Tumor Treatment by A Combination of Chemotherapy and Radiotherapy with Methotrexate Drug

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Abstract

Many ways investigated for cancer treatment, including surgery, chemotherapy, and radiotherapy. In our study new method was performed as photodynamic laser therapies with nanoparticles (NPs), Methotrexate drug (MTX), direct electric current (DEC) and combinations of them which give an interesting combined therapeutic effect. The Primary Purpose of this work was to investigate the ability of NPs to transform laser energy and DEC into therapeutic heating. Characterization of prepared NPs, tumor weight, volume, and histopathology investigated. The results showed that the tumor capsule infiltrate by inflammatory cells. Mice treated with NPs and MTX shows a similar degree of infiltration areas of necrosis and newly formed blood capillaries are also noticeably. Mice treated with NPs, MTX and low-level laser therapy (LLT) shows increased tumor cell necrosis with aggregates and sheets of invasive malignant cells. Mice treated with NPs, MTX and DEC shows slightly increased tumor cell necrosis and increased mononuclear inflammatory cells. Mice treated with NPs, MTX, LLT and DEC shows massive tumor cell necrosis with only minimal aggregates of malignant cells. It concluded that treat tumor with LLT, DEC, MXT, and NPs give us the most inhibition for it.

Keywords: Ehrlich tumor, Laser therapy, Electric current, MTX, Nps, SEM, TEM, XRD, Histopathology

Introduction

Cancer is a major challenge worldwide, contributing to nearly 9.6 million deaths in 2018[1] also cancer is one of the most common causes of death worldwide[2]. Chemotherapy and radiotherapy are the most effective and extensive approaches for cancer management. The most common therapeutic approaches to cancer treatment include immunotherapy and targeted therapy, chemotherapy, radiation, and surgery. Of these modalities, chemotherapy remains one of the most effective methods[3]. However, the efficacy and application of available anticancer chemotherapeutic drugs often fail to achieve complete cancer remission owing to the heterogeneity of cancer cells, show limited efficacy due to dose-limiting toxicity to the patients, and development of multidrug resistance[4].

Nanoscience and nanotechnology hold a great promise for many potential applications, including biomedical uses[5]. At a nanoscale size, noble metals nanoparticles (NPs) have received a wide spread interest in molecular imaging, molecular diagnosis and targeted therapy of malignant tumors due to their easy and fast synthesis, ability to be taken up into the cell, inherently low toxicity, and the unique optical and electronic properties that are not observed on the bulk scale[6,7].

Hyperthermia, is the process of increasing the temperature of tumor tissue to 40–43°C, it’s used as associate therapy with many confirmed cancer treatments like chemotherapy and radiotherapy[8]. In thermal therapy, hyperthermia treatment can be done without damaging the healthy tissue and decreasing the side effect to it either. Hyperthermia can be used to cure the entire body or accurate tumor locations, in local hyperthermia, it’s used to raise the temperature inaccurate area that
contains a tumor location, the heat is applied with external applicators to treat tumors near the surface of the skin or with probes for deeper cancerous tissue. Regional hyperthermia is used for various tumor locations or to treat cancer propagation in many regions. Here, a wide part of the body is heated to ruin the cancerous cell. This treatment is usually used with chemotherapy or radiation therapy.

In hyperthermia treatment, the high frequency electromagnetic energy is applied to the tissue using either external or internal applicators depending on the tumor location. Internal applicators use a needle or probe to release the energy directly into the tumor, and external applicators radiate the tissue from outside. Healthy tissues can also absorb electromagnetic energy and get heated leading to burns, blisters, and discomfort. Thus, it is critical to monitor temperatures in healthy tissues during hyperthermia treatment.

