due to a decrease in the level of glutathione, as indicated above, which is the main substance for the enzyme glutathione peroxidase [30]. And that oxidative stress leads to lipid peroxidation and the generation of free radicals, and since the enzyme glutathione peroxidase defends cell membranes and its components from oxidative damage by donating electron, it leads to its depletion and low rate [10]

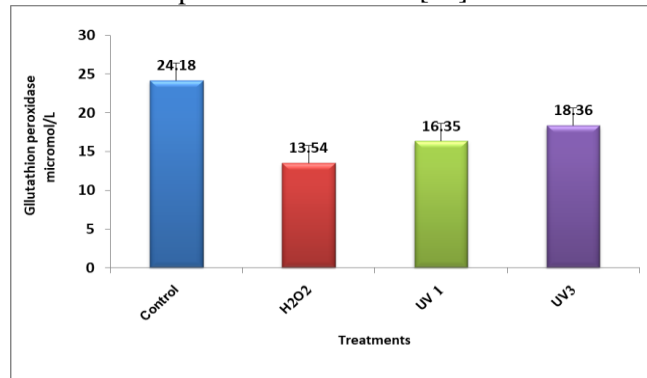


Figure 4 The effect of (18 watt) and (6 watt) ultraviolet rays for a period of (50 days) on Serum Glutathione peroxidase on the blood of male rats

- The number of animals is six for each group.
- Values are expressed as the mean \pm the standard deviation.
- The different letters mean that there are significant differences at the level of significance ($P \leq 0.05$)

3.5.Hematological Tests:-

The effect of different parameters on the percentage of accumulated blood cell volume and hemoglobin concentration in the blood of healthy white male rats with oxidative stress:

The results in Figs. 5 and 6 showed a significant decrease ($P \leq 0.05$) in the volume of agglutinous blood cells and the hemoglobin value in the blood serum of white rats treated with hydrogen peroxide, as well as the ultraviolet treatment by (18 watts) when compared with the healthy control group.

It also showed a significant decrease ($P \leq 0.05$) in the group treated with one wax by (6 watts), when compared with the group treated with hydrogen peroxide, as well as when compared to the group treated with three candles, and it was noticed that there was a significant increase in the two treatments of hydrogen peroxide compared with the H_2O_2 peroxide group. .

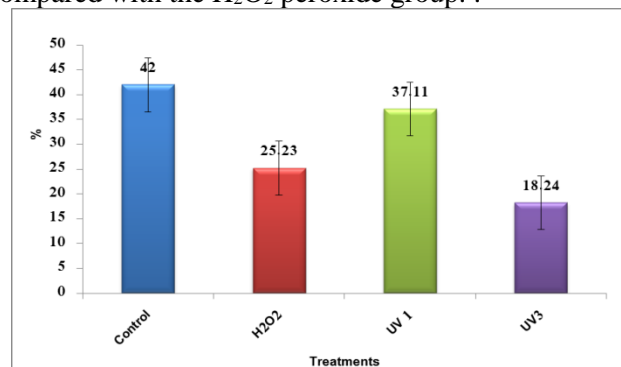


Figure 5 The effect of treatment with ultraviolet rays (18 watts) and (6 watts) for a period of (50 days) on the number of red blood cells in the blood of male rats

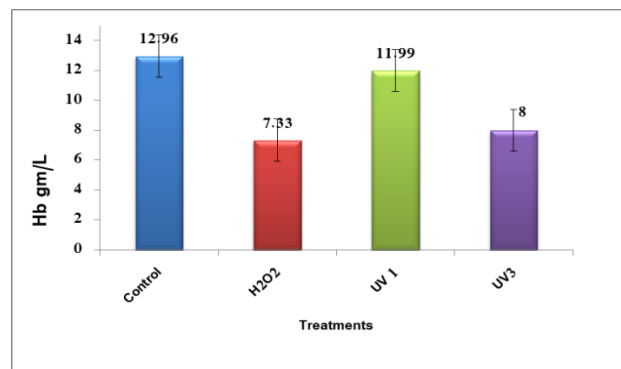


Figure 6 The effect of treatment with ultraviolet rays (18 watts) and (6 watts) for a period of (50 days) on the number of red blood cells in the blood of male rats

- The number of animals is six for each group.
- Values are expressed as the mean \pm the standard deviation.
- The different letters mean that there are significant differences at the level of significance ($P \leq 0.05$)

The results of the present study showed that the oxidative stress caused by hydrogen peroxide in rats led to a decrease in hemoglobin quantity and compact cells volume compared with the control group.

This decrease is due to the active oxygen varieties that result in oxidation of sulfur groups (-SH) in the peptide chain of hemoglobin protein, as well as the free radicals attacking the red blood cell membranes, destroying these membranes and oxidizing the fats that make up these membranes [32].

