IJIRR

International Journal of Information Research and Review
Vol. 08, Issue, 01, pp.7182-7192, January, 2021

RESEARCH ARTICLE

NEW IMPACTS OF *GARCINIA CAMBOGIA* AS SONO/PHOTOSENSITIZER ON EHRLICH ASCITES CARCINOMA BEARING MICE

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ARTICLE INFO

Article History:

Received 16th October, 2020
Received in revised form
29th November, 2020
Accepted 07th December, 2020
Published online 30th January, 2021

Keywords:

Garcinia cambogia, Breast cancer, Ehrlich Ascites Carcinoma, Sonodynamic Therapy, Photodynamic Therapy.

ABSTRACT

Breast carcinoma is a major health problem that threatens the life of women with an increasing need for anticancer therapy. The present study designed to examine the efficiency of using sono/photodynamic therapy (SPDT) in combination with *Garcinia cambogia* as sono/photosensitizer, for Ehrlich Ascites Carcinoma (EAC) therapy. The biochemical markers, hepatorenal injuries markers besides histopathological investigation were harnessed to examine the experimental groups after treatments. This work revealed that tumors from various treated groups of mice bearing the tumor showed different percentages of necrosis and tumor proliferation based on the type of treatment. Tumor excised from mice receiving SPDT with *Garcinia cambogia* treatment showed a significant increase of necrosis compared to other groups without *Garcinia cambogia* as a sensitizer. The group of mice subjected to the combination of SPDT in the presence of *Garcinia cambogia*, showed large foci distinct necrosis areas of large distinct foci of necrosis in the tumor and no tumor proliferation. In conclusion, this study unveiled the possible anti-tumor activity of *Garcinia cambogia* as sono/photosensitizer in combination with SPDT for EAC bearing mice.

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INTRODUCTION

Globally in 2016, cancer was in charge of 9 million deaths with 22% [1]. The number of deaths was escalated in 2018 to 9.5 million with 17 million new cases worldwide. Females breast cancer is observed as one of the most diagnosed cancers. It was noticed as 11.6% of the total cases as well as 6.6% of the total cancer deaths. It was forecasted to be about 2.1 million new cases in 2018, regarding almost 1 in 4 cancer cases among women [2]. In Egypt, the breast neoplasia prevalence represents 19% of total cases with elevation from 1% to 2% per year. Unfortunately, the breast cancer was diagnosed in end stages [3,4]. The most of cases were diagnosed at ages ranged from 30s to 60s. In contrary of Europe and North America countries, women breast carcinoma were diagnosed at 49 years old [3]. Geographically, the Egyptian women in 2014 showed 33.8% for Lower Egypt, 26.8% for Middle Egypt and 38.7% for Upper Egypt of breast carcinoma [5]. Based on the GLOBOCAN 2018, there were 23 081 at 35.1% new diagnosed women cases. Regarding the age-standardized (world) incidence and mortality rates, the incidence rate that was 52.4 cases per 100,000 in opposite to mortality rate that was 21.3 deaths per 100,000 [6].

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In the 1960s, Lipson and Baldes reported the photodynamic therapy (PDT) term. This was after observing fluoresce of porphyrin mixture as photosensitizer under ultraviolet light irradiation in cancerous tissue [7]. Photosensitizer drug is essential for PDT. It is a photosensitive molecule that localizes to a target cell and/or tissue. The light, at a specific wavelength, activates the photosensitizer that transfers energy from light to molecular oxygen O_2, to generate reactive oxygen species (ROS). The exposure to light activates the photosensitizer only in the particular areas of tissue [8]. There were number of studies revealed potential impacts of PDT in early stage breast carcinoma patients [9–11]. The sonodynamic therapy (SDT) term is described as the non-thermally related therapeutic ultrasound (US) applications. Yumita and Umemura were the first discovered the phenomenon of sonochemical activation of photosensitive materials by US for cancer therapy [12]. Also, SDT relies on the generation of ROS through the simultaneous combination of US, O_2 and a sonosensitizer drug [13]. In contrary, the SDT offers significant advantages because US is widely accepted as low cost with high safety [14]. Sonophotodynamic therapy (SPDT), also known as a next generation photodynamic therapy (NGPDT), is the approach of combining SDT with PDT. This combination increases the therapeutic potentials. SPDT approved its efficiency on three breast carcinoma patients who had been exhausted from conventional therapy.

