**ORIGINAL ARTICLE**

# An In-Vivo Study of Sonodynamic Therapy with Encapsulated Hematoporphyrin

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## Abstract

**Purpose:** According to the side effects of invasive cancer treatments, Sonodynamic Therapy (SDT) as a noninvasive method for breast adenocarcinoma was considered. Sonosensitizer agents' encapsulation can improve the accumulation of these drugs in the tumor tissue and reduce treatment side effects. Hence, mice breast adenocarcinoma SDT with Hematoporphyrin (HP) and HP-encapsulated Mesoporous Silica Nanoparticles (HP-MSNs) was carried out.

**Materials and Methods:** 96 female breast adenocarcinoma grafted Balb/C mice were randomly divided into 16 groups (n = 6): control, sham, HP, HP-MSN, Ultrasound (US), SDT+HP, and SDT+HP-MSN groups. Sonosensitizer agents were injected intraperitoneally (2.5 or 5 mg/kg, 0.2 ml) 24h before an US radiation (1MHz, 1 or 2 W/cm$^2$, 60 sec). The tumor growth parameters were evaluated 30 days after SDT.

**Results:** The inhibition ratio was enhanced by 23, 18, 18, and 16% relative to the control group in HP-MSN (5 mg/kg), HP-MSN (2.5 mg/kg) HP (5 mg/kg) and US (2 W/cm$^2$) groups, respectively, at 18 days after the injection time; whereas, the analysis of findings revealed an antitumor effect in SDT with HP-MSN groups. The Tumor Growth Inhibition (TGI) percentages were 45, 42, and 42% for the SDT (2 W/cm$^2$) + HP-MSN (5 mg/kg), SDT (1 W/cm$^2$) + HP-MSN (5 mg/kg), and SDT (2 W/cm$^2$) + HP (2.5 mg/kg) groups, respectively, on the 18th day post-injection, and T2 and T5 times were higher than that of control and sham groups ($P<0.05$). The estimated 44-day survival time in the Kaplan-Meier test was 95% in the SDT (2 W/ cm$^2$) + HP-MSN (5 mg/kg) treated group, which had moderately differentiated cells in tumor grading.

**Conclusion:** Based on the findings, SDT with HP-encapsulated MSNs (5 mg/kg) has an antitumor effect on breast adenocarcinoma.

**Keywords:** Breast Adenocarcinoma; Sonodynamic Therapy; Hematoporphyrin; Mesoporous Silica Nanoparticles.


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## 1. Introduction

Breast cancer is currently one of the most important reasons for death in women [1]. It accounts for about 22% of all cancers, and 70% of breast cancer patients die due to late diagnosis. Breast cancer is often curable in the early stages. Therefore, considering the stage and the spread of the disease at the time of the onset of treatment is very essential [2, 3]. Surgery, radiotherapy, and chemotherapy are the main treatments for cancer. Although these methods are effective in the early stages of cancer, the traditional procedures have numerous weaknesses, such as systemic toxicity and drug resistance. However, the limitations of these therapies have led researchers to explore new minimally invasive cancer treatments [4]. Today, modern methods of gene therapy, immunotherapy, photodynamic therapy, and Sonodynamic Therapy (SDT) have been introduced to the medical community [5, 6]. In 1989, SDT was introduced as a non-invasive method. In this technique, Ultrasound (US) waves and a sensitizing agent are used to damage cancer cells [4, 7]. Since US can penetrate deeply, it could effectively activate the sensitizer [8]. The creation of transient cavities is an effective factor in performing sonochemical responses to low-energy US waves that can be effective in the treatment of tumors [9-12]. Yet several kinds of sonosensitizers include porphyrin-based sonosensitizers, xanthene-based sonosensitizers, and non-steroidal anti-inflammatory drug-based sonosensitizers announced [13]. Sonosensitizers (Hematoporphyrin (HP) and its derivatives) can be stored in cancer tissue and are provoked by sonication that produces activated oxygen and induces cell killing [14].

Antitumor drugs enter the circulatory system and involve all healthy and malignant cells. To overcome this problem, the targeted drug delivery system has been designed to carry effective doses of the drug to target cells [15]. Numerous categories of nanoparticles were used to reinforce the performance of tumor management in combination with sonotherapy [16]. Mesoporous Silica Nanoparticles (MSNs) are useful inorganic nanoparticles that have good loading capacity [17, 18]. The Food and Drug Administration (FDA) authorized MSN for drug transfer procedures, owing to amorphous silica nanoparticles (Cornell dots) [19]. MSN has been employed for many biological purposes, such as gene transport and expression, bio-marking, bio-signal probing, imaging, detecting agents, and drug delivery [20]. The results of an in-vitro study indicated that HP encapsulated MSNs decrease the viability and proliferation of breast cancer cell lines (MCF7) [21]. Besides, the data of an in-vivo investigation showed that HP-MSN injection with 60sec sonication 3 MHz (2 W/cm$^2$) has a temporary effect on mice breast adenocarcinoma [22]. Hence, the main goal of this study was to estimate the therapeutic effect of lower frequency US (1 MHz, 1 or 2 W/cm$^2$) and MSN (2.5 or 5 mg/kg) in the management of mouse breast adenocarcinoma. The outcome of this research was assessed by several tumor growth factors.