Hyperthermia is known to induce apoptotic cell death in many tissues and has been shown to increase local control and overall survival in combination with radiotherapy and chemotherapy in randomized clinical trials. Hyperthermia is normally used in combination with other treatments, including radiotherapy, and can be delivered externally, interstitially or endoluminal with heat generation by radiofrequency waves, microwaves or ultrasound. While normal tumor vasculature dilates to aid heat dissipation, tumor vasculature constricts, providing some tumor selectivity. However, overall, a lack of specificity for tumor tissue, difficulties in heating deep tumors to constrict, providing some tumor selectivity. However, overall, a lack of specificity for tumor tissue, difficulties in heating deep tumors to therapeutically increase temperature in the surrounding microenvironment. This elevated temperature is sufficient to induce irreversible cellular damage through protein denaturation and coagulation and cell membrane destruction.

It has been shown that NPs, activated by light with the appropriate wavelength, can act as efficient photothermal agents mediating cancer therapy. The absorbed photon energy by NPs is rapidly converted into thermal energy through nonradioactive processes leading to an increase in temperature in the surrounding microenvironment. This elevated temperature is sufficient to induce irreversible cellular damage through protein denaturation and coagulation and cell membrane destruction.

In fact, the strategy of treating cancer using thermal therapy has previously been investigated as an alternative to conventional methods of oncological approaches including surgery, chemotherapy, and radiotherapy. Although a variety of heat sources has been employed in cancer hyperthermia such as microwave, ultrasound, and laser photothermal therapy, they all encounter a problem of destroying the tumors selectively. For a successful thermal therapy, hyperthermia should be confined to the target tumors without harming surrounding healthy tissues.

Low-level laser (light) therapy (LLLT) has in recent years become one of the fastest growing fields of medicine using laser in therapies of medical treatments that use focused light not like most light sources, light from a laser (which stands for light amplification by stimulated emission of radiation) is tuned to specific wavelengths that can be adjusted to the specific requirements of the procedure done this enables it to be focused into powerful beams with high levels of accuracy and precision because it focuses on a small area damaging less of the surrounding healthy tissue. Laser therapy is so intense it can be used to shrink or destroy tumors, polyps, precancerous growths, relieve symptoms of cancer, remove part of the prostate, repair a detached retina, improve vision, treat hair loss resulting from alopecia or aging and treat pain including back nerve pain.

The electric field is used to transfer the electromagnetic energy to the tissue, with one electrode placed below and one above the patient. The energy deposition depends on the sizes of electrodes and the electric field. It is seen that there is a high power deposition in the subcutaneous fatty tissue. The disadvantage of this device is that the energy distribution will depend only on the electrodes position and size and hence the treatment needs to be stopped to make any change in electrodes. The blood perfusion plays an important role in the tumor heating.

Direct electric current (DEC) has been reported as having antitumor activity in different tumor models and in human clinical trials. In spite of this, the electrochemical treatment is not established and mechanisms are not well understood. In order to explain the antitumor effect of DEC some mechanisms have been proposed, among them, there are changes in trans-membrane potential, pH local changes tissue ionization and electrochemical reactions.

On the other hand, the role immune system has still not been clarified. However, some researchers have proposed that DEC enhances the immune system of the patient electrochemical treatment produces direct alterations and chemical changes in the tumor, causing the cell metabolism and its environment to be severely disturbed. These alterations result in changes in the cellular homeostasis. One of the threats for cellular homeostasis is the reactive oxygen species (ROS), which in high concentrations cause the oxidative burst. These ROS could arise from the electro-chemical reactions produced around the electrodes and from the immune system.

Nanoparticles (NPs) are microscopic particles with dimensions of less than one hundred nm. They possess a good kind of potential applications and are currently used for diagnosis and for treatment. Fluorescent semiconductor NPs, identified as quantum dots, are used for imaging of biological entities and superparamagnetic nanoparticles are used as contrast agents for resonance imaging (MRI) to assist diagnose numerous diseases. The multifunctional characteristic of nanoparticles makes them very good candidates for targeted drug delivery and cancer treatment, and that they are presently in use as anti-cancer drug and gene carriers for dominant cancer.