This study revealed significant breast tumor mass reduction in all three patients [15]. Another study revealed that there is significant tumor destruction under SPDT and patients live longer in 75% of cases [16]. Both photosensitizers and sonosensitizers are essential for PDT and SDT. Their therapeutic effects depend on their distribution and uptake in cells and tissues. This is due to their very short lifetime and very short diffusion distance of some radical products derived from the sensitizers [17]. The suggested mechanism of photo / sonosensitizers activities is binding of negatively charged lactate in the neoplastic cell that produced through an anaerobic metabolism to the positively charged sensitizer molecule. This is achieved when light or US activation increases the energy level of the sensitizer, producing ROS. This collapses the organic matter, destroying the cell structure and killing or damaging the cell [18]. *Garcinia cambogia* (*G. cambogia*) or the Malabar tamarind is native plant to Southeastern Asia. The fruit rind is typically used as a food preservative, flavoring agent or food bulking agent [19], and as a traditional remedies to treat several diseases and infections [20]. Its phytochemistry contains many bioactive chemical compounds. It has various organic acids [21], xanthones [22] and benzophenones [23]. The *G. cambogia* benzophenone is the common nucleus of garcinol (also known as camboginol or guttiferone E) and isogarcinol (also known as cambogin) [22] whereas guttiferones I, N, J, K and M, as well as the polyisoprenylated benzophenones [24,25]. The major constituents have indicated biological activity such as antiobesity [26], lipids lowering agent [27] and anticancer activity [28] including breast neoplasia [29]. Our study was tailored to point out the anticancer activity of *G. cambogia* extract as photosensitizer and sonosensitizer on Ehrlich ascites carcinoma (EAC) bearing Swiss albino mice. The treatment was associated with PDT and SDT. The aim of the work was assessed through examined biochemical and pathological studies.

Experimental

***G. cambogia* extract:** In the present work *G. cambogia* extract was used as sono/photosensitizer; chemically active by absorption of light and/or US. The extract was prepared by liquid-liquid extraction method [30]. The extract administered to EAC bearing mice intraperitoneally (i.p.) for 14 days 18-20 hours before exposure to either photo and / or sonodynamic treatment modalities.

Animals and Ehrlich Tumor Cells: The male Swiss albino mice with age 60 – 65 day, weighing 20 ± 2.0 g, were purchased from National Cancer Institute, Cairo University. EAC cells, $2x10^6$ cells/mouse mammary in origin, diluted approximately in 0.9 % saline were inoculated subcutaneously (s.c) on the left dorsal limb region of mice. The animals were housed in plastic cages and were kept under natural light with diet and water at available. When the tumor had grown to about 10 mm in diameter at day 10 after inoculation, the treatment study was started. Use of experimental animals in the study protocol was carried out in accordance with the ethical guidelines of the Medical Research Institute, Alexandria University (Guiding Principles for Biomedical Research Involving Animals, 2011). Mice were divided into group I: (30 mice); a) 10 mice: Normal mice without treatment. b) 10 mice: EAC without treatment. c) 10 mice: EAC treated with (*G. cambogia* extract only).