## 2. Materials and Methods

### 2.1. Chemicals and Drug-Loaded

HP 50% (Sigma-Aldrich, Canada) was dissolved in Phosphate-Buffered Saline (PBS, pH = 7.4) and preserved in a dark place at 4 ˚C. Based on the Vazquez *et al.*, protocol, the synthesis of MSNs was performed by a sol-gel process [23]. This process implicates the development of the mesoporous structure [20, 24] using Tetraethyl Orthosilicate (TEOS) as alkoxide precursor and Cetyltrimethylammonium Bromide (CTAB) as surfactant. The particles were stored at room temperature overnight and then were calcined at 550˚C for 3h. Organic components (CTAB and polystyrene) are removed by the calcification process to form mesoporous silica particles. The particles exhibit a homogeneous spherical shape in a low concentration of CTAB. With changing the water amount, the spherical morphology modifies, and rod-like particles were attained [23]. In general, the addition of CTAB produces MSNs with a

specific surface area (585 $m^2/g$), pore volume (0.49 $cm^3/g$), pore diameters 2.5-2.8 nm, and the size distribution range of 300-1000 nm (mean particle volume 600 nm) [23]. Conventional MSN can load a dose of the therapeutic drug with 200-300 mg (maximally about 600 mg) drug/1g silica [17, 20]. Because of the passively loading process, the HP solution was homed adjoining to synthesized nanoparticles; hence, HP enters the MSN cavities [25]. Due to the weight of mice, Inbred Balb/C (20 ± 2g), sonosensitizers (HP or HP-MSN) were injected intraperitoneally (2.5 or 5 mg/kg, 0.2 ml) 24h before sonication [26].

### 2.2. Tumor Model

In order to use a syngeneic mouse tumor model of spontaneous breast adenocarcinoma, the primary Balb/C mice with spontaneous breast adenocarcinoma were provided by Tarbiat Modares University (Iran). The histopathological samples of tissue confirmed tumor type and malignancy. After injection of ketamine/xylazine (30 mg/kg, IP) into the primary mouse, the tumors were extracted and were cut into 2-3 mm diameter pieces in PBS. A part of mass tissue was inserted in the inguinal area of the female receptor animals (20gr weight, 6-8 weeks' age) [26]. Suture clips were used to close the grafted site and to prevent infection, Cefazolin (200 mg/Kg) was adjoined to mice's water.

### 2.3. Treatment Groups

The treatment method started when each of the tumor diameters reached 7-10 mm. 96 female Balb/C mice were distributed randomly into 16 groups (n = 6): control, sham, 2 groups of HP injection (2.5 or 5 mg/kg), 2 groups of HP-MSN (2.5 or 5 mg/kg) injection, 2 groups of 1MHz US (1 or 2 $W/cm^2$), 4 groups of 1MHz SDT + HP injection, and 4 groups of 1MHz SDT + HP-MSN injection.

### 2.4. Sonication

For sonication, each experimental animal was anesthetized and placed to move less in the near field of ultrasonic waves (30 cm) in a rectangular water tank (Plexiglas, 25 × 25 × 35 $cm^3$). The sonication demand waves (frequency of 1MHz, intensities of 1 or 2 $W/cm^2$) were engaged by an ultrasonic treatment system (215A model, Novin Medical Engineering, Isfahan, Iran). The time of the sonication process was 60 seconds.

### 2.5. Evaluation of the Antitumor Effect

Every 3 days to 30 days after SDT, as shown in Figure 1, the length (L), width (W), and depth (D) of tumor mass were determined by a digital caliper for estimating tumor volume using Equation (1):

$$V=0.5 \times L \times W \times D \quad (1)$$

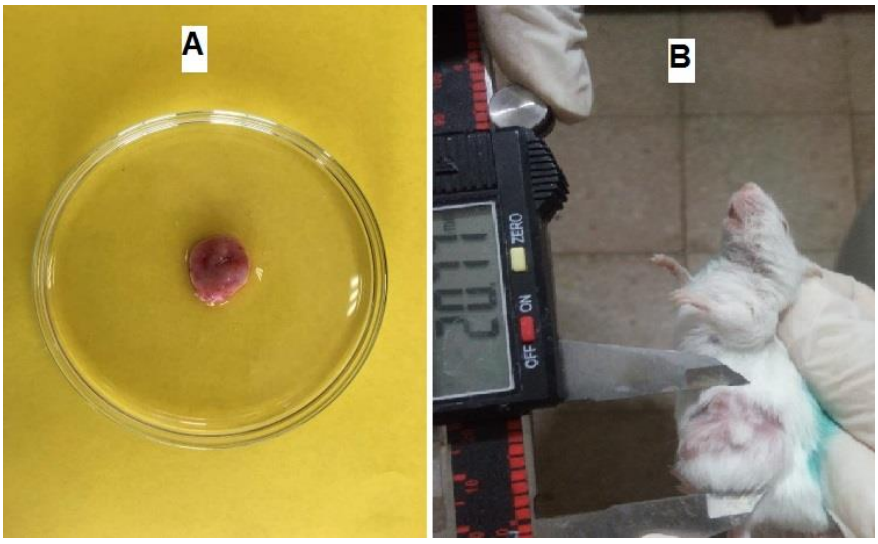


**Figure 1.** Transplanted tumor: reference tumor in Phosphate-Buffered Saline (PBS) solution (A), and grafted tumor dimensions (B)

Tumor growth factors like relative volume, Tumor Growth Inhibition ratio (TGI), and the time required for each tumor to reach two (T2) and five times (T5) the primary tumor volume were estimated by the obtained volumes (V). Tumor relative volume (percent of volumetric change relative to the primary tumor volume) and TGI were assessed by the Equations (2) and (3) [27]:

$$\text{Tumor relative volume} = [(V_P - V_o) / V_o] \times 100 \quad (2)$$

$V_P$ = Tumor volume in a distinct day

$V_o$ = Primary tumor volume

$$TGI\% = [1 - (V_T / V_c)] \times 100 \quad (3)$$

$V_T$ = Normalized tumor volume in a distinct day / Primary day tumor volume in the treatment group

$V_c$ = Normalized tumor volume in the same day / Primary day tumor volume in the control group

In an analysis of cancer mass growth with tissue images, tumor sections from sacrificed mice (ketamine / xylazine, 30 mg/kg, IP) were obtained and stained with hematoxylin/eosin 30 days after treatment.

