

Treatment of cancer by low intensity laser radiation therapy

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Abstract: Cancer treatment is one of the main challenges that face the scientific centers all over the world. The cancer incidence percentage increases year after year. The most used treatment for cancer is chemotherapy which is an application of systemic cytotoxic drug inside the patient. There are two main problems in chemotherapy that the cytotoxic drug is given systematically and cannot be localized in the tumor region in most cases that lead to killing the cancer cells as well as normal cells; and that cause the chemotherapy side effects like anemia, decrease in all blood cells count and platelets, nausea, vomiting, diarrhea, hair and nails loss and may cause a kind of blood toxicity. Photodynamic therapy (PDT) is a relatively new approach for the treatment of malignancies with only minimal side effects for the patient. It is based on the administration of a tumor-localizing dye (photosensitizer, PS) that becomes toxic to neoplastic cells when is activated by light (usually from a laser) at a specific wavelength in the presence of O₂. In this work photodynamic therapy modality was used. Porphyrin derivative was used as a photo sensitizer drug and one source of energy were used; namely infrared laser with three frequency levels (1000, 2000, and 3000 Hz). Tumor- bearing animals were divided into the following groups each of 10 animals. The 1st group consists of two control groups: the 1st one was untreated group and the 2nd one was injected with photosensitizer alone. The 2nd group consists of 30 mice divided into 3 sub-groups and tumor site was irradiated with laser light as follows: 1000 Hz, 2000 Hz and 3000 Hz for 5 min. 3rd group consists of 30 mice injected with HPD 12.5 mg/Kg, then divided into 3 subgroup and tumor site was irradiated with laser light at the same conditions of 2nd group. The results show decrease in tumor size treated with drug (HPD) which is used as a photo sensitizer, laser, and combined treatment, both in the presence and absence of photosensitizer. Combined treatment is more effective on tumor cells than using of laser and drug alone. Also the presence of photosensitizer increases the effect of laser on tumor size. However, from histopathology of ehrlich tumor using 3000Hz of laser irradiation in the presence of the photosensitizer drug approximately has the most effect than using the photosensitizer drug alone and exhibit totally necrotized tumor. It could be concluded that the use of the photosensitizer drug alone has no effect on the ehrlich tumor. However, the presence of the drug gave maximum effects with exposure to 3000 Hz infrared laser irradiation. The work needs to be extended to reach more and powerful effects using the photodynamic therapy with exposure to other different energies of infrared laser radiation.

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1. Introduction

Cancer remains leading cause of death globally which is a multigenic and multicellular disease that can arise from all cell types and organs with a multi-factorial etiology. Cancer treatment is one of the main challenges that face the scientific centers all over the world. The cancer incidence percentage increases year after year. The most used treatment for cancer is chemotherapy which is an application of systemic cytotoxic drug inside the patient. There are two main problems in chemotherapy that the cytotoxic drug is given systematically and cannot be localized in the tumor region in most cases that lead to killing the cancer cells as well as normal cells; and that cause the chemotherapy side effects like anemia, decrease in all blood cells count and platelets, nausea, vomiting, diarrhea, hair and nails loss and may cause a kind of blood toxicity.

The goal of cancer treatment is to produce cure by removing the cause of the disease while producing as little harm to the patient as possible. Most conventional cancer treatments, e.g., surgery, radiation and chemo-hormonal therapy are possible, but they are usually highly morbid with low success rates ⁽¹⁾. The dilemma of cancer therapy lies in selectively targeting and destroying only the cancer cells and leaving the normal cells unharmed. For this reason, new areas of cancer research have explored more specific methods of targeting only cancer cells. Such methods include development of new classes of drugs that are directed to unique sites on cancer cells, as well as techniques that specifically tag only cancer cells for destruction. The destruction of solid tumors using photodynamic therapy and laser therapy has been under investigation for this purpose ⁽²⁻³⁾.

