دراسة التألق الكيميائى لتأثيرات الطاقات العالية للامواج الراديوية النبضيةعلى الدم

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الخلاصة

يعد التألق الكيميائي (CL) (الضوء المنبعث خلال تفاعل كيميائي)احد الطرائق الدقيقة للكشف عن تراكيز الجذور الحرة ، ولاسيما جذور الاوكسجين . ويتم الكشف عن هذه الجذور باستعمال محاليل تعرف بمحاليل التألق الكيميائي .في هذا البحث استخدم كاشف اللمينول للتعرف على تركيز جذور الاوكسجين المتكونة في الدم نتيجة لتعريضه الى طاقات نبضية لامواج مايكروية .

ان شدة ضوء التألق يعتمد بصورة مباشرة على تركيز الجذور الحرة الموجودة في محلول التألق والتغيرالذى يحصل فى تركيزهذه الجذور يرجع الى حصول تغيرات فى تركيب محلول التألق.فى حالة كون هذا المحلول جزءا من نسيج حيوى، فأن ذلك يعنى حدوث تغير فى وظيفة النسيج. ومن جانب اخر يمكن دراسة التأثيرات البايلوجية للاشعاع وذلك عن طريق ملاحظة التغيرات التي تحصل في شدة التألق الكيميأئي. والاخيرة تعطي مؤشرات على جملة من العوامل التي تطرأ على الخلية بسبب تعرضها للاشعاع ، مثلا" ما تأثير هذه الجذور الحرة على الصفات الكهربائية لمكونات الخلية وتأثير الاشعاع كذلك في نفوذية جدار الخلية . وللاجابة عن هذه الاسئلة تم الاستعانة بمعادلة ماكسويل– ويحنر وحسبت قيم التوصيلية الكهربائية للساتيوبلازم وهي 18.3 ms/cm وللوسط المعلق 56.1 ms/gm(اى بزياده قدرها 0.1ms/cm للسايتوبلازم و 0.26ms/cm وللوسط المعلق) ان معدل الامتصاص النوعي للطاقة 1kw/gm . وكانت الطاقة الكلية المترسبة لكل غرام لكل نبضة هي 50 mJ اما مقدار الارتفاع في درجة الحرارة بسبب امتصاص هذه الكمية من الطاقة هو $0.003\ ^0C$, 0.017^oC 0.004^0C لكل من الوسط المعلق ،كريات الدم الحمراء و البيضاء على التوالي . ان هذه االزيادة في التوصيلية الكهربائية والارتفاع في درجة الحرارة سببه تكوين تراكيز من بيروكسيد الهيدروجين الذي ينتج من اتحاد الجذور الحرة للاوكسجين . ان التراكيز القليلة من بيروكسيد الهيدروجين تؤدي الى تحفيز قابلية الالتهام وتكوين الاذرع الوهمية للمفوسايت.اما عدد المفوسايت فأنه يزداد عند الجرعات الواطئة ثم يأخذ بالنقصان للجرع العالية. ولكن عند زيادة تركيز بيروكسيد الهيدروجين عن 10^{-8} M فأنعملية تحلل كريات الدم ستحصل ويسبب اضطراب في عملية الانتشار عبر جدران الخلية أيضا" .

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Chemiluminescence Study of High Power Microwave Pulses Effects on Whole Blood

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Abstract

Chemiluminescenc (CL), light emitted during chemical reaction, is one of the accurate methods used to detect directly oxygen free radicals. In this study, luminol was used as CL detector, to detect the concentration of free radicals formed in whole blood exposed to high power microwave pulses. The changes in the intensity of CL signal gives a clear relation between the concentration of free radicals formed by radiation in blood and changes in blood properties such as hemolysis of blood cells. This is done by measuring the electrical sytoplsimic electrical properties, the results are substituted in Maxwell-Wagner equation, to obtain electrical conductivity of cytoplasm, which is 18.3 ms/cm, while at suspension medium was 56.1 ms/cm, with specific absorption rate 1Kw/ gm and the total deposited energy per gm per pulse of 50 mJ.The over all temperature rise per pulse in suspension, red and white blood cells are, 0.017 ^{0}C, 0.004 ^{o}C and 0.003^{o}C respectively. All these changes lead to increase the peak of CL signal compared with unexposed blood. This also indicates that, the formation of different reactive oxygen species in blood would increase and leads to the formation of hydrogen peroxide, which causes to enhance phagocytes behavior of leukocytes at the early time of exposure, but then causes blood hemolysis for later duration and causes membrane perturbation.

