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Effect of magnetic nanoparticles on Ehrlich solid tumor in mice using thermal ablation

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ABSTRACT

Recently, nanotechnology plays a significant role in cancer prohibition and treatment. Various kinds of iron oxide are naturally occurring and may be synthesized under laboratory conditions. This research was intended to estimate the histological and biochemical changes induced by Ehrlich Solid Carcinoma (ESC) in the skeletal muscle of mice treated with Epirubicin, and Fe_3O_4/MPA NPs with hyperthermia. The experimental animals were equally allocated into 4 groups (50 mice/group). Mice of the first group were benefit as normal control. The second group were intramuscularly injected with ESC. The ESC affected mice of the third and fourth groups were treated with Epirubicin and Fe_3O_4/MPA NPs with hyperthermia, respectively. The present investigations displayed that treatment of ESC mice with Epirubicin and Fe_3O_4 NPs/MPA revealed a significant reduce ($P \leq 0.05$) in the creatinine kinase (CK), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activity. The histological changes in the skeletal muscles in the first treated group showed reduction of cancer cells, cirrhosis and destruction of tissue cells, also, the group administrated by Fe_3O_4/MPA with hyperthermia showed disappearance of a large number of cancer cells compared to the first group of treatment and the return of life to the fabric affected without touching or affecting the normal cells of the tissue. In conclusion, the *in vivo*

cancer treatment test could cure the cancer tumor, protecting the skeletal muscle tissues and ameliorated CK, AST and ALT enzyme activity.

Keywords: Histopathology, Magnetic nanoparticles, biochemical parameters, Hyperthermia, Ehrlich, Cancer

1. INTRODUCTION

Nanotechnology is in prospect make a significant help to cancer prohibition, discovery, finding, imaging, and treatment, directed medication treatment, tissue regrow, cell culture, biosensors, aimed treatment, magneto-fiction and different tools in the scope of molecular biology. Among these nanoparticles, the superparamagnetic iron oxide nanoparticles (SPIONs) showed unique properties that distinguish them from other nanomaterials; this is owing to their excellent magnetic features, high chemical steadiness, excellent colloidal stability, and good biocompatibility (Shubayev et al., 2009). Altogether, SPIONs are potential candidates for improving contrast enhancement in magnetic resonance imaging (MRI) and hyperthermia. Magnetic iron oxide NPs represents the greatest intensively studied nanomaterial in biomedical processes, because of their superior biocompatibility relative to alternative magnetic substances, (oxides or elemental metals). Now a day; the utilize of nanomaterials lower than 100 nm has due to important progress in in vitro and *in vivo* diagnosis and therapy of several maladies as cancer (Neuberger & Schopf, 2005). Key factors influencing the performance of iron oxide lattices in diagnostic and therapeutic procedures include degree of crystallinity and dispersal of nanoparticles to provide the ability to act as hyperthermia have described how these factors are highly linked with the production methodology implemented (Maenosono et al., 2008).

Nanomedicine is presently achievable to give treatment at a molecular scale with the assistance of these devices, in this manner treating sickness and aiding investigation of pathogenesis of infection. Nano medicine includes the use of nanotechnology to assist human wellbeing and prosperity (Abdeen & Praseetha, 2013). Solid Ehrilch Carcinoma tumors are undifferentiated solid tumors that are commonly employed in tumors investigations, both to study tumors development and chemotherapy as reported by Silva et al. (2006). After subcutaneous injection of Ehrlich tumors cells, a 1 cm diameter tumor can be observed in around one week. As a highly virulent tumor it exhibits a mortality rate approaching 100% of experimental subjects within a short time scale. High virulence, rapid growth and development and the infiltrative mode of action of this tumors type are all indications of its high-grade malignancy (Sakai et al., 2010). Currently, Epirubicin is considered as one of the anthracycline derivatives of antibiotics used commonly in chemotherapy (Casazza & Guiliani, 1984; Formelli & Pollini, 1989; Young, 1989; Robert, 1993; Robert & Gianni, 1993; Chen et al., 2011). Hyperthermia is the way toward raising the temperature of a tissue area higher than this worth. Hyperthermia extend between 42.0°C and 45.0°C has been appeared to modify the activity of numerous organelles and enzymatic proteins inside cells, which prompts putrefaction and necrotic cell demise, hyperthermia may prompt reductions in cell development and separation and can encourage apoptosis. The synopsis of hyperthermia is a malignant growth treatment wherein disease tissue or the entire body is presented to temperatures from 41 to 43°C using electromagnetic vitality for a characterized interval to harm and destroy disease cells. Over this temperature, heat has an immediate cytotoxic impact on both ordinary and cancer cells and is point out to as warm removal (Franckena & van der Zee, 2010). Serum levels of skeletal muscle enzymes are biomarkers of the functional levels of muscle tissue as well as differ generally in both histopathological and physiological circumstances. An elevate in these proteins can be a guide of cell harmful or tissue injury coming after severe with incurable muscle lesions (Mokuno et al., 1987; Szumilak et al., 1998; Cervellin & Comelli, 2010). The object of this investigation was to detect the influence of magnetic oxide nanoparticles on Ehrlich solid carcinoma in mice using thermal ablation.