Photodynamic therapy (PDT) is a relatively new approach for the treatment of malignancies with only minimal side effects for the patient. It is based on the administration of a tumor-localizing dye (photosensitizer, PS) that becomes toxic to neoplastic cells when is activated by light (usually from a laser) at a specific wavelength in the presence of O₂. PDT is a two-step procedure: first a photosensitizer is administered, and then the region where the photosensitizer accumulates is exposed to light of a specific wavelength, which activates the photosensitizer⁽⁴⁻⁵⁾, PDT is based on the fact that photosensitizers are absorbed by all cells, but are selectively retained by malignant tissue⁽⁶⁾. PDT kills tumor cells *via* apoptosis or necrosis (or both), both *in vivo* and *in vitro*. The particular mode of cell death in response to PDT depends on experimental conditions, such as the dose of PDT and the subcellular localization of the photosensitizer⁽⁷⁾. Furthermore, PDT-mediated apoptosis may have different mechanisms depending on the type of cells being treated, the type of photosensitizer being used, the light delivery protocols employed, and the time lag between the photosensitizer and light treatment^(8,9).

PDT is a promising modality for the treatment of solid tumor and other non-malignant conditions. PDT is a minimally invasive technique that avoids many of the side effects typical for radiation and chemotherapy, since the drug and light by themselves are not cytotoxic. It has the ability to irradiate only tumor, the possibility of treating multiple lesions simultaneously and the ability to retreat a tumor in order to improve the response⁽¹⁰⁻¹¹⁾.

The treatment involves administration of a photosensitive drug to the target site and after a suitable time period, to allow the drug to accumulate within the tumor, the drug is activated by specific energies of visible light. Laser, near-infrared region, is typically used to produce the activating light, which has greater penetration through tissues and can be selected to lie beyond the longest single-photon absorption band⁽¹²⁾. When the photosensitizer is illuminated with light of appropriate wavelength, the molecule becomes excited. Photo excitation of photosensitizer (PS) leads to the formation of singlet oxygen (¹O₂) and it was suggested that, this highly active oxidant is the main damaging agent in PDT⁽¹³⁾. However, there are some indications that besides ¹O₂ other reactive oxygen species (ROS) such as hydrogen peroxide (H₂O₂), superoxide (O₂⁻) and hydroxyl (OH) radicals might be involved in the PS-PDT induced tumor eradication⁽¹⁴⁻¹⁵⁾.

Investigations on the mechanism of action of PS-PDT showed that this treatment modality may include not only a direct damaging of organelles within malignant cell, but also destruction of blood vessels in

the tumor locus resulting in reduced oxygen supply, nutrients^(16,17) and possibly activated the immune system⁽¹⁸⁾.

Some drugs, referred to as photosensitizers, have synergistic effect with red light irradiation. The majority of photosensitizers are porphyrin and their derivatives (Hematoporphyrin, photofrin II), which have been well investigated in PDT. Hematoporphyrin (HP) is known to enhance the cell- killing effect of light irradiation at a dose at which the chemical alone has unknown biological effect. The present work aims at studying the effect of a combined treatment of near-infrared laser with and without photosensitizer e.g. (Haematoporphyrin Dihydrochloride) on tumor growth rate. In this work photodynamic therapy modality was used. Porphyrin derivative was used as a photo sensitizer drug and one sources of energy were used; namely infrared laser with three frequency levels (1000, 2000, and 3000 Hz).

2. Material and Methods

2.1. Laser unit

Fig.1. show Infrared diode low intensity laser therapy (Mustang, 2000, Germany) was used. The unit has large menu of preset pathologies. For each pathology, all parameters are pre-set and stored in the memory, but can always be modified by therapist

- * Power: 2mWatt.
- * Emission frequency: adjustable from 10Hz and 3000Hz.
- * Large number of pre-set protocol that can be changed at any moment.
- * Digital timer of 40 minutes with memory and acoustic signal.
- * Visualization of the parameters on LCD screen.
- * Two out puts, two laser emitters can be connected simultaneously.