Introduction

The electrical conductivities of the cytoplasm and extracellular fluids of biological cells are similar at microwave frequencies but not identical[1]. The temperature differences created across the cell membrane are due to differential absorption of pulsed microwaves with the same average power, which can create larger transient temperature differences. The magnitude of these transients would be calculated. Particular attention would be paid to red blood cells (RBC), leukocytes and hemolysis due to exposure of pulses at 2GHz.

The RBC and leukocytes was motivated by predication that small transmembrance temperature differences could cause large osmotic pressure differences across the membrane. Also changes in membrane permeability of RBC and leukocytes had been reported, when exposed to microwaves [2]. These changes related primarily to the passage of small molecules across the membrane and may have been caused by heating. More recently, it has been reported of effects in nerves [3], ocular lenses [4] and rabbit erythrocytes [5]. Hydroperoxide – initiated CL has been used to detect decreased level of endogenous antioxidants in lever homogenate of ethanol treated rates [6].And cardiac homogenate of ischemia reperfused rabbit [7].

Because of the extreme instability of these molecules the measurements must be done by indirect method. One of these methods, CL technique was tried in this work. Luminol as a CL probe was used for measuring the reactive oxygen species (ROS) production.

For the past thirty years many researches have been done in various aspects of interaction between microwaves interaction with biological systems. All these researches agree that the conversion of the absorbed energy into kinetic energy is the only significant mechanism involved in such an interaction [8]. The thermoregulatory response in warm-blooded animals is well suited for the detection of bio-effects of continuous waves [9]. However, discrepancies

between empirical observations and theoretical explanations suggest that non-thermal effects of the microwave exposure may also play a role in post-irradiation events, although there is no measurable increase in tissue temperature [10]. Non-thermal effects are presently not completely understood [11]. Based on the theoretical study done by Gartner to measure thermal gradient across red blood cells[12], this study try to measure thermal gradient in other blood contains, and also gives an experimental explanation by means of chemiluminescene probes.

The aim of this study is to observe the effects of pulsed microwaves on the hematological parameters in exposed rabbits, and also to study the thermal effects of this radiation on blood by using chemiluminescence probe.

Materials and Sample preparation

Fourteen rabbits weighting 2 - 2.5 kg were used in this study as blood donners . The animals were housed in temperature, humidity and light controlled environment. For all rabbits whole blood samples were obtained with heparinzed plastic syringes immediate before exposing to microwave pluses as control blood samples. Series of blood samples were obtained immediate after taking exposure.

The rabbits exposed to pulsed microwave radiations for 30 days, 5 days a week, 4 hours a day. The animals were irradiated in Plexiglas cages. During each exposure session, the animals received no feed or water. The exposure conditions and average absorbed power are shown in table (1).

The measurement of blood luminol amplified t-Butyl hydroperoxide (TBHP), initiated CL as described below. Briefly 0.2 ml of luminol in phosphate buffer saline (PBS), buffer (concentration 5 mg/l, pH 7.4), was added to 0.4 ml of sample (heparinzed blood) in a stainless steel cell (5 cm in diameter). The mixture of sample was then incubated in 37^0C for 10 min. The CL was then measured in an absolutely dark chamber of the CL reader system shown in fig.(1). This system is extremely sensitive to detect as 10^{-15} w of radiation energy according to the manufacturers specifications [13].

Photon emission from the whole blood was counted at 10 sec intervals at 37^0C and under atmospheric conditions. After 100 sec 0.1 ml of TBHP in PBS (PH=7.4) was injected into the cell. CL in the sample was continuously measured for total of 1000 sec. The total amount of CL was calculated by integrating the area under the curve and subtracting it from the background level. The assay was performed in duplicated for each sample and was expressed as CL counts/10 sec for blood. A mean (ISE, standard error) of CL level of each sample was calculated.

Statistical analysis

Differences between groups were evaluated by a repeated measurement analysis of variance statistical method. The post hoc test performed was Bonferronis t-test. The level of statistical significance is defined as $p < 0.05$.