Parameters like their comparatively tiny size leading to longer circulation times and their ability to take advantage of the tumor characteristics as an example, NPs less than twenty nm in size are able to pass through blood vessel walls and such tiny particle size permits for intravenous injection as well as intramuscular and subcutaneous applications. The little size of the NPs minimizes the irritant reactions at the injection place as compared to standard cancer treatments. Moreover, NPs size permits for interactions with biomolecules on the cell surfaces and inside the cells without changing the behavior and nd for a specific response by the body to cancer treatment. The major goal is to make the nanoparticles reach the sites of tumor, after administration, with low loss to their volume and activity in blood circulation and to attach the tumor cell without damaging the healthy tissue. This may be done by two strategies: passive and active targeting of drugs.

Passive medication targeting consists in the transfer of NPs through tumor capillaries by convection or passive diffusion between endothelial cells and compromised lymphatic drainage. In this case, NPs could be released in the extracellular matrix and then diffused through the tumor tissue. Inactive targeting, targeting ligands are connected at the surface of the nanoparticle for binding to acceptable receptors expressed at the tumor site. The ligand is chosen to bind to a receptor of specific tumor cells and not expressed by traditional cells.

Magnetic Fe3O4 nanoparticles (MNPs) are a category of NPs that may be manipulated using magnetic fields. Such particles normally consist of 2 elements, a magnetic material, often iron, nickel and cobalt, and...
a chemical element that has functionality. While NPs are smaller than one micrometer in diameter (typically 1-100 nanometers), the larger microbeads are 0.5-500 micrometers in diameter. MNPs clusters that are composed of a variety of individual MNPs are referred to as magnetic nanobeads with a diameter of 50-200 nanometers.

The MNPs are the target of a lot of analysis recently because they possess enticing properties that may see potential use in catalysis including nanomaterial-based catalysts, biomedicine and tissue specific targeting.

Hematite Fe$_3$O$_4$ nanoparticles are the most important polymorph existing in nature. Hematite has a centered hexagonal structure with a close-packed oxygen lattice and it is believed to be a specific candidate for applications, such as sensors, catalysts, data storage materials, fine ceramics, pigments, and photoelectrochemical cells.

Methotrexate drug, formerly known as amethopterin, is a chemotherapy agent and immune system suppressant. It is used to treat cancer, autoimmune diseases, ectopic pregnancy, and for medical abortions. Types of cancers it is used to include breast cancer, leukemia, lung cancer, lymphoma, and osteosarcoma. Types of autoimmune diseases it is used for include psoriasis, rheumatoid arthritis, and Crohn’s disease. It can be given by mouth or by injection. Common side effects include nausea, feeling tired, fever, increased risk of infection, low white blood cell counts, and breakdown of the skin inside the mouth. Other side effects may include liver disease, lung disease, lymphoma, and severe skin rashes. People on long-term treatment should be regularly checked for side effects as it is not safe during breastfeeding. In those with kidney problems, lower doses may be needed. It acts by blocking the body’s use of folic acid.

The primary purpose of this study was to evaluate the capability of nanoparticles to transform laser energy and direct electric current into therapeutic heating. Targeted nanoparticles, in conjunction with laser irradiation and connecting the direct electric current, can increase the temperatures of the targeted area over the peripheral region. Therefore, to become clinically viable, the laser can act as efficient photo thermal agents mediating cancer therapy. The absorbed photon energy by NPs is rapidly converted into thermal energy through nonradioactive processes leading to an increase in temperature in the surrounding microenvironment. This elevated temperature is sufficient to induce irreversible cellular damage through protein denaturation and coagulation and cell membrane destruction.