2. EXPERIMENTAL

Synthesis of magnetic nanoparticles

In a typical synthesis, about 0.11 mM of iron chloride was dispersed in 20 mL of ethylene glycol and stirred until the iron chloride was completely dissolved. This mixture added to a mixture of mercaptopropanol (4 mL), trisodium citrate (4 mL, 30 mM) and sodium borohydride (120 mL, 100 mM). The reaction kept refluxed under nitrogen for 30 min at 90 °C. Then a trimethylamine N-oxide (0.15 mM) was added dropwise. The precipitates were gathered by centrifuge at 14000 rpm/min for 10 min. (Hariani et al., 2013; Peternele et al., 2014; Majeed et al., 2016).

The developed magnetic nanoparticles have been studied via XRD-Regaku-Ultima-IV. The morphology was evaluated by JEOL-JEM-2100F Field Emission Transmission Electron Microscope at 200 KV (Nor et al., 2018). The natural structure of iron oxide nanocrystals was recognized by EDX-S-3000N at 20 KeV (Dukenbayev et al., 2019). The biological studies were performed as follow. Innate adult male albino mice (N= 200) of strain SWR, 20-25g in weight were utilized in this investigation. Whole experimental mice

were 6–8 weeks old and acclimation one week and benefited from their standard pellet and water advertisement. Lib. Also, acquired from the animal house of biological Sciences department, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia. The mice were classified into four groups (50 mice/group) and were housed in standard plastic pens at an ecologically controlled (23°±2, 12-h light/12-h dull cycle). The methods utilized in this investigation are endorsed by Animal Ethics board of trustees of King Abdul Aziz University.

For the in vivo study, four groups were formed. Each group comprised of 50 adult male albino mice and the size range (20-25 Kg). Formed groups are as follows:

Group 1: The normal mice (Control): 50 mice

Group 2: The ESC-bearing mice (ESC): 50 mice, affected group means the Ehrlich solid tumor, in which cancer cells are characterized by rapid diffusion and rapid tumor size in the affected area, which have not yet been treated with any nanoparticles or any other chemical.

Group 3: The ESC-bearing mice treated by multiple doses of the Epirubicin (EPI) chemotherapy: 50 mice. Means the group injected with the 0.1 mg/mice Epirubicin was injected in 6, 9 and 12 days after EAC inoculation, respectively.

Group 4: The ESC-bearing mice treated 50 mice: by single dose Fe_3O_4/MPA nanocubes around Ehrlich solid carcinoma, AC magnetic field intensity of 370 G with frequency of 100 KHz for 15min. with the hyperthermia technique (HT).

To make Ehrlich solid carcinoma in male mice, 0.15 mL of EAC cells (2 $X10^6$ cells/mouse) was injected intramuscularly in the right thigh of the lower limb of mouse.

The size of solid carcinoma was detected by the Vernier caliper after injected with Ehrlich ascites cells. The carcinoma size was estimated by the next equation: tumor size =1/2 (length X $width^2$), where length is the extreme longitudinal diameter and the width is the greatest cross diameter (Jensen et al., 2008).

A tiny slice of the skeletal muscle was rapidly taken away from mouse, and then fixed in formalin. After fixation, sample was dehydrated, prepared paraffin inserting, after that sectioned to 5-7 micrometer thick slices were stained with eosin and hematoxylin Finally the specimens examined under Olympus trinocular microscope (BX-51), and photographs were acquired under various magnifications (Drury & Wallington, 1981; Levison, 1997).