Results

1. Exposures of pulsed microwave radiation to leukocytes and RBC:

Blood was obtained from rabbits by cardiac puncture using heparin as the anticoagulant. The RBC was suspended in 25 mM hydroxyethylpiperazine ethane sulfonic acid buffer containing 140 mM NaCl, 5 mM glucose, and 3% albumin at PH 7.4. While leukocytes were isolated according to (Bauer, 1980) method [14].

The temperature of the blood was kept at 37 ^{0}C by means of Haake bath and water jacketed tubing. Using this bath to keep temperature constant during 30 min exposure period. Samples were centrifuged and amount of hemoglobin was determined by using the method discussed by Morris and Davey [15] and measuring the optical density at 421 nm.

Initial experimental tests, RBC and leukocytes were isolated from cardiac arteries were exposed to pulsed microwave radiation at 2.0 GHz, supplied by an Epsco model PG5KB source and a Hewlett Packard model 436A power meter was used to monitor the power. The

pulse duration and pulse repetition rate were measured on a 1.5GHz Tektronix DM 44 Oscilloscope.

Pulse duration and pulse repetition rate were altered in series from (20 to 2000) pulses per second (pps) and pulse duration from (0.5 to 50) μs as in

Absorbed power was calculated by subtracting the transmitted and reflected power from the incident power. Using this method of calculation, an average incident power of 1.35w resulted in 0.29w of absorbed power; approximately 21% of the incident power was absorbed by the sample. The 0.29w of absorbed power produced an average specific absorption rat (SAR) of 1.75w/g.

2. Dielectric measurements and transmembrane thermal gradient:

The temperature differences to be expected across leukocytes and RBC membrane by calculating the cytoplasmic conductivity in the following manner. As an initial step, the dielectric properties of the leukocytes, RBC suspension and the suspending medium were measured at 2 GHz in 37^0C using Maxwell-Wagner equation shown below [16]:

$$(\sum_s - \sum_a) / (\sum_s + 2\sum_a) = F\ (Y - \sum_a) / (Y + 2\sum_a) \qquad (1)$$

Where $\sum_s$, $\sum_a$ are the complex conductivities of the suspension and the medium respectively. Y is the complex electrical conductivity of cytoplasm. F is volume fraction of cell. The complex conductivities are of the form:

$$\sum = \sigma + j\omega\varepsilon_0\varepsilon \qquad (2)$$

where;

σ = electrical conductivity, ε_0 = permittivity of space, ε = relative dielectric constant, $\omega = 2\pi f$ (angular frequency) and f = frequency.

The cytoplasmic electrical conductivity was, 18.3 ms/cm, while for medium was 56.1 ms/cm. It was clear that the cytoplasmic conductivity was about 1/3 less than that for the medium.

It is well known that during a pulse the transmembrane temperature difference must depend almost entirely on the heat flow across the thermal resistance provided by the membrane [12], and very little on heat storage [17]. The temperature difference rises within a fraction of microsecond to its limiting value at the beginning of the pulse, The heat flow depends on the volume of the cell, the area of the membrane, the membrane thermal conductance per unit area, and the power absorption difference between the cytoplasm and the medium [18]. For the dimension of RBC as a dish of 2μm thick and 8μm in diameter and for the leukocytes as a sphere of 12μm in diameter and 4μm thickness. Using transmembrace temperature difference equation [19] below:

$$\Delta T = VP\gamma / hA \qquad (3)$$

where;

ΔT = temperature drop across membrane,
V = volume of cytoplasm,
h = thermal conductance per unit area of membrane,
γ = fractional difference in power absorption,
P = specific absorption rate during peak pulse,
A = area of the cell.

$V = \pi R^2 d$ and $A = \pi R^2$, where, R,d are the radius and thickness of RBC and leukocytes. Using:

P = 1.75 Kw/gm, d = 2μm, γ= 1/3 and h = 12 x 10^6 w/m^2k,[19], then $\Delta T = 10^{-7}\ ^0$C. Even though this is very small temperature difference it corresponds to a substantial transmembrane thermal gradient. If the temperature difference were divided by 10 nm width of the membrane, the gradient would be about 100 ^{0}C / cm.

