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RESEARCH PAPER

In-vivo effects of Green laser radiation on hematological parameters of albino rats using direct exposure methods.

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A B S T R A C T:

Laser beam has a thermal effect on the exposed body through oscillation of molecules. Rate of the vibrations depend on the time of exposure, intensity of the laser beam, the quantity of the absorbed dose, and the laser wavelength. Impacts of green laser beam on the hematological parameters of Albino Rats investigated in an in-vivo irradiation process. Green laser (200mW, 532nm) was used as a source of non-ionizing radiation to irradiate albino rats, the exposure carried out to heart position (in-vivo) of the rats directly. Fifteen female rats participated. Main blood components: white blood cells (WBC), red blood cells (RBC), and platelet (PLT) counts were evaluated using a direct exposure method for low and high radiation doses. High doses of laser beam affected the WBCs and PLTs significantly (P-value < 0.05), and the low doses were not significant (P-value > 0.05). Laser beam radiation affected on the main blood components at a limited time of exposure. Optimum radiation doses that had high effectiveness on blood component density depended on the type of blood components. As well as, high impacts (high significant) were on the density of PLT not RBCs.

KEY WORDS: Rat, hematological parameters, green laser, nonionizing radiation, blood components.
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1.INTRODUCTION:

Ionized radiation has effects on biochemical and hematological parameters on the basic of ionization of the blood molecules (Ismail and Abdulla, 2021), and the non-ionizing radiation is not make an ionizing of molecules of the blood (oscillations is the essentials of it). Laser source is considered as a non-ionized radiation. It was used widely in medicine regarded to tissue type (exposed organic types). Laser beam has a thermal effect on the exposed body through oscillation of molecules (Thaihammer et al. 2003). Rate of the vibrations depend on the time of exposure, intensity of the laser beam, the quantity of the absorbed dose, and the laser wavelength. Wavelength dependent on the interaction mechanism deviated into two forms: Photochemical interaction and Photo thermal interaction.

At low laser intensities, irradiation of cells at certain wavelength can activate some of the native components in bio substance (biochemical reactions). The term of thermal interaction refers a large group of interaction type, where the increase in local temperature is the significant parameter change (Markolf 1996; vanenko & Hering 1998). Health risk of laser comes from producing an evaporation of the cell's water (breaks down and destabilizes the molecules) for the exposed tissue, and then producing a damage of the cells. (Farhad et a., 2011).

Throughout an incident on a tissue, some basic physical phenomena can occur: reflection and refraction, absorption, scattering and transmission (Markolf 1996; Kutsch 1993). The relative and absolute magnitudes of these phenomena depend on the wavelength of the laser light and the physical properties of the tissue. Investigations of the effects of the laser on hematocrit (packed cell

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volume) (PCV), and the viscosity of red blood cells have been done for in-vitro studies. (Zahra,a 2014). Impacts of the laser beam on the blood parameters such as Erythrocyte Sedimentation Rate (ESR), and blood components considered as an interesting subject for medical therapy (Raad Sh. Alnayli et al., 2017). The effects of low power violet laser irradiation on human blood rheological factors such as mean red blood cell volume (MCV) and ESR investigated for in-vitro (Mustafa et al. 2016).

Low-level laser irradiation has significant effects on cultured human lymphocytes in-vitro, yet, a little is known about such effects on whole blood. Results of many researchers investigated the low level laser irradiation has high impacts on the lymphocyte count in human whole blood in-vitro (Mustafa et al. 2017). Therefore, no in-vivo investigation has been carried out. The present research is aimed to investigate the impacts of green laser (low dose) on the blood components for in-vivo studies at different intensity of exposure.

2. Research Methods

Source of Green Laser

High power green laser pointer it has calibrated and its watt was 200mW,and wavelength of 532nm used as an exposure source. It is small and exquisite, portable and with richer applications. Extremely bright green laser, power-saving, continuous wave, has a small wavelength of 532nm, and compact reliable.