Mice were anesthetized by diethyl ether and blood specimens were obtained from orbital venous plexus in the animal house, Biological Sciences Department, Faculty of Science, King Abdulaziz University. Samples for biochemical parameters analysis of the skeletal muscle of mice are creatinine kinase (CK), aspartate aminotransferase (AST) and ***alanine aminotransferase (ALT***). Blood specimens were centrifuged at 3000 rpm for 10 min then blood serum was collected and keep at -80°C (Hoff, 2000).

This technique for estimation of all out making kinase movement fixation utilizes the International Federation of Clinical Chemistry (IFCC) CK 37°C essential reference strategy methodology; adjusted to the manor Dimension Vista® CKI results are institutionalized to concur with outcome from the IFCC 37°C procedure (Siekmann et al., 2002).

The aspartate aminotransferase technique is an agreement of the process prescribed by IFCC (Bergmeyer et al., 1978). The strategy utilizes the coenzyme pyridoxal-5-phosphate (P5P) to operate the apoenzyme and lactic corrosive dehydrogenase (LDH) to remove of pyruvate obstruction (Tietz, 2006). The Dimension Vista ALT technique is an adjustment of the prescribed alanine aminotransferase methodology of the IFCC as explained by Bergmeyer et al. (1978). This method depends on the standards layout by Wroblewski & Ladue (1956).

Qualities are indicated as the mean ± SD. Information was examined utilizing a single factor (ANOVA) trailed by LSD as a multi correlation test. The degree of significance between mean qualities was set at $P \leq 0.05$. The whole statistical investigation was performed utilizing SPSS programming (version 20) (IBM Corp, 2011).

Ethical approval

Reference No. 328-19, Date: Sunday April 28, 2019

3. RESULTS

Characterization of Fe_3O_4 nanostructures

Figure 1 depicts the XRD patterns of the prepared Fe_3O_4 NPs. The XRD showed diffraction peaks at 2θ= 18.2°, 30.3°, 35.6°, 43.2°, 53.4°, 57.2° and 61.8° which are corresponding to the reflection from the planes (111), (220), (311), (400), (422), (511) and (440) respectively of the cubic spinel Fe_3O_4 crystal structure according to the standard card (JCPDS-75-0033). Figure 2a showed the TEM image of the prepared Fe_3O_4 nanocrystals. It is obvious the Fe_3O_4 nanocrystals have monodispersed nanocubes with mean size of 15±2 nm. Figure 2b revealed histogram for the interplanar distance distribution of the nanocubes. It is note worthy that the mean interplanar distance is around 0.23 nm, which corresponding to the diffraction from the (311) plane of the magnetite nanostructure.

Figure 2c showed the EDS spectra of the prepared Fe_3O_4 nanocubes. All peaks are indexed to iron and oxygen, implying the purity of the sample.

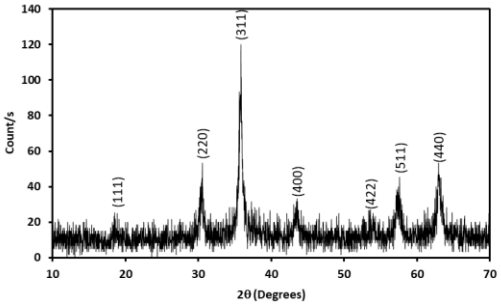

Figure 1 The XRD patterns for the prepared Fe_3O_4 nanoparticles.

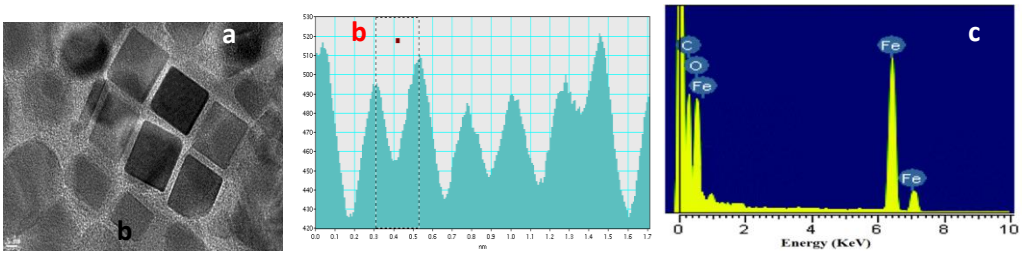

Figure 2 (a) TEM image, (b) Interplanar distance distribution histogram and (c) The EDS spectra of the Fe_3O_4 nanocubes.