Fig. 1. Photograph of Low Intensity laser Therapy Unit

2.2. Experimental Procedures Of Photodynamic Therapy

2.2.1. Photosensitizer

Haematoporphyrin Dihydrochloride was purchased from Sigma Chemicals Co. USA. The photosensitizer obtained as powder with dark red color store of in dark Bottle at 0°C temperature and with Purity: 95% pure by HPLC analysis C34H38N4O6.HCl MW 671.6.

2.2.2. Cell Culture and Tumor Inoculation

Ehrlich ascites tumor was chosen as a rapidly growing experimental tumor model⁽¹⁹⁾ where various experimental designs for anticancer agents can be applied. Ehrlich ascites carcinomas cells (1×10^6 cells), obtained from National Cancer Institute “NCI”– Cairo University, were intraperitoneally injected into albino mice. Ascites fluid was collected on the 7th day after injection. The Ehrlich cells were washed twice and then re-suspended with 22–25 g body weight and 6–8 weeks old (obtained from the animal house of NCI) were then injected subcutaneously in their left flanks where the tumors were developed in a single and solid form. Tumor growth was monitored post-inoculation until the desired volume was reached. All animal procedures and care were performed using guidelines for the Care and Use of Laboratory Animals⁽²⁰⁾ and approved by the Animal Ethics Committee at Cairo University⁽²¹⁾.

2.2.3. Tumor cells and animals

A total of 80 male Swiss albino mice with age 60–65day, weighing 20 ± 2.5 gm, were purchased from National Cancer Institute, Cairo University. Ehrlich ascites carcinoma cells 2×10^6 mammary in origin, diluted approximately in 0.9% saline were inoculated subcutaneously on the left dorsal limb region of mice. The animals were housed in plastic cages and were kept under natural light with diet and water at available. When the tumor had grown to about 10 mm in diameter at day 7 after inoculation, the treatment study was started.

2.2.4. Preparation of Hematoporphyrin Dihydrochlorid (HPD) dye

Hematoporphyrin Dihydrochlorid was dissolved in a sterilized saline solution 0.9% saline and administered to tumor-bearing mice at a dose of 12.5 mg/kg by intraperitoneally injection for 15 day 24 hours before exposure to different treatment modalities.

2.3. TREATMENT METHODS

Tumor- bearing animals were divided into the following groups each of 10 animals.

1- 1st group two control groups: the 1st was untreated group and the 2nd was injected with photosensitizer alone.

2- 2nd group consists of 30 mice divided into 3 sub-groups and tumor site was irradiated with laser light as follows:

a- 1000 Hz, for 5 min.

b- 2000 Hz for 5 min.

c- 3000 Hz for 5 min.

3- 3rd group consists of 30 mice injected with HPD 12.5 mg/Kg, then divided into 3 subgroups and tumor site was irradiated with laser light at the same conditions of 2nd group

2.3.1. Laser Treatment

The mice were anesthetized with ethyl ether. The hair over the tumors was shaved off. The mice were fixed on a board with the tumor upwards. The probe was placed tightly on the tumor, which was irradiated with laser for five minute at the different conditions as mentioned before.

2.4. Tumor size measurements

Due to the high growth rate in Ehrlich tumor model, change in tumor volume (V_{mm^3}) was monitored over a 14day period for laser-photosensitizer treated groups and control group. Ellipsoidal tumor volume was assessed every day and tumor volume (V) was calculated using the formula

$$V = (\pi/6) (D)2 (D) \quad (1)$$

Where D and d are the long and short axes respectively. Tumor diameters were measured with a digital Vernier calipers. Mice were selected for treatment when the tumors reached the desired volume ($0.7-1\text{cm}^3$). This size was chosen as a convenient treatment size that matches the laser spot size. Multivariate analysis of variance (MANOVA) was carried out to investigate the effect of both time (from day 0 up to day 15) and type of treatment on tumor growth. T-TEST (least significance difference) multiple-comparison test was also conducted to check the significance between group pairs⁽²¹⁾.