Group II: (20 mice, laser irradiated group) a) 10 mice: were exposed to 4000 Hz Infra-Red Laser (IRL), for 3 minutes. b) 10 mice: were exposed to 7000 Hz IRL, for 3 minutes. Group III: (20 mice, ultrasound group); a) 10 mice: were exposed to pulsed ultrasound (US) for 3 minutes. b) 10 mice: were exposed to continuous US for 3 minutes. Group IV: (20 mice, *G. cambogia* extract, IRL group); The mice of this group were injected i.p. with *G. cambogia* extract, then the EAC site was irradiated to IRL at same conditions of group II. Group V: (20 mice, *G. cambogia* extract, US group); The mice of this group were injected i.p. with *G. cambogia* extract, then the EAC site was irradiated to US at same conditions of group III. Group VI: (20 mice, US/IRL group); a) 10 mice: The tumor site was irradiated to IRL for 3 minutes, followed by US for 3 minutes. b) 10 mice: Injected i.p. with *G. cambogia* extract, then the EAC site was irradiated to IRL for 3 minutes, followed by US for 3 minutes.

IRL and US Modalities: For IRL and/or US exposure, the mice were anesthetized with diethyl ether. The hair over the tumors was shaved off. The mice were fixed on a board with the tumor upwards. The probe was placed nearly on the tumor, which was irradiated with IRL and/or US for 3 minutes at the different conditions as mentioned before. Animals were maintained in the dark to avoid skin irritation after PDT, SDT and SPDT. Exposure of mice tumor to the IRL beam was carried out using an infrared diode laser, model LAS 50- Hi-Tech fysiomed, Germany operated at a wavelength of 904 nm and a peak power of 50 W a frequency up to 7000 Hz. Exposure of mice tumor to the continuous and pulsed US was carried out using an US therapy instrument, model CSl Shanghai, No. 822 Factory. China. This instrument uses electronic tube to generate an electric oscillation with frequency 0.8 MHz and power output which converted to US mechanical energy by means of US transducer (calcium zirconate etitanate). The mechanical US energy has a beam power density which can be adjusted from 0.5 to 3 W/cm^2. This instrument operates at both continuous wave mode with output power from 0.5 to 3 W/cm^2 adjustable in 11 steps and pulsed mode (pulse frequency 1000 Hz, duty ratio 1/3 and average power density from 0.15 to 1 W/cm^2).

For assessment of the impacts to all studied mice groups the biochemical, molecular, and histopathological investigations were done:

Tumor Assays: During treatment session, tumor growth was examined regularly every day. Length and width of tumors were measured with a caliper and tumor volume (TV) was calculated by the use of the following equation [31].

$$TV\ (mm^3) = \frac{22}{7} \times \frac{4}{3} \times \frac{\text{Lenghth}}{2} \times \left(\frac{\text{Width}}{2}\right)^2$$

Evaluation of Sonophotosensitizer and SPDT: Two weeks after the treatment, the mice were killed and the tumors were dissected out, weighed (in grams), their volumes (in $millimeters^3$) were measured using cylindrical measuring flux. The tumor mass inhibition ratio (TMIR%) [32] and tumor volume growth ratio (TVGR%) [33] were calculated as following equation.

$$TMIR\% = \left(1 - \frac{\text{Average Tumor Width of the Treated Group}}{\text{Average Tumor Width of the Control Group}}\right) \times 100$$

$$TVGR\% = \frac{\text{Final Volume} - \text{Initial Volume}}{\text{Initial Volume}} \times 100$$

Blood Samples Preparation: Blood sample (2.5 mL of venous blood) was withdrawn from all mice groups. These blood samples were clotted thoroughly for 20 minutes then centrifuged at 3000 ×g for 20 minutes for separating serum for biochemical examinations.

Oxidative Stress and Antioxidant Panel: Lipid peroxidation (LPx - malondialdehyde, MDA), total antioxidant capacity (TAC), glutathione reductase (GR) activity, glutathione-S-transferase (GST) activity, superoxide dismutase (SOD) activity and Catalase (CAT) activity assay kits were purchased from (Biodiagnostic diagnostic and research) manufacturer and used according to the manufacturer's instructions.