Lux meter

A Lux meter used to measure the incident photon energy per time of exposure. It has a wide range to 20,000 Foot Candles or Lux with high resolution of 0.01 Fc/Lux, remote light sensor on 305mm (12") coiled cable-expandable to 609mm (24"), a large LCD display with analogue barograph with complete with 9V battery

Setup of Laser exposure technique

Laser exposure technique designed and calibrated its light intensity with the distance using Luxmeter. The technique based on the change of the distance between green laser sources to get different values of light intensity (photon energy per time of exposure) for each point. Average light background was measured and it was considered as an out of data within irradiation process. The calibration results of the exposure technique of green laser rate listed in Table 1. The intensity variation versus the distance is shown in figure 1.

Table (1) Variation of light intensity with the source - distance results.

Distance /cm	Intensity of laser/klx
5	1300.67 ± 2.5
20	1015.13 ±5.11
50	986.334 ± 12.64
75	948.334 ± 2.75

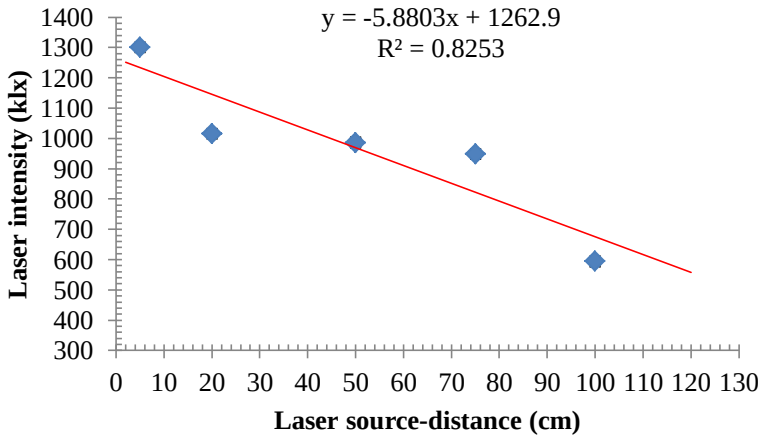

Figure 1: Incident of green laser intensity inversely proportioned with the distance (x).

Albino Rats

Fifteen Albino rats (weight about 200gm) prepared ; as cases (12 exposed to green laser beam) and control (3 without exposure) from the animal house of the biology department. The rats

were raised in suitable environmental conditions; eating and drinking (Rat's Feed), ventilation rate (air moving), light rate (12hr. light and 12hr. dark), and kept in plastic cages.

Exposure procedures

Twelve of female rats prepared to direct exposure to the sources of green laser and 3 rats left as control group. The exposure carried out to heart position (in-vivo) of the rats directly (figure.2). Impact of nonionizing radiation doses on the density of blood component investigated using different doses of green laser as the distance of exposure variated. Light background taken into account.

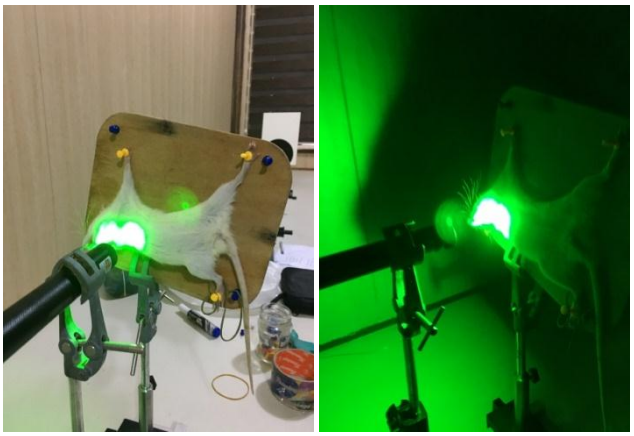

Figure 2: Exposing of the rat using green laser source.

Blood sample collection

After irradiation process, animals anathemised with a mixture of ketamine and xylazine (Xu et al., 2007). Blood samples were collected directly from the rats' heart after dissection and about 10ml of blood sample was obtained. Complete blood counts (CBC test) were taken to investigate the blood count variations.