Materials and Methods

Cell Culture and Tumour Inoculation

Ehrlich ascites tumor was chosen as a rapidly growing experimental tumor where various experimental designs for anticancer agents can be applied. Ehrlich ascites carcinoma cells, obtained from Medical Research Institute - Alexandria University, were intraperitoneally injected into male albino mice. Ascites fluid was collected on the 7th day after injection. We took 1cm of Ehrlich cells and then re-suspended in 9ml saline. Male albino mice with 20-35g body weight and 6-8 weeks old obtained from the animal house of Pharos University in Alexandria were then injected subcutaneously in their right 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 and approved by the Animal Ethics.

Experimental Animals

In the present work a total of 56 male mice weighing 20-35gm of 5-7 weeks age in all the experiments have used. Suspension of 106 cells/mL isolated from Ehrlich ascites carcinoma was prepared in mice. The animals will be injected with 0.25 mL of this suspension in the right flank. When tumors reach about 4-10 mm in diameter, mice will be randomly divided into the following groups, each of 7 mice as follows:

G1: untreated mice bearing tumor.
G2: mice bearing tumor injected with magnetic NPs.
G3: mice bearing tumor injected with hematite NPs.
G4: mice bearing tumor injected with methotrexate drug.
G5: mice bearing tumor injected with hematite NPs, magnetic NPs and drug.
G6: mice bearing tumor exposed with low-level laser therapy and injected with hematite nanoparticles, magnetic NPs and drug.
G7: mice bearing tumor exposed with direct electric current therapy and injected with hematite NPs, magnetic NPs and drug.
G8: mice bearing tumor exposed with low-level laser therapy and direct electric current and injected with hematite NPs, magnetic NPs, and drugs (mixed group).

Tumor Growth

Due to the high growth rate in Ehrlich tumor model, change in tumor volume (V) was monitored over a 15 days period for all groups every three days and tumor volume (V) was calculated using the formula: V= d$^2$/d/6, where D and d are the long and short axes, Tumor diameters have measured with caliper and the following used formula to estimate the tumor weight: $\text{Tumor weight (mg)} = \text{Length (mm)} \times (\text{width (mm)}^2) \times 0.5$

Manufacture Method of hematite NPs

Fe$_3$O$_4$ NPs have been synthesized via a wet chemical method based on co-precipitation methods. This method based on alkaline the co-precipitation of ferric and ferrous salts in aqueous solution. Briefly, a solution containing Fe III at a predetermined concentration was prepared followed by the addition of a base, such as NaOH under vigorous stirring at a temperature within the range of 60-80°C. Subsequently, the pH of the solution was carefully adjusted and a dispersing element was used to stabilize the particles.

Preparation and properties of Hematite Fe$_3$O$_4$ (HNPs)

Hematite {Iron (III) Oxide}Fe$_2$O$_3$ (HNPs) was prepared by adding 1g of Fe$_2$O$_3$ powder dissolved in 50mL sterile saline, then we dissolved it in solution for 15 minutes until the color of the solution turned brown. Its color was appearance brownish-red and solid in form, it dispersed and solubility in water/ethanol. Needles & Rods like shape, and its average size by TEM 500±50 (L) and 1525 (W) nm.

Transmission electron microscopy (TEM) of HNPs

The physical size and shape of the prepared iron oxide particles were determined by TEM (JOEL JEM-2100) at an accelerating voltage of 200 kV. For this purpose, a drop of this HNPS solution loaded on to a 400 mesh carbon coated copper grid and the solvent allowed to evaporate in the air, and then screened under TEM.

Characterization of HNPs & MNPs

Properties of magnetite nanoparticles were characterized using High-Resolution TEM (HR-TEM) & Scanning Electron Microscope (SEM), X-ray Diffraction (XRD), Vibrating Sample Magnetometer (VSM).

Manufacture Method of Magnetite nanoparticles

MNPs have been synthesized via co-precipitation method. This method based on alkaline co-precipitation of ferric and ferrous salts in aqueous solution. Briefly, two solutions containing Fe II and Fe III at a pre-determined concentration ratio were mixed followed by the addition of a base. Subsequently, the pH of the solution was carefully adjusted and a dispersing element was used to stabilize the particles.