2.5. Inhibition ratio percent of the tumor size

Two weeks after the treatment, the mice were killed and the tumors were dissected out, weighed (in grams). The inhibition ratio was calculated as follows⁽²²⁾.

Tumor Volume

$$= \left[1 - \frac{\text{Average tumor weight of treated group}}{\text{Average tumor weight of the control}} \right] \times 100\% \quad (2)$$

2.6. Tumor extraction

The tumor mass was collected and its measure at its longest and shortest axis was determined using a digital caliper. Then the solution was maintained at 10% formaldehyde for one day and transferred to 70% alcohol. After that the samples were embedded in paraffin, cut at $5\mu\text{m}$ thick and stained with hematoxylin-eosin (HE) for light microscopic observation⁽²³⁾.

2.7. Histopathological examination

At the time of sacrifice, the tumors were excised from the animals samples from each tumor tissues were fixed in 10% formalin and embedded in paraffin. Sections of tissues were stained with Hematoxylin and eosin ⁽²³⁾.

2.8. Statistical Analysis of Data:

The data were analyzed using one-way analysis of variance (ANOVA). Results were expressed as mean ± standard error (SE) and values of P>0.05 were considered non-significantly different; while those of P < 0.05 and P < 0.01 were considered significant and highly significant, respectively probability expresses the general effect between groups.

3. Results

Tumor size variation curves are illustrated in Fig. (2-3). The figures show decrease in tumor size treated with drug (HPD) which is used as a photo sensitizer, laser, and combined treatment, both in the presence and absence of photo sensitizer. Combined treatment is more effective on tumor cells than using of laser and drug. Also the presence of photosensitizer increases the effect of laser on tumor size.

As shown in Fig.4. At the end of treatment period, measurement of the tumor size of different groups indicate that by increasing of the laser intensity decrease the tumor size in the presence of photo sensitizer suffer more decreasing.

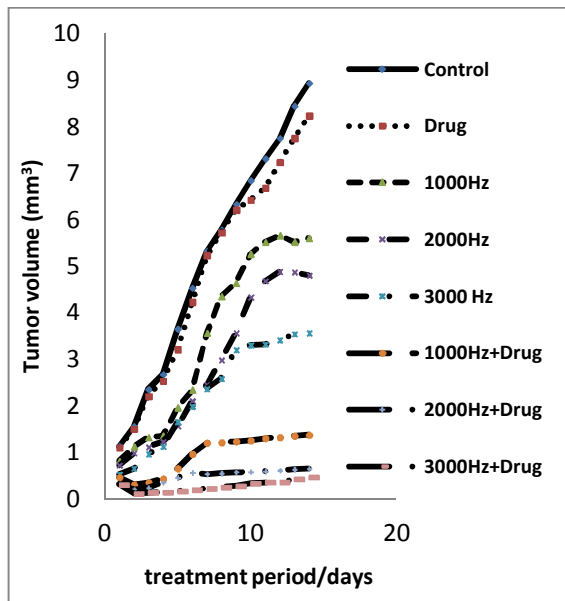


Fig. 2. Comparison between the normalized tumor volume and period for the different modes of treatments.

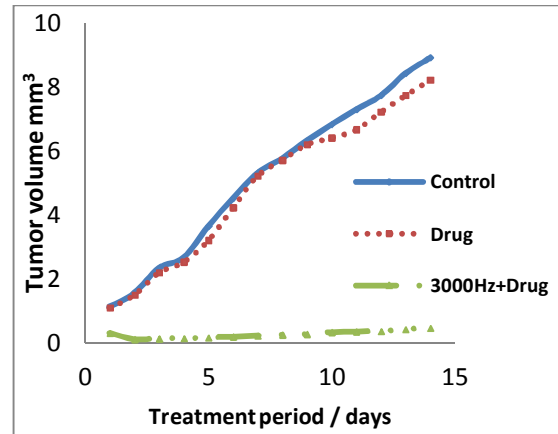


Fig. 3. Relation between the normalized tumor volume and the period after starting the treatment with combination of laser and in the presence of the drug.