***In vivo* cancer treatment by hyperthermia**

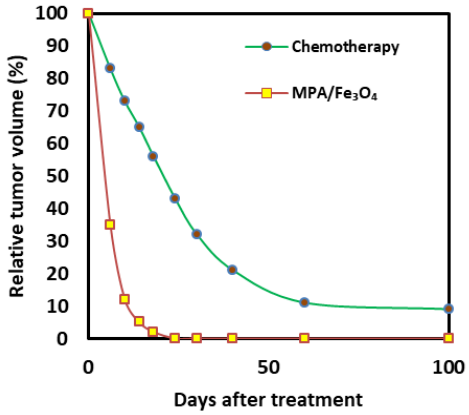

Figure 3 The variation of the relative tumor volume versus time under various treatment conditions.

Table 1 (a) shows the duration of survival of the treated mice by epirubicin and the number of days they lived in and (b) shows the duration of survival of the mice treated and the number of days in which they lived.

Survival rate	50	35	29	19	14	9	4	0
EPI-treatment (days)	0	10	20	30	40	50	60	62
Survival rate	50	48	46	45	43	41	40	39
Hyperthemia-treatment (days)	0	25	50	100	150	200	250	290

Figure 3 showed the variation of the tumor volume of mice during treatment period *via* the injection of Fe_3O_4/MPA nanocubes under the subjection to AC magnetic field and without treatment for comparison. It is noticeable that the tumor volume is increased for un-treated mice. Mice treated by Fe_3O_4/MPA nanocubes impacted that the tumor volume decreased and completely

disappeared after 24 days. However, the survival rate of the treated mice with functionalized magnetic nanocubes after 290 days of treatment is 78%, while the non-treated mice died within about 62 days as presented in (Table 1).

Histological changes in Muscle

Figure 4 a-h showed the normal skeletal muscle. It is made up of muscle bundles and each bundle consists of muscle fibers surrounded by plasma membrane called sarcoplasm. The sarcoplasm and muscle fibroblast is called sarcolemma. The muscular fibula is composed of muscle fibers and the single fiber consists of contiguous muscle segments and muscle parts made up of protein lines, actin and myosin, the cross sectional and the longitudinal section study of the muscles were examined and compared in two different phases: pre-treated mice bearing ESC and treated groups which in turn are divided into two groups (groups related to mice bearing ESC group treated by epirubicin and group correlated to mice bearing ESC group treated by Fe_3O_4/MPA NPs with hyperthermia). In ESC group the fragmentation of the muscle fibers is manifested by the spread of vascular cancer cells and the degeneration of the general form of fibrosis as described in white and the absence of peripheral nuclei which Ehrlich carcinoma cells making solid tumor notice the pleomorphic shape of cell nuclei. In the treatment phase group ESC+EPI decreased appearance of decomposed areas in muscle fibers resulting from necrosis of healthy cells and initiation of necrosis exclusively for cancer cells. The group SEC+ Fe_3O_4/MPA NPs and HT showed large disappearance of areas of fiber erosion because cancer cells die and cells remain intact.