Preparation of Magnetite Fe$_3$O$_4$ (MNPs)

Magnetite{Iron (III) Oxide} Fe$_3$O$_4$ MNPs was prepared by adding 1g of Fe$_3$O$_4$ powder dissolved in 50 mL sterile saline, then we dissolved it in solution for 15 minutes until the color of the solution turned black. Its
color was appearance black and solid state it dispersed and solubility in water/ethanol and stability will remain 6 months in solid-state and its average size by TEM 20±10 nm.

**Transmission electron microscopy (TEM) of MNPs**

High-Resolution TEM (HR-TEM) images were carried out in Nanotech Company for photo-electronic, Dreamland, 6-October, Egypt. HR-TEM is JOEL JEM-2100 operating at 200kV equipped with Gatan digital camera Erlangshen ES500.

**Scanning electron microscope (SEM)**

SEM produces pictures of a sample by scanning the surface with a beam of electrons. The electrons interact with atoms in the sample, producing different signals that contain information about the surface topography and composition of the sample.

**X-ray Diffraction (XRD) analysis**

MNPs were analyzed for the crystalline nature by XRD (Shimaduz, XRD 7000, Maxima, and Japan). The Crystallite domain size was calculated from the width of the XRD peaks from Scherrer’s equation.[27]

**Vibrating sample magnetometer (VSM)**

The hysteresis loop of HNPs & MNPs at room temperature was performed by VSM.

**In vivo Laser Therapy Irradiation**

The experiments started after four weeks of tumor injection when a palpable mass of tumor tissue appeared, and the average volume of the tumor 0.5 ± 0.2 mm³. The mice of G6 & G8 were exposed to low-intensity laser therapy unit (Mustang, 2000, Germany) the maximum power is 2mw, the emission frequencies ranged from 10Hz to 3000Hz and two outputs. First, we injected the Magnetic and hematite NPs to produce local heating of labeled cells without harming surrounding healthy tissues. After that we injected it with the methotrexate drug then the two groups were exposed to the laser for 20 minutes for each by laser therapy device (mustang 2000+) at the power of 2mJ and frequency 3000 HZ[25], the period of exposure was 30min.

**Application of Direct Electric Current In vivo**

Tumor treatment based on the application of a low intensity direct electric current to the tumor tissue through electrodes placed within the tumor zone or in the surrounding areas. The Electrochemical treatment produces direct alterations and chemical changes in the tumor, causing cell metabolism and its environment to be severely disturbed. These alterations result in changes in cellular homeostasis. This treatment is noted for its very good effectiveness, minimal Invasiveness, and local impact. The Electrochemical treatment of cancer utilizes direct electric current (DEC) to produce direct alterations and chemical changes in tumors. However, the DEC treatment isn’t established and mechanisms don’t seem to be well understood In vivo studies were conducted to evaluate the effectiveness of DEC on animal tumor models[28, 29]. Ehrlich tumor was injected subcutaneously in seven mice. When the tumor size of NPs reached to 0.5 ± 0.2 mm³, the temperature of the cancerous tissue was raised without damaging the healthy tissue surrounding the tumor, then methotrexate drug was injected and two platinum electrodes were inserted into the tumors of 2.5volelectric field was applied for 20 minutes after being anesthetized.

**Analysis of Histopathology**

Tumors specimens from different groups were excised and fixed in 10% phosphate-buffered formalin solution at room temperature and dehydrated in graded ethanol (70–100%), cleared in xylene, and embedded in paraffin. Paraffin-embedded tissue sections (5 µm thick) were ready, mounted on slides, and in-room temperature. Thereafter, slides were dewaxed in xylene, hydrated using graded ethanol, and stained by hematoxylin and eosin (H&E) dyes. The sections were examined under a light microscope and photographed with a digital camera (Canon, Japan)[30].