As a result of this temperature gradient across the membrane cell the amount of hemoglobin released was increased, as shown in fig. (2)

The ratios vary above 1.0; a value of 1.0 indicating no difference in hemoglobin release between exposed and control samples. These results show that as the pulse repetition rate of the exposing field increases, then hemoglobin release will increase too. This is because of membrane alteration and lead to cell hemolysis. But as seen from the fraction, even for this relatively high microwave power the hemolysis caused by the thermal gradient was no large enough to cause a large amount of hemoglobin release. Perhaps, more intense pulses could cause more membrane alteration. Now one may ask, what causes that membrane alteration? .The answer is given by the CL study for the blood samples treated with pulsed microwave signals as discussed in the flowing section.

3. Luminol –amplified TBHP-initiated CL:

CL studies gave good evidence to the relation between pulse duration and cell membrane alteration. It was well known that ionized radiation would cause bounds breaking for tissue components [20]. But for non-ionized radiation the effects of interaction differ. It was seen, from the above calculation that heat is one of these effects. Also, recent evidence suggests that oxygen derived free radicals may play a role in the pathophysiology of thermal treatments for the tissue [21]. As demonstrated above that luminol, TBHP initiated blood CL was evaluated in Table (2),the effect of lumionl –amplified TBHP-initiated tissue CL flowing exposure for the conditions (pulse repetition = 500pps, pulse duration= 4μs and absorbed power= 291mw).

Also CL of RBC and leukocytes were increased as the duration of microwave exposure, as shown in fig. (3).

This is because, as in table (1), the absorbed power from the incident field increases for low pulse duration. This is also an indication for the formation of reactive oxygen products from activated blood leukocytes increase compared with control (Zero exposure leukocytes). Also fig's.(4a,b and c).show that,the numbers of RBC,s increase as the exposure increase, this because of the thermal action of radiation [18].While for lymphocytes, there is an induced phagosytosis ability because of specific low exposures of microwave radiations during the first week. But then the numbers of lymphocytes would be reduced as the exposure become heigh. Fig.(4c) shows the total numbers of leukocytes behave as lymphocytes, but there are some differences because of the contribution of other components of leukocytes.

Fig.(5) shows how CL signal for RBCs and leukocytes reduce after 4h after 20 hours of irradiation, but still more than background. This also indicates that hemoglobin is still released through 4h after irradiation, for the 16 μsec and 4μsec pulse durations. That is an expected result, because of the large amount of ROS generated during irradiation, as clear from fig.(6), that the destruction in cell membrane is because of the ROS .

For low pulse durations, cell may return to its previous condition, but for high pulse duration the cell membrane will be destroyed. These data suggest that high pulsed microwave radiation leads to systemic leukocyte activation and the activated leukocytes lead to the production of toxic oxygen products.

The release of these free radicals and their toxic metabolites results in destabilization of cell membrane, peroxidation of enzyme and antienzyme, causes increases in capillary permeability and changes in vascular reactivity. All of these modifications strongly resemble some of the prominent characteristics of circulatory burn shock [22]. The generation of free radicals in the presence of defective scavenging defense might be the cause of the distant organ failure after high intense pulsed microwave radiation absorption.

The microwave exposure in environment is about 10 mw/cm^2 on the order 1 mw/cm^2 at 2000 MHz [23]. It is clear that the peak value of exposure used in this work is about 10^3 times grater than that in nature. Thus it seems unlikely that homolysis occurs in animals under ordinary condition of exposure. Also the peak temperature difference that had been calculated is of order 10^{-4} ^{0}C. While naturally it about 10^3 times less than the calculated value i.e. the temperature difference is about 10^{-4} ^{0}C. So this difference could have any effects.

Conclusion

Heat cannot be stored across the leukocytes and red cell membrane during a microwave pulse.ThereforE, The transmembrane temperature difference during a pulse depends almost entirely on heat flow across the membrane.Hemolysis increase as the peak power increases.

The temperature difference of only 10^{-4} ^{0}C created by pulsed with peak SAR of 1.75 kw/g, in this study it may seem to be very small, but it corresponds to a substantial thermal gradient across the membrane of 100 ^{0}C/cm. A gradient of this size is enough to cause hemolysis and cell membrane perturbations.