As the free radicals formed as a result of oxidative stress induced hydrogen peroxide leads to lipid peroxidation in the cell membranes of red blood cells, leading to the decomposition of unsaturated fatty acids by a series of reactions to form malondaldehyde MDA that reflects the state of increased generation of free radicals and oxidative stress [33], and These oxygen free radicals lead to damage to hemoglobin, and the formation of deposits inside the red blood cell called Heinz Body, and these bodies lead to the degradation of red blood cells [27].

Or, the reason may be attributed to the degeneration of red blood cells and thus the decrease in hemoglobin concentration along with elevated liver enzymes [10], which may be the cause of the short life of the red blood cells, which may be due to the increased chances of being attacked and eaten by phagocytes and endothelial tissues [34].

3.6.Red Blood Cells Count

3.6.1.Total number of red blood cells:

The results in Fig. 7 showed that there was a significant decrease in the number of red blood cells in the group treated with H₂O₂ as well as the group treated with UV rays (18 watts), compared with the control group. Compared to the treatment group (6 W) with the peroxide group, and the control compared to the control.

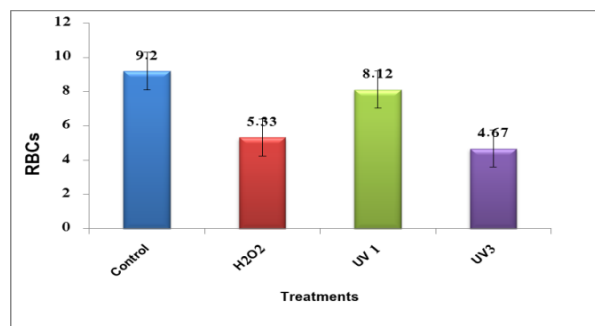


Figure 7 The effect of treatment with ultraviolet rays (18 watts) and (6 watts) for a period of (50 days) on the number of red blood cells in the blood of male rats

- The number of animals is six for each group.

- Values are expressed as the mean \pm the standard deviation.
- The different letters mean that there are significant differences at the level of significance ($P \leq 0.05$)

Exposure of red blood cells to hydrogen peroxide has led to negative effects on them, such as breaking them down due to oxidative damage, which is usually due to the effect of free radicals that are produced in large numbers, which affects the membrane of these cells and oxidizes the fats that form them, and then increased lipid peroxidation and a large depletion of oxidizing substances. It causes a decrease in the elasticity of the membrane and thus its ability to change shape, which leads to a shortening of its life. The reason for the decrease in hemoglobin may also be due to the lack of need for it due to the lack of oxygen demand due to the low metabolism rate [11], and the reason for the decrease in the percentage of the volume of compressed blood cells is due to the small size of the red blood cells and the breakdown of the membranes of the large red cells or the short duration of their survival This variable is considered a helpful factor in diagnosing anemia [35].

And it can be increased as a result of stimulating or activating the $\text{Na}^+ - \text{H}^+$ pump by its effect on some hormones through its effect on the hormone aldosterone, which leads to an increase in the entry of the Na^+ ion into the cell and the exit of the hydrogen ion H^+ outside the cell [27], and it may return to the iron percentage. Inside the animal's body, iron is one of the most important compounds of hemoglobin

3.6.2. To study the effect of different parameters on the total and differential number of white blood cells:

Fig. 8 shows that there was a significant increase ($P \leq 0.05$) in the total number of white blood cells and for each of Ly%, as well as GR% in the UV group (18 watts) and the hydrogen peroxide group compared with the control groups. Noting the presence of a significant decrease in MO.%

The increase in the total number of white blood cells in the blood of rats may be due to the hepatotoxicity caused by hydrogen peroxide and radiation, which leads to inflammatory conditions, which causes the white blood cells to be found at high levels in the blood greater than their level in the usual cases as is the case in infections Other.

However, this increase was accompanied by an increase in the differential number of types of leukocytes in general, as there is a special increase in the number of lymphocytes, which indicates that the weakness of the animal as a result of oxidative harm has stimulated the immune system to form defense cells, which represented an increase in the total number of cells White blood in general and the special increase in lymphocytes in particular [38] The decrease in the protein content in the body in stressed animals may cause a decrease in insulin and thus an increase in sugar in the body fluids, which constitutes a good medium for the growth of microorganisms, which may lead to an increase in the numbers of defense cells, [27] And that the prominence of these cells was in the increase in cells from the lymphoid types and then in their elevation in the peripheral blood of animals [11]