**Results**

The TEM photo indicates that the prepared Hematite NPs (Fig.1) and MNPs (Fig.2) almost spherical shapes. The particles have a tendency to aggregate because of their magnetic properties. The SEM observations of the synthesized HNPs & MNPs (Fig.3 & Fig.4) show an equal distribution of NPs on the surface. From this image, it was determined that the Fe₂O₃ & Fe₃O₄ is spherical in shape, SEM can achieve resolution 5µm.
As represented in Table 1, there was no difference in mortality rate between untreated tumor-bearing mice and mixed group 8. Only one mouse died from the following groups G2, G3, G4 and two mice died from groups G5, G6, G7. Relative tumor volume and weight variation data obtained for each group at the end of the treatment period, 9 days are analyzed statistically, the results obtained indicated in Table 2. It is clear that there was extremely statistically significant decreased in the normalized tumor volume and weight with the different treatment modalities as compared with control group. Also note significant decreased for mixed treatment was indicated i.e. the treatment of Ehrlich tumour with laser + electric field + Drug + Fe$_3$O$_4$ + Fe$_2$O$_3$) give us the most inhibition for tumour. The electron diffraction shows that the samples of HNPs & MNPs are crystalline as confirmed from the XRD Fig. 5& Fig. 6.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Number tested</th>
<th>Survivors/total mice</th>
<th>% Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>7</td>
<td>7/7</td>
<td>0%</td>
</tr>
<tr>
<td>G2</td>
<td>7</td>
<td>6/7</td>
<td>10%</td>
</tr>
<tr>
<td>G3</td>
<td>7</td>
<td>6/7</td>
<td>10%</td>
</tr>
<tr>
<td>G4</td>
<td>7</td>
<td>6/7</td>
<td>10%</td>
</tr>
<tr>
<td>G5</td>
<td>7</td>
<td>5/7</td>
<td>20%</td>
</tr>
<tr>
<td>G6</td>
<td>7</td>
<td>5/7</td>
<td>20%</td>
</tr>
<tr>
<td>G7</td>
<td>7</td>
<td>5/7</td>
<td>30%</td>
</tr>
<tr>
<td>G8</td>
<td>7</td>
<td>7/7</td>
<td>0%</td>
</tr>
</tbody>
</table>

Table 1: percentage of Mortality for different groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Tumor volume (cm$^3$) after 15 day</th>
<th>Tumor weight (gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>0.34</td>
<td>0.500</td>
</tr>
<tr>
<td>G2</td>
<td>0.11</td>
<td>0.100</td>
</tr>
<tr>
<td>G3</td>
<td>0.1</td>
<td>0.920</td>
</tr>
<tr>
<td>G4</td>
<td>0.12</td>
<td>0.290</td>
</tr>
<tr>
<td>G5</td>
<td>0.091</td>
<td>0.167</td>
</tr>
<tr>
<td>G6</td>
<td>0.069</td>
<td>0.170</td>
</tr>
<tr>
<td>G7</td>
<td>0.067</td>
<td>0.168</td>
</tr>
<tr>
<td>G8</td>
<td>0.065</td>
<td>0.120</td>
</tr>
</tbody>
</table>

Table 2: Indicates volume tumor size and weight for all groups as compared with the animal bearing tumor.
The hysteresis loop of Fe$_3$O$_4$ & Fe$_2$O$_3$ at room temperature is showed in (Fig.7 & Fig.8) that the prepared NPs are super paramagnetic and saturation magnetization. This result demonstrates that HNPs & MNPs present excellent magnetic properties to react with an external magnetic field and have good potential for magnetic hyperthermia.
Histopathological examination of the Ehrlich solid tumor (EST) under light microscope stained with hematoxylin and eosin (H&E) Fig.9: show for control group the invasion of subcutaneous adipose tissue and skeletal muscle by the neoplastic cells (yellow arrow); the tumor capsule infiltrate by inflammatory cells. The surrounding tissue show mild or no inflammatory infiltration. Fig.10 mice treated with NPs and MTX, shows a similar degree of infiltration as described with the control group. Similar areas of necrosis and newly formed blood capillaries are also noticed. The inflammatory cell infiltration and necrosis in comparison with EST were slightly increased. Fig.11 mice treated with NPs, MTX and laser therapy, shows increased tumor cell necrosis with aggregates and sheets of invasive malignant cells. Fig.12 mice treated with NPs, MTX and direct electric current shows slightly increased tumor cell necrosis and increased mononuclear inflammatory cells. Fig.13 mice treated with NPs, MTX, laser therapy and electric current shows massive tumor cell necrosis with only minimal aggregates of malignant cells.