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Table (1) below:

Microwave exposure conditions							
Pulse parameter units	Conditions tested						
Pulse repetition (pps)	2000	1000	500	250	125	62	20
Pulse duration (μs)	0.5	1.0	2.0	4.0	8.0	16.0	50
Entergy/ pulse (mJ)	0.67	1.35	2.70	5.40	10.8	21.6	86.4
Absorb. power (mW)	287	293	296	301	311	318	322

Table(2): Effect of luminol amplified TBHP-initiated CL

Tissue	CL(counts/10sec/1mg)		P value
	Control	Treated tissue	
Leukocytes	621	17609	<0.001
RBC	492	7954	<0.001
Cytoplasm	358	6177	<0.001

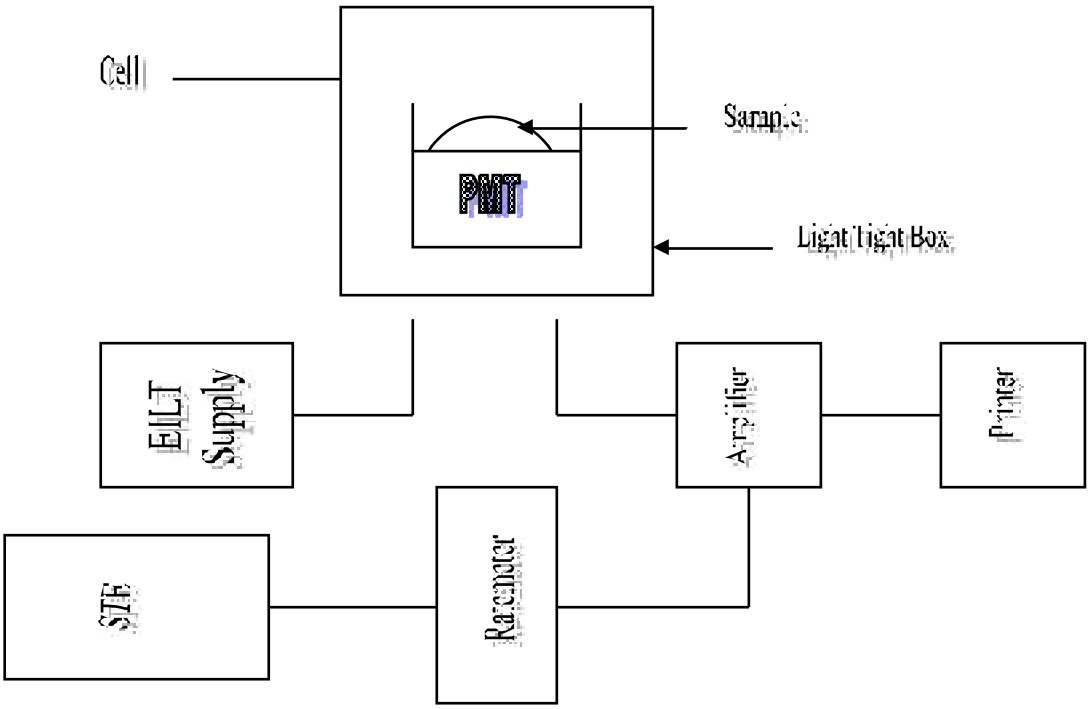

Fig. (1) Chemiluminescenc reader system

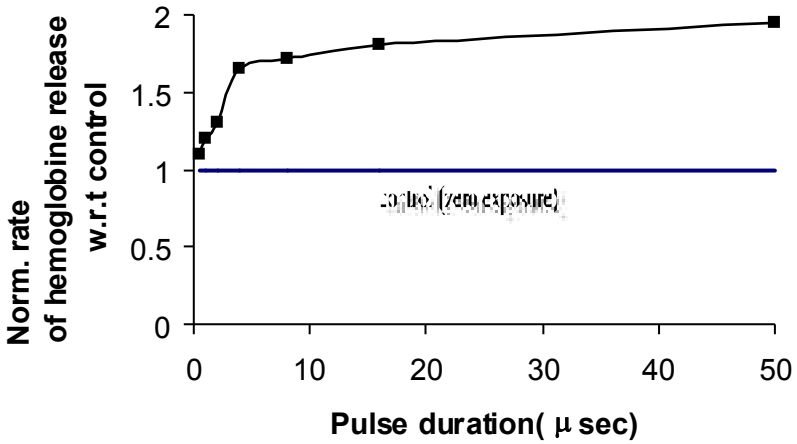

Fig.[2]:Hemoglobine relase in diffrent microwave duration treatment

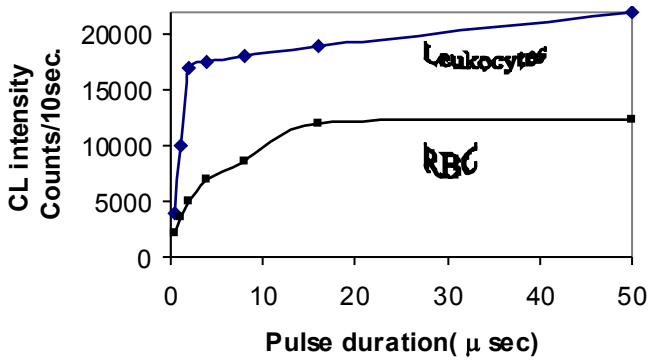

Fig.[3]: CL of leckocyts and RBC at different pulse durations.

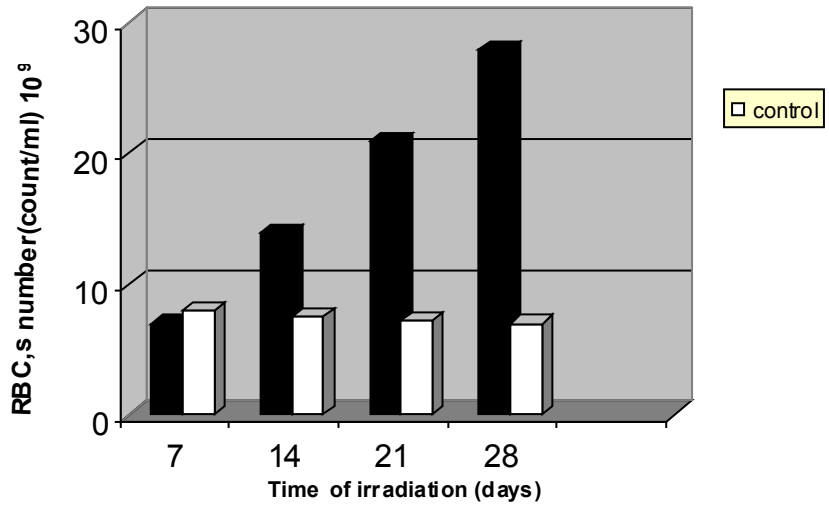

Fig.[4a]:The variation of RBC,s number during period of exposur.

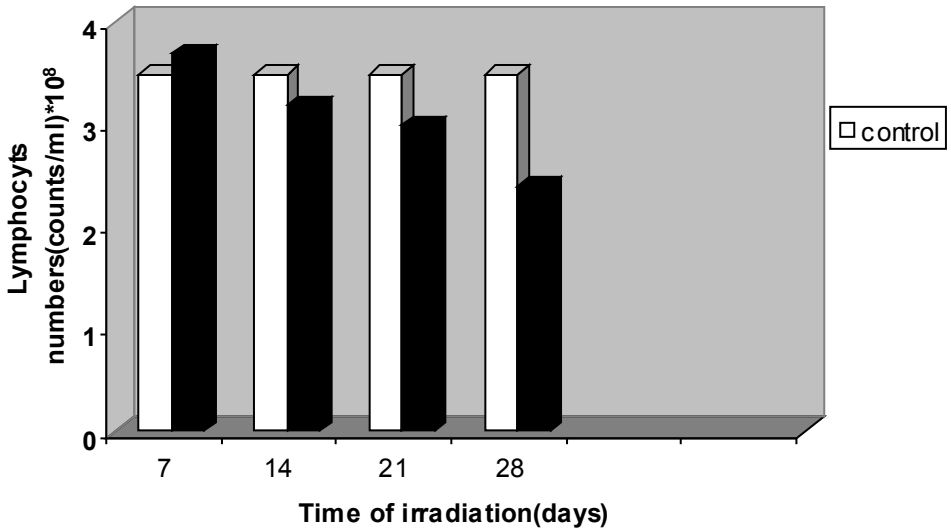

Fig.[4b]:The veration of lymphocytes with respect to control.