Renal and Hepatic Biomarkers: Urea (Ur), creatinine (Cr), alanine transaminase (ALT) activity, aspartate aminotransferase (AST) activity and gamma-glutamyl transferase (GGT) activity assay kit were purchased from BIOLABO SAS, Les Hautes Rives 02160, Maizy, France and used according to the manufacturer's instructions.

Light Microscopy and Transmission Electron Microscopy Examination: Small pieces of EAC tissues of the experimental groups were processed and examined by hematoxylin and eosin method. The small pieces of EAC tissues were fixed at 10% formaldehyde. Then dehydrated in ascending grades using alcohol. Embedded in paraffin to produce paraffin block. Then the blocks were cut into 3-4 µm thick sections and floated in water bath. Cleaned with xylene, then rehydrated in descending grades of alcohol. Stained with hematoxylin and eosin stain. Cleaned again ethylene. After all, covered by covering slides, thus the slides were prepared to be examined by light microscopy.

Statistical Analysis: Analysis of numeric data was performed using one-way ANOVA; it is a parametric statistical test that used to compare the means for certain data of more than two independent groups which follow a normal distribution. The given graphs were constructed using Microsoft excel software. All statistical analysis was done using two tailed tests and alpha error of 0.05. The p value less than or equal to 0.05 was statistically significant.

RESULTS AND DISCUSSION

Tumor Assays: Effects of Treatment Modalities on Tumor Volume and Tumor Weight. The relationships between tumor volume (TV) and between tumor weight (TW) during treatment period for various treatment modalities either treated with IRL-PDT or pulsed/continuous US-SDT in the presence or absence of *G. cambogia* extract are presented in Figure (1). TVs or TWs were normalized to volumes before starting the treatment. Treatment with *G. cambogia* extract only has little or no effect on either TV or TW. Up to one week, all treatment modulates have little effect on the TV or TW.

After one week, treatment with IRL and US (pulsed or continuous wave) in the presence or absence of *G. cambogia* extract, become more effective. Results obtained indicated that presence of *G. cambogia* extract increases the effect of both IRL and US. Pulsed US wave is more effective than continuous US wave in the presences of *G. cambogia* extract. Pulsed wave US was selected to combine with IRL at 7000 Hz. The combination between IRL and US treatment modality is efficient on tumor cells more than using them separately.

Tumor Volume Growth Ratio (TVGR%)

The Table (1) showed the TVGR% for only EAC and treated groups. *G. cambogia* extract alone had minimal inhibitory effect on TVGR%. IRL alone at 7000 Hz had lower growth rate than that of US wave (pulsed or continuous). IRL operated at 4000 Hz and 7000 Hz combined with *G. cambogia* extract showed synergistic antitumor effect than US with *G. cambogia* extract. Combination of IRL at 7000 Hz, pulsed wave US and *G. cambogia* extract showed high suppression of tumor growth rate.

Table (1): Tumor volume and tumor volume growth ratio in the different studied groups at the end of treatment in absence of dieldrin

Groups	Tumor volume (mm^3)	Tumor growth rate (%)	F (p)
Only with EAC	7.155+1.21	100	
Garcinia Only	6.83±1.30	95.458	
IRL			
4000 Hz	4.65^{ab}±0.22	64.989	
7000 Hz	3.53^{ab}±0.35	49.336	
IRL + Garcinia			
4000 Hz+ Garcinia	2.72^{ab}±0.42	38.015	
7000 Hz+ Garcinia	1.54^{ab}±0.34	21.523	
Ultrasound			28.013 (<0.001*)
Continuous US	4.12^{ab}±0.47	57.582	
Pulsed US	3.51^{ab}±0.55	49.057	
Ultrasound + Garcinia			
Continuous US+ Garcinia	3.15^{ab}±0.36	44.025	
Pulsed US+ Garcinia	2.61^{ab}±0.32	36.478	
IRL + Ultrasound			
7000 Hz + Pulsed US	2.22^{ab}±0.14	31.027	
IRL + Ultrasound + Garcinia			
7000 Hz + Pulsed US+ Garcinia	1.35^{ab}±0.02	18.868	