Relative tumor size variation data obtained for each group at the end of the treatment period, 14 days, are analyzed statistically, the results obtained are presented in Table (I).

It is clear from this table that there was highly significant changes in the normalized tumor size with the different treatment modalities except on using the drug alone which has no any effect on the tumor size along the total period of treatment. However, using 3000Hz of laser irradiation in the presence of the photo sensitizer drug approximately has the most effect than using the photo sensitizer drug alone.

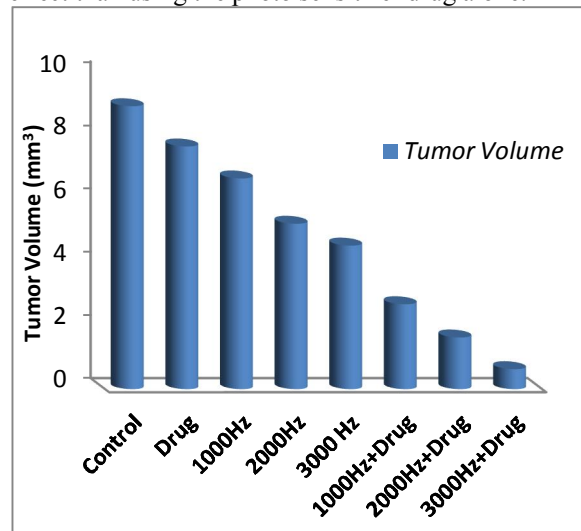


Fig. 4. Relation between tumor growth at day 14 of treatment and Frequency (Hz) of laser.

On the other hands, table II illustrates the effect of the different treatment modalities at the end of the treatment period, 14 days. It is clear from this table that the inhibition ratio percent increases according to the order arrangement giving in the table.

Table (I): Tumor growth rate after 14 days of starting the different treatment modalities

Number of mice	Control (mm ³ /day)	Drug only (mm ³ /day)	3000Hz (Laser)+Drug (mm ³ /day)
1	57.8	50.9	0.025
2	63.3	55.2	0.027
3	68.4	61.2	0.033
4	73.1	68.5	0.035
5	77.5	72.3	0.036
6	84.4	74.5	0.056
7	89.3	76.6	0.063
Mean	73.4	65.6	0.0393
±SEM	4.257	3.771	0.0055
Significant Level		Not. Sig.	Very High Sig. P< 0.0001

Table (II): Inhibition ratio percent of the tumor size at the end of 14 days starting from the day of treatment.

groups	Tumor weight (g) ^a	Inhibition ratio (%)
Control	1.37 ±0.034	0
Drug (Hp)	1.33 ±0.054	2.9
Laser		
1000 Hz	1.27±0.053	7.3
2000 Hz	1.24 ±0.011	9.49
3000 Hz	1.18 ±0.013	13.87
Laser + Drug		
1000 Hz	1.14 ±0.044	16.79
2000 Hz	0.75 ±0.011	45.26
3000 Hz	0.25±0.068	81.75

It must be mentioned that, in comparing the effects of using 3000 Hz infrared laser in the presence of the photosensitizer drug (3000Hz + Drug) on both the normalized tumor volume and the inhibition ratio percent, there is a slight difference between them in case of tumor volume and in the inhibition mass ratio percent. This may be due to the difference in the water content of the tumor which results from the exposure of the 3000 Hz infrared laser in the presence of the photo sensitizer drug. Decreasing in the tumor size was observed either on using infrared laser alone with its three frequencies or in the presence of the drug. However, the decline in the tumor volume was maximum in case of using 3000 Hz infrared laser in the presence of the drug.