Fig.8: Vibrating sample magnetometer of Fe$_3$O$_4$ NPs

Fig.9: Histology of Untreated control tumor bearing-mice Scale bar: 400µm, stained with hematoxylin and eosin (H&E).

Fig.10: Histology of group treated with NPs and drug (MTX) (Scale bar: 400µm), stained with hematoxylin and eosin (H&E).
Discussion

Cancer is one of the leading causes of death in the world. New procedures for the treatment of cancer that have been implemented have been successful, but the results are still limited. The use of hyperthermia along with conventional cancer treatment such as chemotherapy and radiation has been successful in many cancer treatments. In this approach, the cancer tumors are heated to a temperature between 40 to 45°C for a specific period of time which renders tumor cells more sensitive to radiation and chemotherapy. The increase in temperature stimulates blood flowing the tumor, increases oxygen at ion and hence makes the treatment more effective. The main concern in deep hyperthermia is the powered exposition in tumors and the temperature monitoring during treatment. The current procedures have a limitation on the body target sites that are too difficult to treat. There is a large need for alternative effect vie options in hyperthermia for providing deep seated power deposition to various regions of the body, without affecting healthy surrounding tissues and organs. Hence, in this research we investigate an enhanced hyperthermia treatment through the use of magnetic NPs. TEM micrograph demonstrates the formation of homogeneous spherical hematite and magnetite NPs with an average size of approximately 22±3nm for hematite NPs (Fig.1) and 15±2 nm for magnetite NPs (Fig. 2). Particles within this size range are suitable for laser heating as it enhances thermal diffusivity in the surrounding medium.[31,32].

The SEM observations of the synthesized HNPs & MNPs (Fig.3 & Fig.4) show an equal distribution of NPs on the surface. From this image, it was determined that the FeO3&Fe3O4 is spherical in shape.

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Administration of NPs in neoplastic tissues followed by Photoactivation by laser irradiation can offer the advantage of less-invasive and effective modality over the conventional treatment methods, as it combines a minimal systemic toxicity, rapid and effectiveness of heat deposition with highly selective destruction of tumor cells. For laser applications in medicine and biology, it is necessary to reduce the energy level below the medical safety standard (2mJ in our research) in order to avoid the detrimental effects of high power lasers[31].

On the other hand, the tumor growth rate in mice was monitored over 15 days for different groups. The average change in tumor volume with time for each group was indicated in Table 2. Under our experimental conditions, a pronounced inhibition in tumor growth was demonstrated in the animal group that treated with both laser, EC, MNPs and MXT drugs (mixed group) compared with the control one. Within 3 days, a detectable difference in tumor growth between the mixed group and control group was noticed. Statistical analysis demonstrated that laser in combination with EC, MNPs and MXT drugs has a high significant suppression on tumor growth rate up to 15 days (p < 0001). Since the coherent oscillations of the conduction band electrons are in strong resonance with frequencies of visible light, spherical MNPs absorb light strongly in this region ensuring an efficient and rapid conversion of the absorbed photons into heat.

With the increased interest in nanoparticles and owing to their peculiar properties, magnetic nanoparticles (MNPs) of various compositions, sizes and shapes have attracted considerable attention during the last four decades and have become an active research field in the area of magnetism. NPs have many potential different applications in electronics and data storage industries and as materials in catalysis and the field of biological applications. Owing to their ease of preparation, strong ferromagnetic behavior and oxidative stability (less sensitive to oxidation than magnetic transition metals such as Co, Fe and Ni), major interest has recently been devoted to the synthesis of iron oxide MNPs such as magnetite and hematite that have received the most attention for biomedical applications[32,33].