F: F value for ANOVA test

a: Significant with EAC group

b: Significant with garcinia only group

*: Statistically significant at $p \leq 0.05$

Tumor Mass Inhibition Ratio (TMIR%)

The Table (2) illustrated the effect of different treatment modalities at the end of the treatment period of 14 days. It is clear from this table that the TW after IRL or US in the presence of *G. cambogia* extract were reduced compared with that after IRL or US alone. The maximum inhibition ratio percentage was noticed in the treated group with IRL and US in the presence of *G. cambogia* extract.

Oxidative Stress and Antioxidant Panel: In the underlying study, the increase in LPx was reported in control group which carried EAC. In all the irradiated groups and that irradiated and treated without *G. cambogia* extract, a significant increase in the levels of MDA was observed. Animals in groups irradiated with IRL or US or both with *G. cambogia* extract exhibited significantly low levels of MDA, as compared with the cancer control group or with treated mice without activation of *G. cambogia* extract, as illustrated in Table (3). There were statistically significant differences between groups at $p \leq 0.05$.

Table 2. Tumor weights and tumor mass inhibition ratio in the different studied groups at the end of treatment in absence of dieldrin

Groups	Tumor weight (gm)	Tumor mass inhibition ratio (%)	F (p)
Only with EAC	5.545±1.33	0.00	
Garcinia Only	5.23±1.32	5.681	
IRL			
4000 Hz	1.82^{ab}±0.35	67.178	
7000 Hz	1.71^{ab}±0.49	69.161	
IRL + Garcinia			
4000 Hz+ Garcinia	0.92^{ab}±0.43	83.408	
7000 Hz+ Garcinia	0.70^{ab}±0.33	87.376	
Ultrasound			20.678
Continuous US	1.51^{ab}±0.53	72.768	(<0.001*)
Pulsed US	1.15^{ab}±0.67	79.261	
Ultrasound + Garcinia			
Continuous US+ Garcinia	1.05^{ab}±0.33	81.064	
Pulsed US+ Garcinia	0.90^{ab}±0.36	83.769	
IRL + Ultrasound			
7000 Hz + Pulsed US	0.63^{ab}±0.16	88.638	
IRL + Ultrasound + Garcinia			
7000 Hz + Pulsed US+ Garcinia	0.54^{ab}±0.03	90.261	

F: F value for ANOVA test
a: Significant with EAC group
b: Significant with garcinia only group
*: Statistically significant at $p \leq 0.05$

Table 3. Activities of antioxidants in the different studied groups in absence of dieldrin