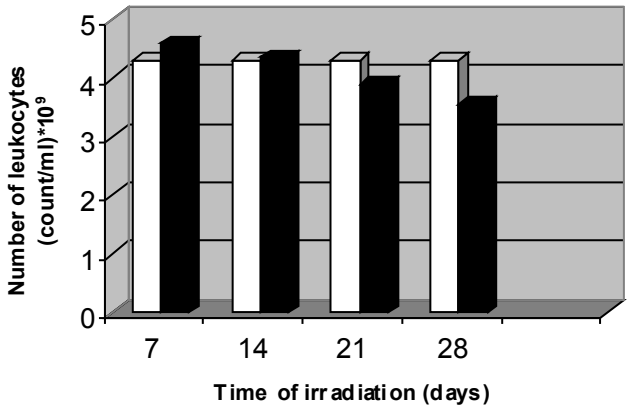

Fig.[4c]: The variation of leukocytes number with respect of contro during period of irradiation.

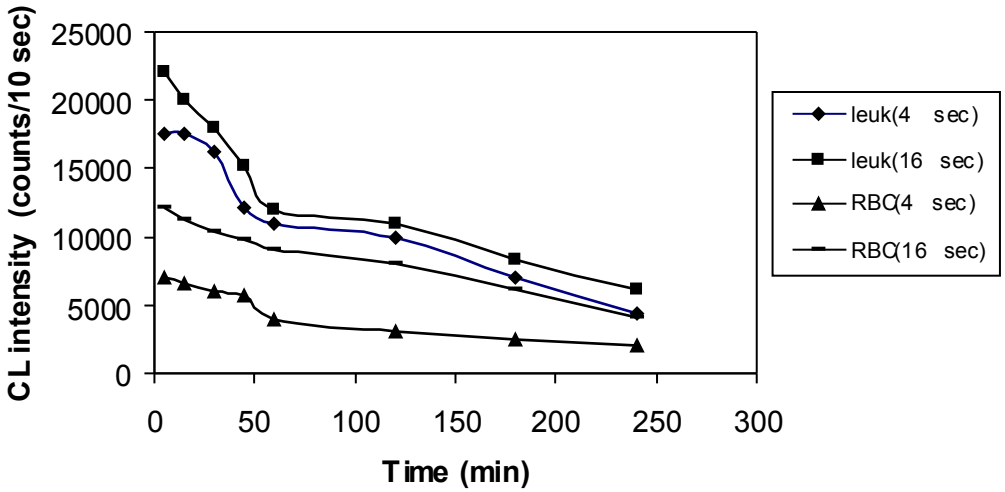

Fig.[5]: CL of leukyoctes and RBC's at pulse durations 4 sec and 16 sec for aperiod of time up to 6h.

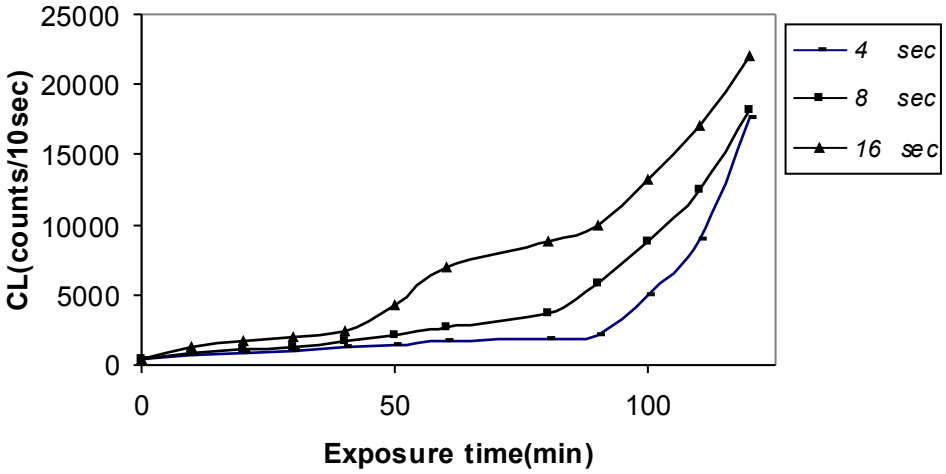

Fig.[6]: CL and exposure time dependance for leukocytes at different pulse duratons.