The tumor grading and malignancy were assessed based on Bloom-Richardson (BR) classification: tumor tubule formation, the number of mitosis/10 high power fields, and nuclear grade. Tumor grading degrees are classified as low (well-differentiated), intermediate (moderately-differentiated, and high (poorly-differentiated) [28]. The number of tumor volumes and pathological examination were carried out blindly.

### 2.6. Statistical Analysis

The normal distribution of records was evaluated by the Tukey test. One-way ANOVA and Kruskal-Wallis tests (SPSS 16.0 software) were employed to check the statistical differences between groups. Statistical differences were deemed significant at the $P < 0.05$ level. Kaplan-Meier survival analysis was used to estimate the survival period of animals.

## 3. Results

To estimate the results of 60-second sonication with sonosensitizer (HP and HP-MSN) on breast adenocarcinoma, the relative tumor volume was evaluated. Figure 2 illustrates tumor growth curves based on the relative volume and post-treatment time (days). These results verified that injection of 5 mg/kg sonosensitizer (HP and HP-MSN) was useful in delaying tumor enlargement after 15 days of injection. Analysis of the data showed non-significant differences between groups prior to 15 days ($P > 0.05$). The desired times for T2 and T5 are prepared in Figure 3. The analysis of findings showed that the time of T2 in the group of HP-MSN (5 mg/kg) injection was higher than that of the control and sham groups ($P < 0.05$). The TGI was shown in Figure 4 over 30 days of post-treatment. The inhibition ratio in the 5 mg/kg sonosensitizer groups (HP and HP-MSN) increased compared with others. The inhibition ratio was enhanced by 23, 18, 18, and 16% relative to the control in the HP-MSN (5 mg/kg), HP-MSN (2.5 mg/kg), HP (5 mg/kg) and US (2 W/cm$^2$) groups, respectively, at 18 days after the injection time. The findings demonstrate that these groups have valuable treatments for breast adenocarcinoma.

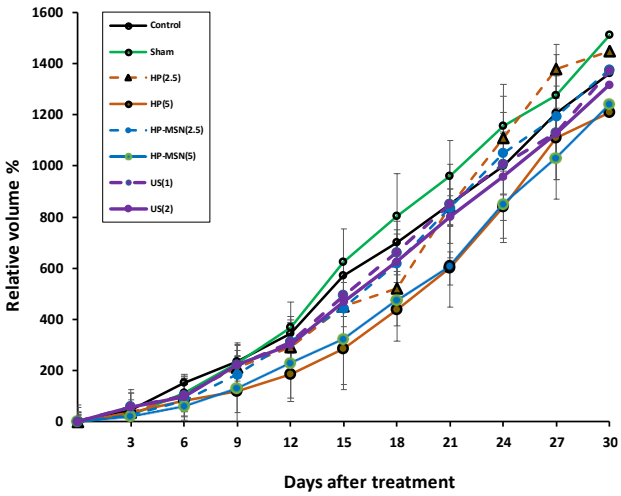


**Figure 2**. The relative volume percent of adenocarcinoma tumors for control, sham, Hematoporphyrin (HP) (2.5 mg/kg), HP (5 mg/kg), HP- Mesoporous Silica Nanoparticles (MSN) (2.5 mg/kg), HP-MSN (5 mg/kg), 1 MHz Ultrasound (US) (1 W/cm$^2$), and 1MHz US (2 W/cm$^2$) groups. The data expressed as mean ± SD

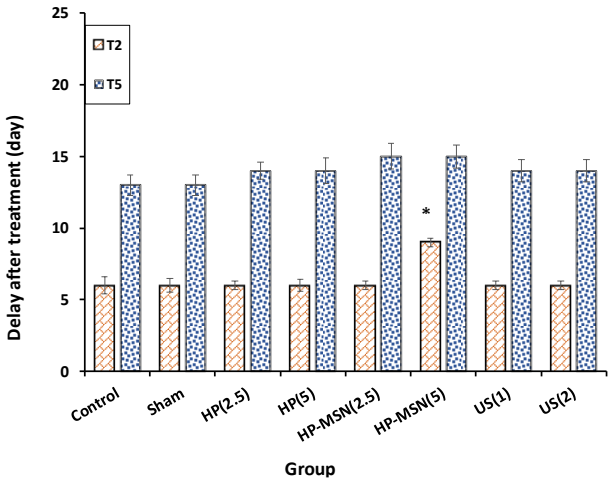


**Figure 3.** The time required for each tumor volume to reach two (T2) and five times (T5) the primary volume in different groups: control, sham, HP (2.5 mg/kg), HP (5 mg/kg), HP-MSN (2.5 mg/kg), HP-MSN (5 mg/kg), 1MHz US (1 W/cm$^2$), and 1MHz US (2 W/cm$^2$) groups. The mean time of T2 in the case of HP-MSN (5 mg/kg) group is greater than that in the sham and control groups. The data expressed as mean ± SD. * ($P < 0.05$)

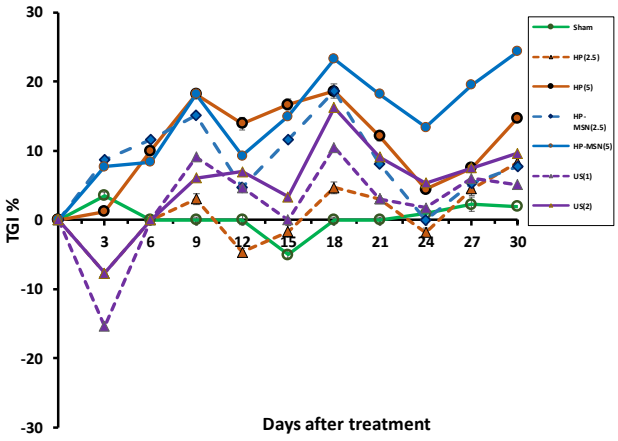


**Figure 4.** The tumor growth inhibition percent (TGI%) in the following treatment groups: sham, HP (2.5 mg/kg), HP (5 mg/kg), HP-MSN (2.5 mg/kg), HP-MSN (5 mg/kg), 1MHz US (1 W/cm$^2$), and 1MHz US (2 W/cm$^2$)

To confirm the findings of this study, we need to assess the results of SDT with HP and HP-MSN on adenocarcinoma growth. Hence, we estimated the antitumor outcomes of US (1 or 2 W/cm$^2$) in cooperation with sonosensitizer (HP and HP-MSN). Figure 5 illustrates the relative tumor volume and post-treatment time. A significant difference was observed between the experimental groups and sham in tumor volume, 15 days after treatment ($P < 0.05$). Evaluating of the data verified that the groups of SDT (2 W/cm$^2$) + HP-MSN (5 mg/kg), SDT (1 W/cm$^2$) + HP-MSN (5 mg/kg) had better results in cancer treatment (from days 18 to 27 after drug injection). The desired times for T2 and T5 are presented in Figure 6, as T2 and T5, respectively.