Groups	GST (U/ml)	GR (mU/ml)	CAT (mU/ml)	TAC (mM/L)	SOD (U/ml)	MDA (nmol/ml)
Normal	3.94^{bc}± 0.81	0.035^{bc}± 0.008	817.03^{bc}± 3.60	0.755^{bc}± 0.001	1759.67^{bc}± 5.29	25.60^{bc}± 0.04
EAC Only	0.35^{a}± 0.07	0.011^{a}± 0.006	91.44^{a}± 0.51	0.022^{a}± 0.004	36.12^{a}± 0.36	251.84^{ac}± 1.66
Garcinia Only	0.41^{a}+ 0.12	0.013^{a}+ 0.001	95.22^{a}+ 0.72	0.029^{a}+ 0.005	38.43^{a}+ 1.57	237.33^{ab}− 1.44
4000 Hz IRL	0.52^{a}⊥ 0.18	0.021^{a}⊥ 0.003	124.51^{abc}⊥ 1.21	0.118^{abc}⊥ 0.011	154.19^{abc}⊥ 1.31	191.31^{abc}⊥ 0.09
7000 Hz IRL	0.61^{a}+ 0.15	0.022^{a}+ 0.005	241.99^{abc}+ 0.69	0.262^{abc}− 0.009	202.87^{abc}+ 1.32	170.55^{abc}+ 0.06
4000 Hz IRL+ Garcinia	0.73^{a}⊥ 0.17	0.023^{a}⊥ 0.005	245.16^{abc}⊥ 2.75	0.267^{abc}− 0.032	209.31^{abc}⊥ 2.04	151.90^{abc}⊥ 0.5
7000 Hz IRL+ Garcinia	0.81^{a}± 0.12	0.026^{a}± 0.004	253.56^{abc}± 2.50	0.282^{abc}± 0.024	282.90^{abc}± 0.14	140.71^{abc}± 0.38
Continuous US	1.13^{a}± 0.08	0.029^{a}± 0.003	273.76^{abc}± 2.77	0.360^{abc}± 0.008	371.70^{abc}± 1.14	131.31^{abc}± 1.08
Pulsed US	1.32^{abc}⊥ 0.10	0.030^{a}⊥ 0.009	361.82^{abc}⊥ 5.48	0.371^{abc}⊥ 0.006	372.32^{abc}⊥ 0.35	120.64^{abc}⊥ 1.44
Continuous US+ Garcinia	2.08^{ab}± 0.12	0.038^{a}± 0.015	391.56^{abc}± 3.85	0.406^{abc}± 0.016	604.90^{ab}± 1.62	99.69^{abc}± 0.62
Pulsed US+ Garcinia	2.19^{abc}⊥ 0.20	0.041^{a}⊥ 0.020	508.29^{abc}⊥ 3.87	0.432^{abc}⊥ 0.01	870.40^{abc}⊥ 1.6	92.36^{abc}⊥ 0.65
7000 IRL + Pulsed US	3.21^{bc}± 0.18	0.042^{a}± 0.031	533.29^{abc}± 3.97	0.532^{abc}± 0.003	1440.70^{abc}± 1.57	87.66^{abc}± 0.03
7000 IRL + Pulsed US+ Garcinia	3.34^{bc}− 0.30	0.053^{bc}+ 0.014	630.46^{abc}+ 3.64	0.542^{abc}− 0.006	1555.30^{abc}+ 1.20	89.90^{abc}+ 0.52
F	52.192	7.333	1492.00	382.052	29080.00	1862.00
P	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*

F: F value for ANOVA test
a: Significant with Normal group
b: Significant with Only EAC group
c: Significant with Garcinia only group
*: Statistically significant at $p \leq 0.05$
Data was expressed by using mean ± SD.

The implanted mice with EAC showed decreased activities of the enzymatic and non-enzymatic antioxidants such as SOD, CAT, GR, GST and TAC in comparison with normal animals. On the other hand, there is a significant increase in the antioxidant guard in the groups irradiated with IRL or US or both with *G. cambogia* extract when compared with cancer control group or with treated mice without activation of *G. cambogia* extract.

Renal and Hepatic Biomarkers: The biomarkers of renal function, namely, Cr and Ur, were estimated. The EAC caused a significant increase in the serum Cr and Ur levels in the studied groups. On the other hand, the *G. cambogia* extract caused decrease in the levels of serum Cr and Ur which is probably an indication of renal protection, as showed in Table (4). This also confirms the protective role of *G. cambogia* extract against renal toxicity. The statistically significant differences between groups at $p \leq 0.05$ were observed. Also, the biomarkers of hepatic function, ALT, AST and GGT, were assessed. The EAC caused a significant increase in the serum activities of ALT, AST and GGT of the tumor treated groups. However, in the EAC treated groups with *G. cambogia* extract a decrease in serum levels of ALT, AST, and GGT, were observed which is an indication of the hepatoprotection by *G. cambogia* extract, i.e., this confirms the protective role of *G. cambogia* extract against hepatotoxicity.