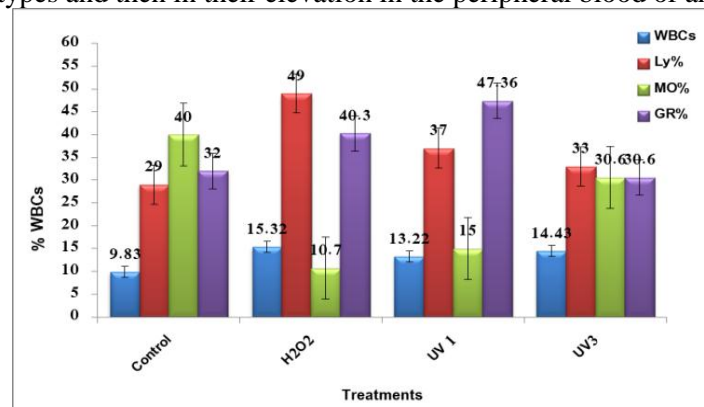


Figure 8 The effect of treatment with ultraviolet rays (18 watts) and (6 watts) for a period of (50 days) on the total and differential number of white blood cells

- The number of animals is six for each group.
- Values are expressed as the mean \pm the standard deviation.

- The different letters mean that there are significant differences at the level of significance ($P \leq 0.05$)

3.6.3. Total number of platelets:

The results in Fig. 9 showed a significant decrease ($P \leq 0.05$) in the value of blood platelets in the blood serum of rats treated with hydrogen peroxide as well as the UV working group when compared with the healthy control group.

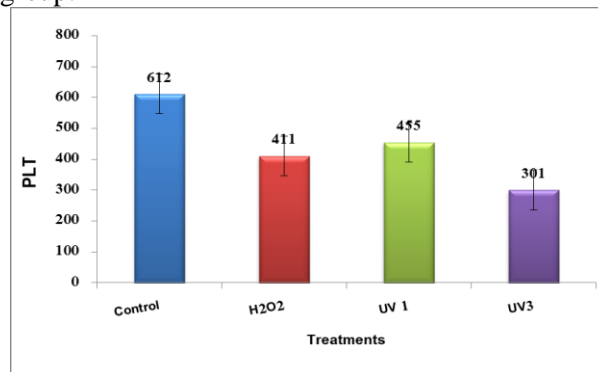


Figure 9 The effect of (18 watts) and (6 watts) ultraviolet radiation for a period of (50 days) on the total number of platelets in the blood of male rats

- The number of animals is six for each group.
- Values are expressed as the mean \pm the standard deviation.
- The different letters mean that there are significant differences at the level of significance ($P \leq 0.05$)

Decreased blood platelets to complications of diabetes, as it was found in cases of diabetes, an increase in the generation of active oxygen varieties and the occurrence of the so-called oxidative stress, which is the difference in the balance between the concentration of the active oxygen varieties and the antioxidants present in the body.

The results shown in Fig. 10 showed a significant increase ($P \leq 0.05$) in the glucose level of animals treated with hydrogen peroxide as well as the group working with ultraviolet rays compared with the control group.

3.7. level of sugars concentration

The high level of sugars in the blood of male rats may be due to an increase in the concentration of certain hormones, such as adrenaline, which stimulates the process of (glycogenesis) due to an imbalance in the secretion of the insulin hormone from beta cells in the pancreatic gland due to the increase in the generation of different active free radicals and this leads to an inability The cells take up glucose and thus stimulate the process of glycogen formation and degradation [27]

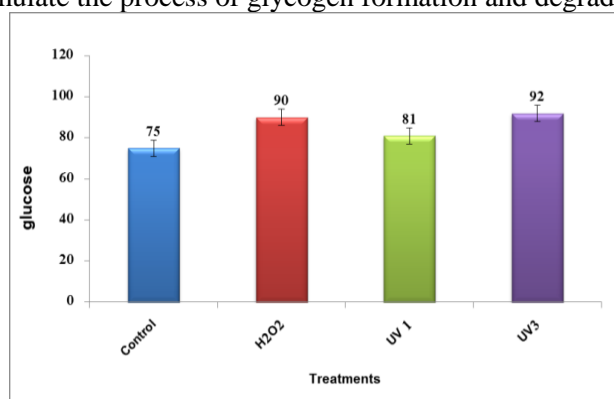


Figure 10 The effect of treatment with ultraviolet rays (18 watts) and (6 watts) for (50 days) on the level of glucose in the blood serum of male rats

- The number of animals is six for each group.
- Values are expressed as the mean \pm the standard deviation.
- The different letters mean that there are significant differences at the level of significance ($P \leq 0.05$)

4. Conclusions:

It was found that the effect of ultraviolet rays had a significant effect on inducing oxidative stress compared to hydrogen peroxide

5.Recommendations: -

Study of oxidative stress in various types of radiation.

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