The time of T2 in the case of SDT (2 W/cm$^2$) + HP-MSN (5 mg/kg) is higher than that in the control and sham groups ($P < 0.05$). The required time of T5 to the initial volume in the groups of SDT (2 W/cm$^2$) + HP (2.5 mg/kg), SDT (2 W/cm$^2$) + HP (5 mg/kg), SDT (1 W/cm$^2$) + HP-MSN (5 mg/kg) and SDT (2 W/cm$^2$) + HP-MSN (5 mg/kg) was higher than that in the control and sham groups ($P < 0.05$). The inhibition ratio along with the treatment time was shown in Figure 7. The TGI percentages were 45, 42, and 42% for the SDT (2 W/cm$^2$) + HP-MSN (5 mg/kg), SDT (1 W/cm$^2$) + HP-MSN (5 mg/kg), and SDT (2 W/cm$^2$) + HP (2.5 mg/kg) groups, respectively, on the 18th day post-injection.

The Kaplan-Meier evaluation demonstrated that the 44-day survival was 95% for the group of SDT (2 W/cm$^2$) + HP-MSN (5 mg/kg). As shown in Figure 8, the survival meantime (with 95% confidence interval) for the control, US (2 W/cm$^2$), HP-MSN (5 mg/kg), SDT (2 W/cm$^2$) + HP (5 mg/kg)

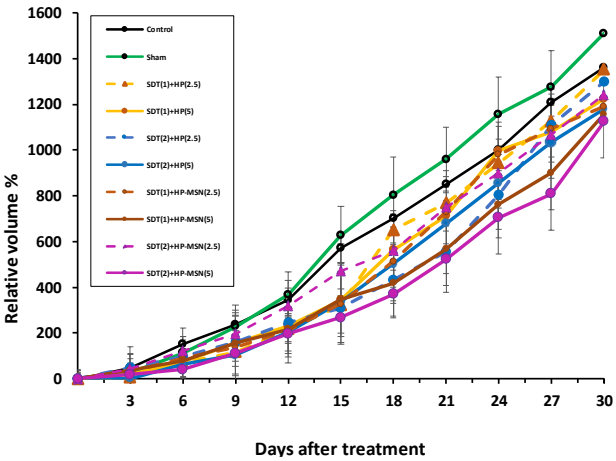


**Figure 5.** The relative volume percent of adenocarcinoma tumors for the following treatment groups: control, sham, Sonodynamic Therapy (SDT) (1 W/cm$^2$) + HP (2.5 mg/kg), SDT (1 W/cm$^2$) + HP (5 mg/kg), SDT (2 W/cm$^2$) + HP (2.5 mg/kg), SDT (2 W/cm$^2$) + HP (5 mg/kg), SDT (1 W/cm$^2$) + HP-MSN (2.5 mg/kg), SDT (1 W/cm$^2$) + HP-MSN (5 mg/kg), SDT (2 W/cm$^2$) + HP-MSN (2.5 mg/kg), and SDT (2 W/cm$^2$) + HP-MSN (5 mg/kg). The data expressed as mean ± SD

and SDT (2 W/cm$^2$) + HP-MSN (5 mg/kg) groups was 32, 32, 39, 38, and 44 days, respectively; overall comparison test of survival equality for the different levels of groups demonstrated the

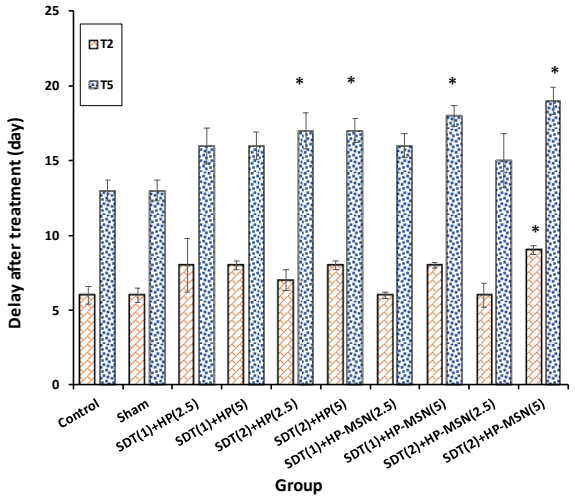


**Figure 6.** The required times for each tumor to reach two (T2) and five times (T5) its primary volume for all treatment groups: control, sham, SDT (1 W/cm$^2$) + HP (2.5 mg/kg), SDT (1 W/cm$^2$) + HP (5 mg/kg), SDT (2 W/cm$^2$) + HP (2.5 mg/kg), SDT (2 W/cm$^2$) + HP (5 mg/kg), SDT (1 W/cm$^2$) + HP-MSN (2.5 mg/kg), SDT (1 W/cm$^2$) + HP-MSN (5 mg/kg), SDT (2 W/cm$^2$) + HP-MSN (2.5 mg/kg), and SDT (2 W/cm$^2$) + HP-MSN (5 mg/kg). The mean time of T2 in the case of SDT (2 W/cm$^2$) + HP-MSN (5 mg/kg) is greater than that in the control and sham groups, * ($P < 0.05$). The required time of T5 in the groups of SDT (2 W/cm$^2$) + HP (2.5 mg/kg), SDT (2 W/cm$^2$) + HP (5 mg/kg), SDT (1 W/cm$^2$) + HP-MSN (5 mg/kg), and SDT (2 W/cm$^2$) + HP-MSN (5 mg/kg) is greater than that in the control and sham groups. The data expressed as mean ± SD. * ($P < 0.05$)