Histopathological examination of the Ehrlich solid tumor (EST) under light microscope (Fig.5) with magnification x100. Showed compact and aggregation of the tumor tissue cells spread within the muscular tissues (↑). In case of group Fig.5 (B) where tumors

were injected with drug without any treatment with laser. In case of sham group (Fig.5(C, D and E) where tumors were exposed to laser with frequency 1000, 2000 and 3000 Hz respectively without drug injection, show viable islands of tumor cells and there is an increase in necrotic. Fig.5F, G and I x100: Section in EST exposed to laser radiation at different frequencies 1000,2000 and 3000Hz respectively in the presence of photosensitizer which shows tumor tissue sections represented high and wide zones of apoptotic cells and other many zones of tumor cells (Fig.5F), pleomorphism, hyperchromatism (Fig.5G), and totally necrotized for tumor (Fig.5H) for groups exposed to 3000Hz of laser radiation after drug injection which give us good improvement.

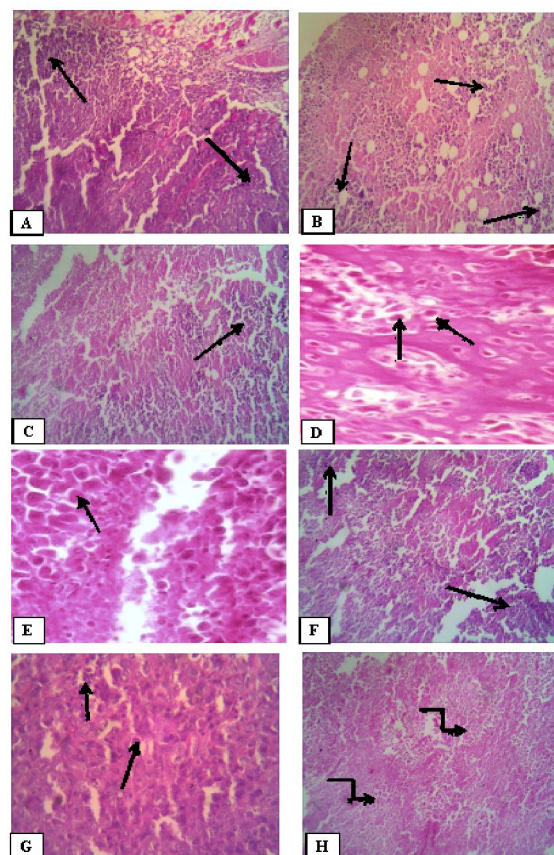


Fig.5. Histopathology of Ehrlich tumor cells with magnification 400x. (A) Photomicrographs of sections in Ehrlich solid tumor (EST) stained by Hx. and E for control group (neither laser nor drug treatment) Fig.5A: Section in Ehrlich solid tumor (EST). Fig.5B: Section in EST treated by photosensitizer. Fig.5 C, D and E x100: Section in EST exposed to laser radiation at different frequencies 1000,2000 and 3000Hz respectively in the absence of photosensitizer. Fig. 5F, G x100: Section in EST exposed to laser radiation at different frequencies 1000,2000 and 3000Hz respectively in the presence of photosensitizer.

4. Discussions

Cancer is a class of diseases or disorders characterized by uncontrolled division of cells and the ability of these cells to invade other tissues, either by direct growth into adjacent tissue through invasion or by implantation into distant sites by metastasis. This unregulated growth is caused by damage to DNA, resulting in mutations to genes that encode for proteins controlling cell division. Many mutation events may be required to transform a normal cell into a malignant cell. These mutations can be caused by chemicals or physical agents called carcinogens, or by certain viruses that can insert their DNA into the human genome. Mutations occur spontaneously, or are passed down generations as a result of germ line mutations⁽²⁴⁾.

Cancer can be treated by surgery, chemotherapy, radiation therapy, immunotherapy, and electro-chemotherapy or other modalities. The choice of therapy treatment method depends upon the location and grade of the tumor and the stage of disease, as well as the general state of the patient.