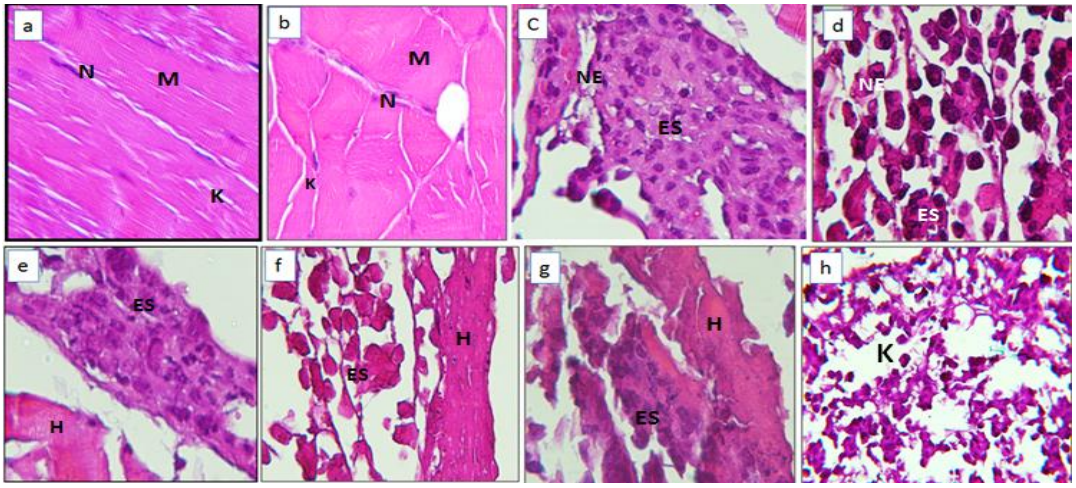

Figure 4 Light microscope images of longitudinal and cross sections of the mice back thigh muscle: (a,b); control. (c, d); Ehrlich solid carcinoma. (e, f); Ehrlich solid carcinoma treated by epirubicin, (g,h); Ehrlich solid carcinoma treated with hyperthermia and Fe_3O_4 NPs. N: end of muscle; M: musculoskeletal fibroblasts; K: sarcoplasm between fibers; NE: irregular granules; ES: cancer cells; H: necrosis of cancer cells.

Blood biochemistry parameters

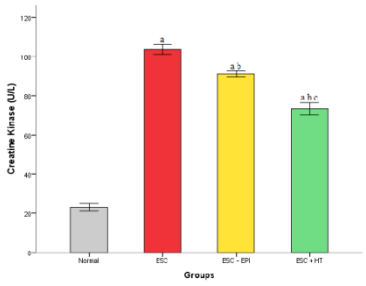

Figure 5a

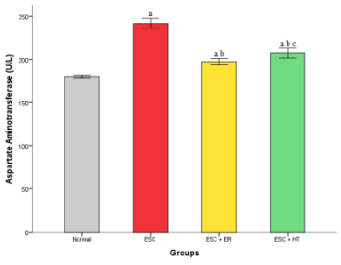

Figure 5b

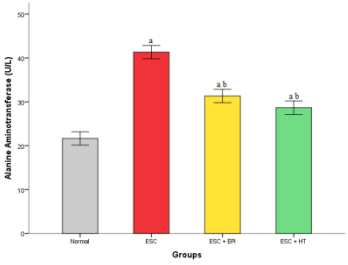

Figure 5c

Figure 5 Difference between all studied groups in adult male albino mice among (a) creatine Kinase U/L (CK), (b) Aspartate Aminotransferase U/L (AST) and (c) Alanine Aminotransferase U/L (ALT).

Data illustrated in figure 5a-c showed that Ehrlich solid carcinoma group (ESC), Ehrlich solid carcinoma treated by epirubicin or Ehrlich solid carcinoma treated with iron oxide nanoparticles/MPA and hyperthermia (ESC+HT) caused in a significant elevate in the activity of the creatinine kinase (CK), Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) relative to the normal group. Though, two treated groups (ESC+EPI) and (ESC+HP) showed high significantly reduced relation to Ehrlich solid carcinoma group. While, ESC+HT was significantly decreased contrasted with ESC+EPI.

4. DISCUSSION

The tumor is the damage of the regular cell cycle, leading in the loose cell development and the absence of discrimination, described as malignant developments. The tumor can happen at any time, in any organ or tissue (Sznarkowska et al., 2017; Wang et al., 2018). Covering of magnetic nanoparticles by 2-mercaptoproponic acid is basic to decrease their collection, enhanced their appropriation and soundness, shields their surface from oxidation, and raises the blood flow time and cell take-up, decrease lethality and gives surface to conjugation of medicines (Wahajuddin & Arora, 2012). According to the XRD, TEM and EDS results, the developed magnetic nanoparticles by our synthetic recipe resulted in cubic crystal structure and nanocubes with high purity. This means the developed synthetic recipe is an efficient method to get nanocarriers and highly magnetic materials for the use for hyperthermia technique for cancer treatment.

The obtained histological findings showed that the ESC group the fragmentation of the muscle fibers is manifested by the spread of vascular cancer cells and the degeneration of the general form of fibrosis as described in white and the absence of peripheral nuclei which Ehrlich carcinoma cells making solid tumor notice the pleomorphic shape of cell nuclei. These results agree with Abdel-Gawad et al. (2016) who found that the skeletal muscle examined by light microscope presented compact and aggregation of Ehrlich cells within the muscle in all wide implanted area. Ehrlich carcinoma showed groups of large, and with pleomorphic shapes. Our outcomes detected that cancer growth was significantly decreased by intratumor magnetic nanoparticles injection contrasted with untreated tumor. These outcomes are supported by Wallimann et al. (1992) who found that the magnetic nanoparticles inhabit cancer cell growth in the muscle.