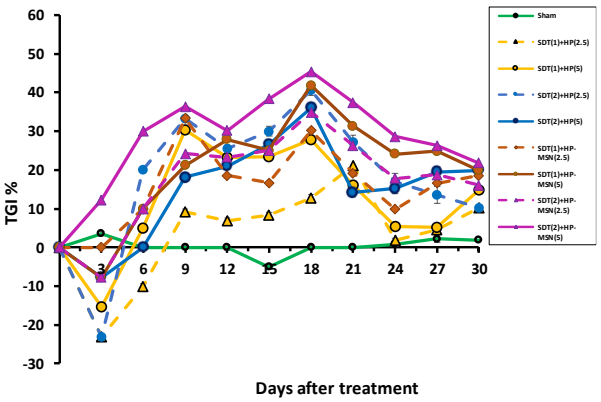


**Figure 7.** The tumor growth inhibition percent (TGI%) in the following treatment groups: sham, SDT (1 W/cm$^2$) + HP (2.5 mg/kg), SDT (1 W/cm$^2$) + HP (5 mg/kg), SDT (2 W/cm$^2$) + HP (2.5 mg/kg), SDT (2 W/cm$^2$) + HP (5 mg/kg), SDT (1 W/cm$^2$) + HP-MSN (2.5 mg/kg), SDT (1 W/cm$^2$) + HP-MSN (5 mg/kg), SDT (2 W/cm$^2$) + HP-MSN (2.5 mg/kg), and SDT (2 W/cm$^2$) + HP-MSN (5 mg/kg)

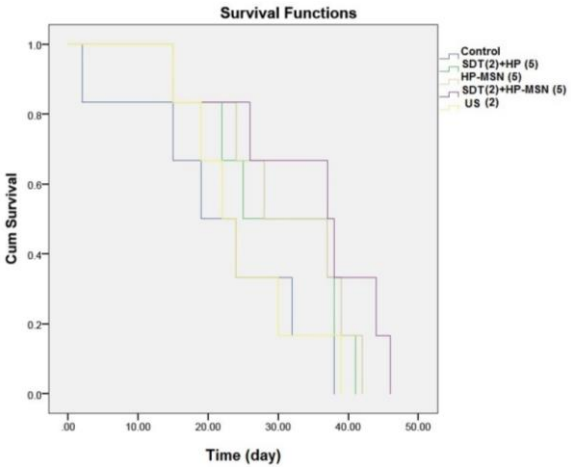


**Figure 8**. Kaplan-Meier curve comparing the cumulative survival fraction (day) between control, 1MHz US (2 W/cm$^2$), HP (5 mg/kg), SDT (2 W/cm$^2$) + HP (5 mg/kg) and SDT (2 W/cm$^2$) + HP-MSN (5 mg/kg) experimental groups at 50 days after treatment, the survival mean time (with 95% confidence interval) was 32, 32, 39, 38, and 44 days, respectively

least differences between experimental groups: Log Rank (Mantel-Cox), P = 0.173. To verify tumor growth analysis, microscopic assessment of cancer samples demonstrated multiple nuclear mitosis and polymorphism in all treated groups. The findings of the histopathological study and total tumor grading according to the BR classification were presented in Table 1. All experimental groups have grade 3 (poorly differentiated), apart from the SDT (2 W/cm$^2$) + HP-MSN (5 mg/kg) group, which has grade 2 (moderately differentiated) in tumor malignancy.

## 4. Discussion

In the current research, the effect of 1 MHz SDT with HP and HP-MSN on mouse breast adenocarcinoma was investigated. Tumor growth parameters were utilized to assess the treatment method. A single dose of HP (2.5 mg/kg) did not affect tumor relative volume, and T2 time was equal in all experimental groups. However, results showed that an increase in HP injection dose (5 mg/kg) is operative on the tumor relative volume and delays tumor growth (T2 and TGI %) after 9 days of treatment. These findings are consistent with the results of Quan-hong *et al.*, and Yumita *et al.*

**Table 1**. The Bloom-Richardson (BR) classification of tumors in the control, sham, HP (5 mg/kg), 1 MHz US (2 W/cm$^2$), SDT (2 W/cm$^2$) + HP (5 mg/kg), and SDT (2 W/cm$^2$) + HP-MSN (5 mg/kg) experimental groups

| Group | Tumor tubule formation | Number of mitosis | Nuclear grade | Total score | BR grade | Grade |
|---|---|---|---|---|---|---|
| **Control** | 3 | 3 | 3 | 9 | Poorly Differentiated | 3 |
| **Sham** | 3 | 3 | 3 | 9 | Poorly Differentiated | 3 |
| **HP-MSN (5 mg/kg)** | 2 | 3 | 3 | 8 | Poorly Differentiated | 3 |
| **US (2 W/cm$^2$)** | 2 | 3 | 3 | 8 | Poorly Differentiated | 3 |
| **US (2 W/cm$^2$) + HP (5 mg/kg)** | 3 | 2 | 3 | 8 | Poorly Differentiated | 3 |
| **US (2 W/cm$^2$) + HP-MSN (5 mg/kg)** | 1 | 3 | 3 | 7 | Moderately Differentiated | 2 |

They concluded that injection of HP had no effect [29, 30]. Injection of 2.5 mg/kg HP-MSN conducted any alterations between experimental groups. Moreover, a dose-dependent response was induced after the injection of 5 mg/kg of HP-MSN [21]. In agreement, Osminkina demonstrated that injection of MSN had no inhibitory effect on Lewis lung carcinoma growth [31]. It is clear that sonotherapy with HP-MSN, is more competent than non-encapsulated HP, owing to an increase in permeability, drug solubility, and more accumulation in the tumor [32]. Checking the outcomes showed that 60sec sonication had less effect at 1 W/cm$^2$, while having a more therapeutic effect at 2 W/cm$^2$ intensity. In agreement with our study, Barati *et al.*, showed that dual sonication (1 MHz + 150 kHz) for 30 min decreased mouse breast adenocarcinoma tumor growth without any thermal effects [33].