Complete removal of the cancer without damage to the rest of the body is the goal of treatment. Photodynamic therapy (PDT)⁽²⁵⁻²⁶⁾, is suggested by a number of researchers as a promising modality for the treatment of solid tumors and other non-malignant conditions. It comprises the use of a photo sensitizer drug to enhance the absorption of the incident irradiation. It avoids many of the side effects of chemo and/or radiation therapy in that it is invasive modality since the photo sensitizer drug used in addition to exposure to light are not toxic by themselves. Also, in this promising modality, it is possible to irradiate only the tumor with a minimum or completely damage to the adjacent normal tissues.

So, it was aimed to use one radiation source, namely laser radiation, with different frequencies with a photo porphyrin derivative as a photo sensitizer, to study the effects of this promising treatment modality on Ehrlich tumor (of human origin) injected to mice.

The experiments performed in this work used a wide range of parameters in order to obtain a valuable conclusion as can as possible. The control group in this work was a group of mice bearing Ehrlich tumor only. So all obtained results were compared to this group as baseline. The other parameters used were; i) photo sensitizer drug (hepatoporphyrin derivative HPD) alone, ii) Laser with three different frequencies, namely, 1000, 2000, and 3000 Hz in the infrared region, iii) laser with its different frequencies in combination with the drug. So, it of interest to explain and discuss the effect of each of these parameters singly to simplify the results and prevent overlapping of results.

The single tumor growth curve describes nearly an ascending curve (line) beginning by nearly zero volume starting from the day of injection of the tumor cells in the normal mice to reach maximum value at day 14. The injection of the photo sensitizer drug in the solid tumor has no effect on the tumor normalized volume as can be observed in fig (2-4).

During the fourteen days following the exposure of the tumor bearing mice to three different frequencies of infrared laser a depression in the tumor volume occurred showing a plateau with the 1000 Hz, followed by that of the 2000 Hz, and ending with the 3000 Hz infrared laser. So, it can be concluded that infrared laser has a depression effect on the tumor volume starting with day eleven with the 1000 and 4000 Hz, and with day nine with the 3000 Hz infrared laser.

However, the combination of the different frequencies of the infrared laser with the drug showed more and significant depression in the normalized volume of the tumor. The combination of the drug and exposure to 1000 Hz infrared laser started depression in the tumor volume at day 7 starting from the drug injection day. The depression describes a plateau for about seven days after that started slight more depression until day 14. For the drug in combination with exposure to 3000 Hz infrared laser show tendency to decrease or depression in the normalized volume, with the overall depression is larger than that in case of 1000 Hz in combination with the drug. It is clear from this part that the exposure of the mice bearing tumor to infrared laser alone or in combination with the photo sensitizer drug has profound effect on the normalized tumor size with the maximum effect is in the presence of the drug and with maximum energy (or frequency) of the infrared laser, i.e. with 3000 Hz. However, from histopathology of Ehrlich tumor using 3000Hz of laser irradiation in the presence of the photosensitizer drug approximately has the most effect than using the photosensitizer drug alone and exhibit totally necrotized tumor. It could be concluded that the use of the photosensitizer drug alone has no effect on the Ehrlich tumor. However, the presence of the drug gave maximum effects with exposure to 3000 Hz infrared laser irradiation. The work needs to be extended to reach more and powerful effects using the photodynamic therapy with exposure to other different energies of infrared laser radiation.

5. Conclusions

From the data of this work, it can be concluded that;

1- The photo-sensitizer drug (HPD) alone has no effect on Ehrlich tumor.

2- The use of infrared laser in the frequency range (1000 – 3000) Hz has an effect on that tumor which is judged by the inhibition ratio of the tumor (\approx 13.87 %) on using 3000 Hz infrared laser.

3- The infrared laser (3000 Hz) in the presence of the drug is more effective than using infrared laser alone which is judged by the inhibition ratio of the tumor (\approx 81.75 %).

4- The combination of exposure to infrared laser (3000Hz) in the presence of photosensitizer is more effective than infrared laser alone.

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