Creatine kinase (CK) isoenzymes accelerate reaction the adjustable relocate of the phosphate group of phosphocreatine (PCr) to ADP, to produce ATP and creatine. The CK/PCr/Cr structure is current mainly in tissues with high and changing energy requests for examples, cerebrum, cardiac and skeletal muscle, also, serves as a transient and altitudinal "energy buffer" that assistances to keeps up a high intracellular phosphorylation effort in circumstances of evaluated metabolic interest (Dinning & Seager, 1951; Wyss & Kaddurah-Daouk, 2000). Writing information on creatine content and CK indication in the cancer cells and neoplasm-carrying animals, though, give some degree unclear form. There are statements, particularly in the last studies of elevates in creatine content in virulent tissues and in tumor-bearing animals (Gazdar et al., 1981; Bassiony et al., 2014). Many proportional researches on the actions of verious CK isoforms in several ordinary and cancer cells as well as, show varied results. A few investigations describe upregulation of several shape(s) of CK in cancer cells (Carney et al., 1984; Zarghami et al., 1996).

The degrees of fructose-biphosphate aldolase, a catalyst engaged with glycolysis, lactate dehydrogenase, which changes over lactate to pyruvate, as well as the AST and ALT can be increased when the degree of creatinine kinase is ordinary in sick persons by incendiary muscle infection (Rosalki, 1989; Meffert et al., 2005). Aspartate transaminase increase speed the reaction of aspartate and α-ketoglutarate to oxaloacetate and glutamate. This response happens between the mitochondria and cytosol and supplies energy to the cells. The enzyme confined mostly in the skeletal and heart muscle, liver and red blood cells, and is primarily an indicator of liver sickness. While numerous hepatotoxic biomarkers can give data correlative to liver and muscle sickness (Pachman, 1995), serum ALT and AST amount and the AST/ALT ratio have revealed excellent diagnostic precision in sick persons with incurable liver illness (Nyblom et al., 2004; Ozer et al., 2008). In chronic muscle damage, AST and ALT were both elevated (Giannini et al., 2002), although AST action is scarcely increased in focuses with no sickness and ordinary CK effectiveness, proposing the existence of AST macroenzyme (Nathwani et al., 2005). Mice with Ehrlich solid carcinoma display elevated carcinoma size increased expression of ALT and AST (Cabrera-Abreu et al., 2008). Alanine aminotransaminase (ALT) and aspartate aminotransferase (AST) were estimated in the mice's blood sera exhibited elevated values in the tumor group contrasted with the normal group.

5. CONCLUSION

A magnetic nanoparticle has been successfully prepared via colloidal technique. The XRD measurement depicted that cubic spinel Fe_3O_4 nanoparticles have been formed. The examined HRTEM showed that the formed Fe_3O_4 nanoparticles have a shape like nanocubes with mean size 15±2nm. The EDS estimate revealed that the formed Fe_3O_4 nanoparticles are highly pure. The effect of Ac magnetic field on the Fe_3O_4 nanocubes showed that a heat generated reached 45ºC at concentration of 50 mg/mL, which is required for cancer treatment via hyperthermia technique. The *in vivo* cancer treatment test showed that the functionalized Fe_3O_4 nanocubes

could cure the cancer tumor of the mice for 24 days and the rate of survival was 80%. The results of the current research observed that the influence of Fe_3O_4/MPA with hyperthermia on the histological alterations of skeletal muscle and muscle biochemical parameters in ESC bearing mice and treated mice, significant decreases were observed in CK, AST and ALT after injected the EAC bearing-mice by Fe_3O_4/MPA with hyperthermia compared with EAC bearing-mice.

List of Abbreviations

ESC: Ehrlich Solid Carcinoma; CK: creatinine kinase: AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; MRI: Magnetic Resonance Imaging; NPs: Nanoparticles; EPI: Epirubicin; HT: Hyperthermia Technique; IFCC: International Federation of Clinical Chemistry; P5P: Pyridoxal-5-phosphate; LDH: Lactic Corrosive Dehydrogenase; EDS: Energy Dispersive Spectroscopy; PCr: Phosphocreatine; ADP: Adenosine Diphosphate; ATP: Adenosine Triphosphate; CK/PCr/Cr: Creatinine Kinase/Phosphocreatine/Creatinine; HRTEM: High Resolution Transmission Electron Microscope; MIONs: Magnetic Iron Oxide Nanoparticles; $Fe_3O_{4::}$ Ferrous Ferric Oxide; Fe_3O_4/MPA NPs: Ferrous Ferric Oxide/ mercaptoproponic acid .Nanoparticles

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Conflicts of Interest

The authors declare no conflict of interest.

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