According to the present results, in a combination of sonication and HP, the SDT (2 W/cm$^2$) + HP (5 mg/kg) group had more useful effects than others, undoubtedly because of US intensity and the higher dose of HP. In agreement, an assessment of the SDT (1 MHz + 150 kHz) effects disclosed that SDT with dual-frequency and HP (5 mg/kg) induced a significant decrease in the relative volume percent of mouse breast adenocarcinoma [27]. Moreover, Yue *et al.* demonstrate that SDT (1 MHz, 1.5 W/cm$^2$) with HP monomethyl ether eliminates the 4T1 murine breast cancer cell line [34]. As Quan-hong *et al.*, Lv *et al.*, and Gao *et al.* reported, when US was employed with HP, high toxicity and inhibitory effects were better than with US and HP [30, 35, 36]. In the present study, 5 mg/kg HP-MSN was injected 24 hours prior to US. From the results, the group of SDT (2 W/cm$^2$) + HP-MSN (5 mg/kg) had higher harmful effects. Analysis of this group's relative tumor volume showed a temporary antitumor effect. In harmony with our results, Zheng *et al.* investigated that sonication with capsulated HP is more useful than noncapsulated HP or sonication [16]. In addition, the combination of MSN and sonication prevented cancer cell increment [31].

Many antitumor drugs involve all healthy and malignant cells. To overcome this problem, the targeted drug delivery system is designed to carry effective doses of the drug to the target cells [29]. A remarkable class of inorganic nanomaterials for medical purposes is MSN. Although most MSNs have diameters of 50-300 nm and narrow pore size of 2-6 nm, the structure, and morphology of MSNs are controllable, yielding high surface area (> 300 m$^2$/g) and large pore volumes (> 0.9 cm$^3$/g) that enable a high cargo carrying capacity [37]. In order to control the anticancer drug delivery in tumor cells with gate-keeping functions, mesoporous silica materials have been widely used. This gate-keeper system enables drug release as a reply to

external stimuli and chemical conditions of cell environments such as pH, oxidation-reduction, enzymatic degradation, temperature, electricity, magnetic fields, and photo-irradiation responses [24]. Hence, MSN lends a new platform for various purposes, such as diagnostic methods for cancer and drug delivery [18, 38].

Sonication as its non-invasiveness and tissue penetration was concerned in cancer treatment [18]. The bio-effects of US (heat, mechanical effects, and cavitation) are dependent on the wave's frequency and intensity. An increase in wave intensity causes heat production, and with the decline in frequency, cavitation has occurred [10]. Hence, an increase in cell damage could be induced by an intensity increment [29, 39]. Apart from the thermal effect, US has the capability to modify biological systems [10]. Cavitation has non-inertial and inertial types. Chemical effects such as pyrolysis of the water and the production of reactive hydroxyl radicals can be produced by inertial cavitation [9].

Sonotherapy, based on the synergy of sensitizer and sonication, comprises several mechanical, chemical, and cavitational activated mechanisms [33]. Yet many types of sonosensitizers, including porphyrin-based sonosensitizers, Xanthene-based sonosensitizers, and non-steroidal anti-inflammatory drug-based sonosensitizers were announced [13]. Sonication by increasing the cell membrane's permeability and improvement of drug release would extend the action of sensitizers [10]. Cavitation is the main process of collaboration between anti-tumor drugs and US exposure. Free radicals that are induced by cavitation can be detrimental to cell membranes, which are followed by membrane permeability. As well, US waves provoking the collapse of microspheres may be employed in drug delivery. Hence, if anticancer drugs are encapsulated, they can go over to the targeted tumor, and then sonication-induced collapse can discharge the drugs. Using this method, anticancer drug molecules are targeted at cancer tissue. In this situation, cavitation bubbles collapse can release the drugs and permeable the surrounding vessel endothelium [10].

These effects may be increased by membrane destabilizing molecules such as porphyrins, which reveal sonication-induced cell destruction [9]. The encapsulation may raise the drug concentration in an aqueous location. In addition, in order to advance the consequences of tumor management, the stimuli-response of drug release methods has evolved. The cargo will be delivered in response to many parameters, such as pH, hyperthermia, light, or US [40, 41]. The interaction of nanoparticles with US waves can generate acoustic cavitation. The collapse of cavitating bubbles can generate sono-mechanical and sono-chemical cytotoxic effects, such as the formation of cytotoxic reactive oxygen species [42], including singlet oxygen and hydroxyl radicals (the further formation of $H_2O_2$ and peroxyl radicals), could kill tumor cells via apoptosis and necrosis [43]. The mitochondria-caspase signaling pathway was activated in the SDT that caused apoptosis of cancer cells, and US stimulates the expression of pro-apoptotic proteins such as Bax and Caspase-3 in cancer cells. US exposure could make cancer cells more sensitive to anticancer drugs, which is a noninvasive physical method to convince the chemo-drug resistance inversion in tumors [12].