3.RESULTS AND DISCUSSION

Table 2 shows the influence of laser light on main blood cells. Figure 3 represents the percentage variation of WBCs in compare to control values. WBCs counts increased from 4.3±0.1(109 /l) to 4.1±0.04(109 /l) and 4.6±0.02(109 /l) under 1300.67 klx and 948.334 klx respectively. Other exposure decreased the WBCs counts. Most of the variations occurred from granulocytes percentage rather than lymphocytes. Figure 4 represents the percentage variation of RBCs, in compare to control values. RBCs was varied from 7.12±0.09 (1012 /l) to 6.37±0.05 (1012 /l) due to 948.334 klx. HGB and HTC percentage showed a similar behaviour as RBCs counts. Red blood cell indices did not indicate a significant variation under all exposure intensities. Red cell distribution width (RDW) has risen highly at the lowest exposure intensity. Figure 5 represents the percentage variation of PLTs, in compare to control values. PLTs were under the most significant variation compare to other blood counts, i.e. from 720.66±9.4 (109 /l) to 142±2 and 304±5.2 (109 /l) due to 1300.67 klx and 948.334 klx respectively. Mean platelet volume (MPV) showed a similar result as control values. Platelet distribution width (PDW) had a significant variation at the lowest exposure intensity.

Table (2) Impacts of nonionizing radiation (green Laser; klx) on the density of WBC, RBC, and PLT for the female Rats.

	Exposure (laser)				
Parameter	BG	1300.67	1015.13	986.334	948.334
WBC(109 /l)	4.3±0.1	4.1±0.04	3.8±0.05	3.2±0.05	4.6±0.02
LYM%	78.9±3.4	66.3±8.5	79.3±8.5	87.1±8.3	71.6±5.3
MID%	13.9±1.3	17.3±2.4	16.4±1.42	8.7±3.9	13.7±1.85
GRA%	7.2±0.9	16.4±3.1	4.3±2.1	4.2±1.1	14.7±4.3
RBC(1012 /l)	7.12±0.09	7.49±0.01	6.52±0.13	7.25±0.01	6.37±0.05
HGB(g/dl)	13.1±3.2	13.6±0.67	12±1.1	13.2±0.46	11.5±1.24
HCT%	39.6±5	44.2±10	35±4.1	39.1±4.87	35.7±15.8
MCV(fl)	55±5.2	59.1±6.3	53.9±6.8	54±12	56±6.3
MCH(pg)	18.3±2.1	18.2±2.06	18.4±1.7	18.2±3.12	18.1±1.21
MCHC(g/dl)	33.2±1.6	30.8±7.3	34.2±5.4	33.8±13	32.3±8.3
RDW%	14.9±0.5	13.8±2.29	15±0.5	14.4±2	15.8±3.56
PLT(109 /l)	720.66±9.4	142±2	682.66±1.5	501.66±3.7	304±5.2
MPV(fl)	6.5±1.01	7.5±0.43	6.5±1.4	6.4±1.8	8±1.5
PDW(fl)	7.6±0.93	8.4±0.03	7.5±0.73	7.4±0.73	9±2
PCT%	0.47±0.02	0.1±0.07	0.44±0.05	0.32±0.04	0.24±0.095
LPCR%	5.2±0.7	12.9±1.4	4.6±0.76	6.1±0.1	12.8±0.7

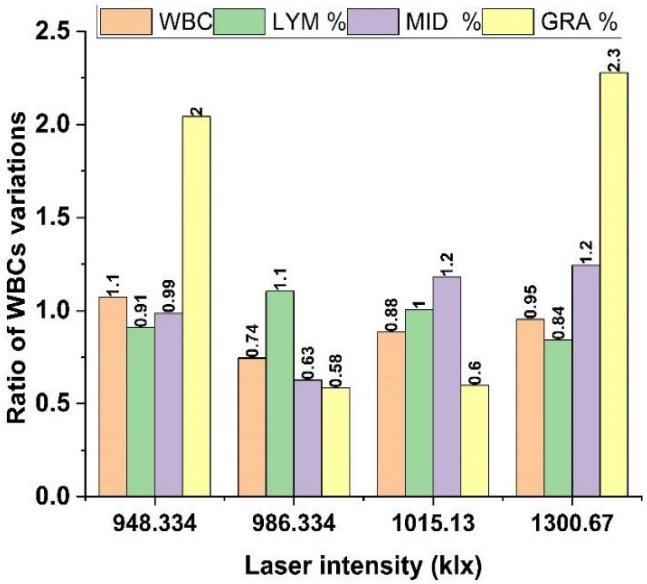

Figure 3: Impacts of NIRDs on the density of WBC for the irradiated rats.