Fig.9. revealed EST under light microscope stained with hematoxylin and eosin for control group which appears compact and aggregation of the tumor tissue cells spread within the muscular tissues also appears the invasion of subcutaneous adipose tissue and skeletal muscle by the neoplastic cells the tumor capsule infiltrate by inflammatory cells. The surrounding tissue show mild or no inflammatory infiltration. Fig.10. revealed tumor tissue of mice treated with NPs and MXT, shows similar areas of necrosis and newly formed blood capillaries are noticed. The inflammatory cell infiltration and necrosis in comparison with EST were slightly increased. Fig.11. mice treated with NPs, MXT and laser therapy, shows increased tumor cell necrosis with aggregates and sheets of invasive malignant cells. Fig.12. mice treated with NPs, MXT and direct electric current shows slightly increased tumor cell necrosis and increased mononuclear inflammatory cells. Fig.13. mice treated with NPs, MXT, laser therapy and electric current shows massive tumor cell necrosis with only minimal aggregates of malignant cells also shows the necrotic area where the tumor cells are eosinophilic and swollen with loss of cellular details. showed an extensive also. From histopathological analysis it can be deduced that the use of laser and electric current in combination with NPs and MTX injection is more effective in the destruction of Ehrlich tumor tissue as compared with sham or control group.

Based on the above results, the pronounced inhibition in tumor growth rate for laser, electric current-NPs and drug treated tumors (Table 3) and the massive area of cell necrosis demonstrated in histological sections of the same group (Figs.13) which verify the high efficiency of photo thermal destruction of tumour cells under the current treatment protocol. It can be proposed that when NPs absorb laser in their Plasmon band, they efficiently convert the absorbed photon energy into heat resulting in cellular death[36,37]. Due to the inability of visible light to penetrate deeply into subcutaneous tissues, this modality of using spherical NPs and MXT drug couples with laser and direct electric current can potentially be useful for in vivo applications of photothermal therapy of surface and/or near surface type tumors. The low power laser in the present treatment protocol can offer the advantage of using low cost lasers in photothermal therapy using NPs and MXT drug which might be more accessible in research as well as in vivo applications with the advantage of avoiding the unnecessary side effects of high laser power.

The primary goal of this work was to investigate the capability of NPs and MXT drug to transform laser energy and direct electric current into therapeutic heating. Targeted NPs, in conjunction with laser irradiation and connecting the direct electric current, can increase the temperatures of the targeted area over the peripheral region. Therefore, to become clinically viable, the laser can act as photothermal agents for cancer therapy. The electric current has anticancer activity and conducts electrochemical treatment causing cell metabolism and chemical changes in the histology of tumor that result in disturbances in the vital functions of the cancer cell.

Conclusion

It was concluded that NPs have obvious effects on cancer cells by killing it with no effect on normal cells. There was highly significant a pronounced inhibition in the normalized tumor size with the different treatment modalities as compared with the control group. Very high significant decreased for combined therapy was indicated i.e. the treatment of tumors with laser therapy, DEC, MXT and NPs give us the most inhibition for tumors.

Photothermal therapy is a minimally invasive treatment method in which laser photon energy is converted into thermal energy sufficient to induce cellular destruction. This modality holds a great promise as selective hyperthermia in cancer treatment via employing MNPs and MXT drug in combination with laser irradiation and DEC.

The present work describes the photothermal therapy of a subcutaneous Ehrlich tumor grown in mice using the easily prepared spherical MNPs in conjunction with visible laser. The principle feature presented in this study is the use of long-term exposure of tumours in order to attain the photothermal damage by applying low laser power and low concentration of nanoparticles.

References