As presented formerly, histopathological results confirmed that US (2 W/cm$^2$) and HP-MSN (5 mg/kg) diminished the tumor malignancy to moderately differentiated (BR intermediate, grade 2), nevertheless the relative tumor volume percent increased during the 30 days and TGI% decreased gradually. This variation shows that injection of 5 mg/kg HP-MSN with 60sec US (1 MHz, 2 W/cm$^2$) has a temporary effect on tumor treatment, and possibly we need fractional sonication and multiple HP-MSN injections for better treatment of breast adenocarcinoma tumors. These data are the same as our previous investigation results with 3 MHz SDT [21], which means that the results of SDT aren't frequency-dependent and are not only determined by US wave power density but also related to HP-MSN injection dose. In both frequencies, the tumor malignancy

declined with an increase in sonication power density and HP-MSN injection dose. On the other hand, improved examinations and further trials should be done to find better tumor treatment methods and clarify the mechanism of these occurrences.

## 5. Conclusion

The results of this study demonstrated that SDT with 60sec 1MHz (2 W/cm$^2$) and HP encapsulated MSNs (5 mg/kg) had an inhibitory effect on mouse breast adenocarcinoma. It can be appreciated that the precise choice of the nanoparticles with sonication will play a significant role in the success of minimally invasive cancer therapies.

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## References

1- G.L. Schattman and J. Navarro, "Breast cancer and fertility preservation." *Placenta*, Vol. 29pp. 147-51, (2008).

2- B.M. Rabatic, "Experimental therapies in breast cancer." *Breast Cancer Biology Radiation Oncologist*, pp. 81-90, (2014).

3- T.J. Dougherty *et al.*, "Photodynamic therapy." *Journal National Cancer Institute*, Vol. 90pp. 889-905, (1998).

4- H. Chen, X. Zhou, Y. Gao, B. Zheng, F. Tang, and J. Huang, "Recent progress in development of new sonosensitizers for sonodynamic cancer therapy." *Drug Discovery Today*, Vol. 19pp. 502-09, (2014).

5- M. Intra *et al.*, "A new option for early breast cancer patients previously irradiated for Hodgkin's disease: intraoperative radiotherapy with electrons (ELIOT)." *Breast Cancer Research*, Vol. 7 (No. 5), pp. R828-R32, (2005).

6- M. Rezaei-Tavirani, E. Dolat, H. Hasanzadeh, S.S. Seyyedi, V. Semnani, and Sobhi S., "TiO2 nanoparticle as a sensitizer drug in radiotherapy: in vitro study." *Iranian Journal Cancer Prevention*, Vol. 6pp. 37-44, (2013).

7- K. Tachibana, Jr. LB. Feril, and Y. Ikeda-Dantsuji, "Sonodynamic therapy " *Ultrasonics*, Vol. 48pp. 253-59, (2008).

8- A.P. McHale, J.F. Callan, N. Nomikou, C. Fowley, and B. Callan, "Sonodynamic therapy: concept, mechanism and application to cancer treatment." *Therapeutic Ultrasound*, Vol. 880pp. 429-50, (2016).

9- I. Rosenthal, J.Z. Sostaric, and P. Riesz, "Sonodynamic therapy- a review of the synergistic effects of drugs and ultrasound." *Ultrasonics Sonochemistry*, Vol. 11pp. 349-63, (2004).

10- T. Yu, Z. Wang, and Mason T.J., "A review of research into the uses of low level ultrasound in cancer therapy." *Ultrasonics Sonochemistry*, Vol. 11pp. 95-103, (2004).

11- H. Shibaguchi, H. Tsuru, M. Kuroki, and M. Kuroki, "Sonodynamic cancer therapy: a non-invasive and repeatable approach using low-intensity ultrasound with a sonosensitizer." *Anticancer Research*, Vol. 31pp. 2425-29, (2011).

12- W.K. Bai, E. Shen, and B. Hu, "Induction of the apoptosis of cancer cell by sonodynamic therapy: a review." *Chinese Journal Cancer Research*, Vol. 24pp. 368-73, (2012).

13- G.Y. Wan, Y. Liu, B.W. Chen, Y.Y. Liu, Y.S. Wang, and N. Zheng, "Recent advances of sonodynamic therapy in cancer treatment " *Cancer Biol Med*, Vol. 13 (No. 3), (2016).

14- M. Kuroki *et al.*, "Sonodynamic therapy of cancer using novel sonosensitizers." *Anticancer research*, Vol. 27 (No. 5), pp. 3673-78, (2017).

15- N. Yumita, R. Nishigaki, and S.I. Umemura, "Sonodynamically induced antitumor effect of Photofrin II on colon 26 carcinoma." *Journal Cancer Research Clinical Oncology*, (2000).

16- P. Tharkar, R. Varanasi, W.S.F. Wong, C.T. Jin, and W. Chrzanowski, "Nano-enhanced drug delivery and therapeutic ultrasound for cancer treatment and beyond." *Frontiers in Bioengineering and Biotechnology*, (2019).

17- B.G. Trewyn, S. Giri, I.I. Slowing, and VS-Y. Lin, "Mesoporous silica nanoparticle based controlled release, drug delivery, and biosensor systems." *Chem Commun*, Vol. 31pp. 3236-345, (2017).

18- J.L. Paris, M.V. Cabañas, M. Manzano, and M. Vallet-Regí, "Polymer-grafted mesoporous silica nanoparticles as ultrasound-responsive drug carriers." *ACS Nano*, Vol. 9pp. 11023-33, (2015).

19- P. Dogra *et al.*, "Establishing the effects of mesoporous silica nanoparticle properties on in vivo disposition using imaging-based pharmacokinetics." *Nature communications,* Vol. 9 (No. 4551), pp. 1-14, (2018).

20- C. Beharti, U. Nagaich, A.K. Pal, and N. Gulati, "Mesoporous silica nanoparticles in target drug delivery system: A review." *International Journal Pharmaceutical Investigation,* Vol. 5 (No. 3), pp. 124-33, (2015).

21- A. Khaleghian, H. Hasanzadeh, A. Moshtaghian, R. Nasr, A. Emadi, and S. Moshfegh, "Inhibitory effects of silica nanoparticles loaded with hematoporphyrin on breast cancer cell line." *Middle East J Rehabil Health Stud,* Vol. 5 (No. 3), (2018).