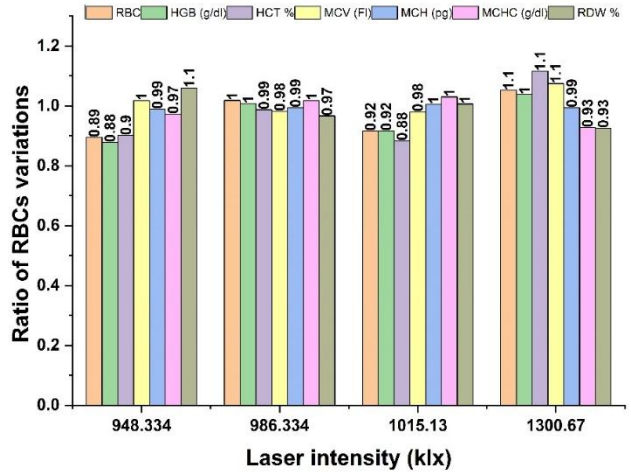

Figure 4: Impacts of NIRDs on the average density of RBC for the irradiated rats.

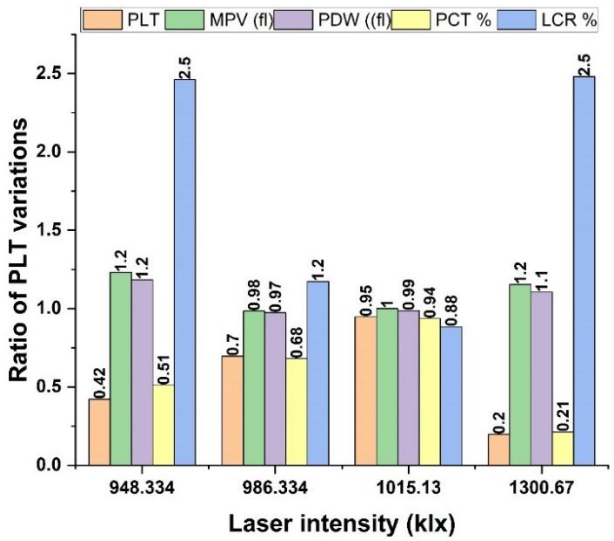

Figure 5: Impacts of NIRDs on the density of PLT for the irradiated rats.

An optimum time to irradiate the rats with the green laser intensity corresponded with other references (Mustafa et al., 2016; Huda, 2017; Siti Sakina et al., 2018). Impacts of nonionizing radiation doses on the blood component density was relativity different, it was higher (P-value <0.05) on PLT density compared with the density of WBC and RBC due to variation in the radiosensitive and biological formation of the blood components. The density of PLT was more effective with the NIRDs due to structure and high radiosensitive of PLT (Ismail et al, 2012)].

At low NIRD (green laser)) (594 ± 2.16 klx), WBC density had not sufficed to affect, most of the effects were at the ranges of (986.334 ± 12.64 and 948.334 ± 2.75klx). So, it is considered as critical NIRDs make sufficient changes to the WBC density. Green laser doses had low effects on RBC density, and the effectiveness ranged between (986.334 ± 12.64klx) to (948.334 ± 2.75) klx look like its effectiveness on WBC density but in inverse changes. At the mentioned ranges, the density of RBC decreased, this means that the rate of NIRDs can do sufficient changes on RBC, especially at high dose (>1300 klx).

CONCLUSIONS

Non-ionizing radiation doses affected relatively on the density of main blood components (PLT, WBC, and RBC) at a lime time of exposure of the female rats, and optimum time of exposure for NIRDs calibrated experimentally in present work. Impacts of high doses for NIRDs had significant effects on the density of PLT, WBC, and RBC. Optimum radiation doses that had high effectiveness on blood component density depended on the type of blood components. As well as, high impacts (high significant) focused on the density of PLT. Cytogenetic effects of green laser are recommended for future work.

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Conflict of Interest: The authors declared that they have no conflicts of interest.

Animal guideline

We carried the experiment out under the university guideline and instructions of animals applied by the biology department at the college

of education (Salahaddin University-Erbil) for researchers.

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