22- S. Jafari, M. Jadidi, H. Hasanzadeh, T. Khani, R. Nasr, and V. Semnani, "Sonodynamic therapy of mice breast adenocarcinoma with HP-MSN." *Iran J Sci Technol Trans Sci,* (2020).

23- Naiara I Vazquez, Zoilo Gonzalez, Begona Ferrari, and Yolanda Castro, "Synthesis of mesoporous silica nanoparticles by sol–gel as nanocontainer for future drug delivery applications." *Boletín de la Sociedad Española de Cerámica y Vidrio,* Vol. 56 (No. 3), pp. 139-45, (2017).

24- M.A. Shahbazi, B. Herranz, and H.A. Santos, "Nanostructured porous Si-based nanoparticles for targeted drug delivery." *Biomatter,* Vol. 2 (No. 4), pp. 1-17, (2012).

25- X. Yu, I. Trase, M. Ren, K. Duval, X. Guo, and Z. Chen, "Design of nanoparticle-based carriers for targeted drug delivery." *Journal Nanomaterials,* (2016).

26- M. Alamolhoda and M. Mokhtari-Dizaji, "Evaluation of fractionated and repeated sonodynamic therapy by using dual frequency for murine model of breast adenocarcinoma." *Journal Therapeutic Ultrasound,* Vol. 3 (No. 10), pp. 1-9, (2015).

27- M. Alamolhoda, M. Mokhtari-Dizaji, A.H. Barati, and H. Hasanzadeh, "Comparing the in vivo sonodynamic effects of dual and single frequency ultrasound in breast adenocarcinoma." *Journal Medical Ultrasonics,* Vol. 39 (No. 3), pp. 115-25, (2012).

28- H.J. Bloom and W.W. Richardson, "Histological grading and prognosis in breast cancer: a study of 1409 cases of which 359 have been followed for 15 years." *Br J Cancer,* Vol. 11pp. 359-77, (1957).

29- N. Yumita, R. Nishigaki, K. Umemura, and Si. Umemura, "Hematoporphyrin as a sensitizer of cell-damaging effect of ultrasound." *Japanese Journal Cancer Research,* Vol. 80 (No. 3), pp. 219-22, (1989).

30- L. Quan-hong *et al.*, "Synergistic anti-tumor effect of ultrasound and hematoporphyrin on sarcoma180 cells with special reference to the changes of morphology and cytochrome oxidase activity of tumor cells." *Journal Experimental Clinical Cancer Research,* Vol. 23 (No. 2), pp. 333-41, (2004).

31- L. Osminkina *et al.*, "Silicon nanoparticles as amplifiers of the ultrasonic effect in sonodynamic therapy." *Bulletin Experimental Biology Medicine,* Vol. 161 (No. 2), pp. 296-99, (2016).

32- S. Gelperina, K. Kisich, M.D. Iseman, and L. Heifets, "The potential advantages of nanoparticle drug delivery systems in chemotherapy of tuberculosis." *Am J Respir Crit Care Med,* Vol. 172 (No. 12), pp. 1487-90, (2005).

33- A.H. Barati, M. Mokhtari-Dizaji, H. Mozdarani, Z. Bathaie, and M.H. Hassan, "Effect of exposure parameters on cavitation induced by low-level dual-frequency ultrasound." *Ultrasonics Sonochemistry,* Vol. 14 (No. 6), pp. 783-89, (2007).

34- Wenwen Yue *et al.*, "Checkpoint blockade and nanosonosensitizer-augmented noninvasive sonodynamic therapy combination reduces tumour growth and metastases in mice." *Nature communications,* Vol. 10 (No. 1), pp. 1-15, (2019).

35- Y. Lv *et al.*, "Antiproliferative and apoptosis-inducing effect of exo-Protoporphyrin IX based sonodynamic therapy on human oral Squamous cell carcinoma." *Scientific Reports,* Vol. 7 (No. 40967), (2017).

36- Hui Gao *et al.*, "pH-Responsive Nanoparticles for Enhanced Antitumor Activity by High-Intensity Focused Ultrasound Therapy Combined with Sonodynamic Therapy." *International Journal of Nanomedicine,* Vol. 17p. 333, (2022).

37- X. Huang, N.P. Young, and H. Townley, "Characterization and comparison of Mesoporous silica particles for optimized drug delivery regular." *Nanomater Nanotechnol,* Vol. 4 (No. 2), pp. 1-15, (2014).

38- T. Wang, L. Zhang, Z. Su, C. Wang, Y. Liao, and Q. Fu, "Multifunctional hollow mesoporous silica nanocages for cancer cell detection and the combined chemotherapy and photodynamic therapy." *ACS Appl Mater Interfaces,* pp. 2479-86, (2011).

39- Q. Liu, X. Li, L. Xiao, P. Wang, X. Wang, and W. Tang, "Sonodynamically induced antitumor effect of hematoporphyrin on Hepatoma 22." *Ultrason Sonochem,* Vol. 15 (No. 6), pp. 943-48, (2008).

40- Shengjie Sun and Meiying Wu, "Sonodynamic Therapy: another “light” in tumor treatment by exogenous stimulus." *Smart Materials in Medicine,* Vol. 2pp. 145-49, (2021).

41- Y. Zheng *et al.*, "Hematoporphyrin encapsulated PLGA microbubble for contrast enhanced ultrasound imaging and sonodynamic therapy." *Journal Microencapsulation,* Vol. 29 (No. 5), pp. 437-44., (2012).

42- G. Canavese *et al.*, "Nanoparticle-assisted ultrasound: A special focus on sonodynamic therapy against cancer." *Chem Eng J,* Vol. 15 (No. 340), pp. 155-72, (2018).

43- Yuki Horise *et al.*, "Sonodynamic therapy with anticancer micelles and high-intensity focused ultrasound in treatment of canine cancer." *Frontiers in Pharmacology,* p. 545, (2019).