Table 4. Renal and hepatic functions in the different studied groups in absence of dieldrin

Groups	Urea (mg/dL)	Creatinine (mg/dL)	ALT (U/L)	AST (U/L)	GGT (U/L)
Normal	11.55^{bc}± 0.66	0.24^{bc}± 0.04	40.59^{bc}± 1.05	36.06^{bc}± 0.24	1.34^{ac}± 0.53
EAC Only	30.13^{a}± 0.47	0.71^{a}± 0.06	202.00^{ac}± 3.53	332.61^{ac}± 0.92	7.83^{a}± 0.47
Garcinia Only	29.11^{a}+ 0.96	0.62^{a}+ 0.03	187.00^{ab}+ 2.88	323.80^{ab}+ 0.63	2.64^{a}+ 0.34
4000 Hz IRL	28.18^{ab}± 0.46	0.51^{a}± 0.039	151.00^{abc}± 1.44	209.9^{abc}± 0.1	2.51^{a}± 0.25
7000 Hz IRL	24.26^{abc}± 0.3	0.42^{b}± 0.19	132.00^{abc}± 0.75	155.55^{abc}± 0.50	2.42± 0.52
4000 Hz IRL+ Garcinia	23.19^{abc}± 0.65	0.37^{bc}± 0.02	95.90^{abc}± 0.64	141.55^{abc}± 0.04	2.23± 0.14
7000 Hz IRL+ Garcinia	22.17^{abc}± 0.56	0.33^{bc}± 0.01	85.90^{abc}± 0.52	125.65^{abc}± 0.60	2.04± 0.46
Continuous US	20.32^{abc}± 0.41	0.31^{bc}± 0.15	81.50^{abc}± 0.36	57.38^{abc}± 0.40	2.01± 0.21
Pulsed US	19.46^{abc}± 0.82	0.30^{bc}± 0.03	72.70^{abc}± 0.18	53.40^{abc}± 0.95	1.95± 0.36
Continuous US+ Garcinia	18.14^{abc}± 0.75	0.29^{bc}± 0.04	60.20^{abc}± 0.23	49.51^{abc}± 0.45	1.74± 0.23
Pulsed US+ Garcinia	17.53^{abc}± 0.55	0.28^{bc}± 0.072	54.20^{abc}± 0.03	47.67^{abc}± 0.83	1.60^{b}± 0.45
7000 IRL + Pulsed US	16.22^{abc}± 0.45	0.27^{bc}± 0.07	50.40^{abc}± 0.67	44.52^{abc}± 0.97	1.53^{b}± 0.33
7000 IRL + Pulsed US – Garcinia	15.17^{abc}± 0.68	0.25^{bc}± 0.03	48.50^{abc}± 0.62	42.68^{abc}± 0.46	1.41^{ac}± 0.45
F	252.128	10.741	435.20	8442.00	4.756
P	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*

F: F value for ANOVA test
a: Significant with Normal group
b: Significant with Only EAC group
c: Significant with Garcinia only group
*: Statistically significant at $p \leq 0.05$
Data was expressed by using mean ± SD.

Light Microscopy and Transmission Electron Microscopy Examinations: Figure (2) showed the histopathologic examination for tumor tissue of all studied mice groups. The histological evaluation revealed that all tumors from the group of EAC mice without treatment work as a control group were highly malignant cells and the tumors showed (0-5%) necrosis. Group of EAC mice treated with *G. cambogia* extract as sono/photosensitizer; nearly similar percentage as only EAC group due to *G. cambogia* extract inactivation. Group of mice treated with IRL group, 4000 Hz, 7000 Hz only, showed significant areas of necrosis (45-60%, respectively). In the group of mice injected i.p. with *G. cambogia* extract then the tumor site was irradiated to 4000 Hz, 7000 Hz showed significant areas of necrosis (65-75%, respectively). Group of mice treated with pulsed or continuous US for 3 minutes

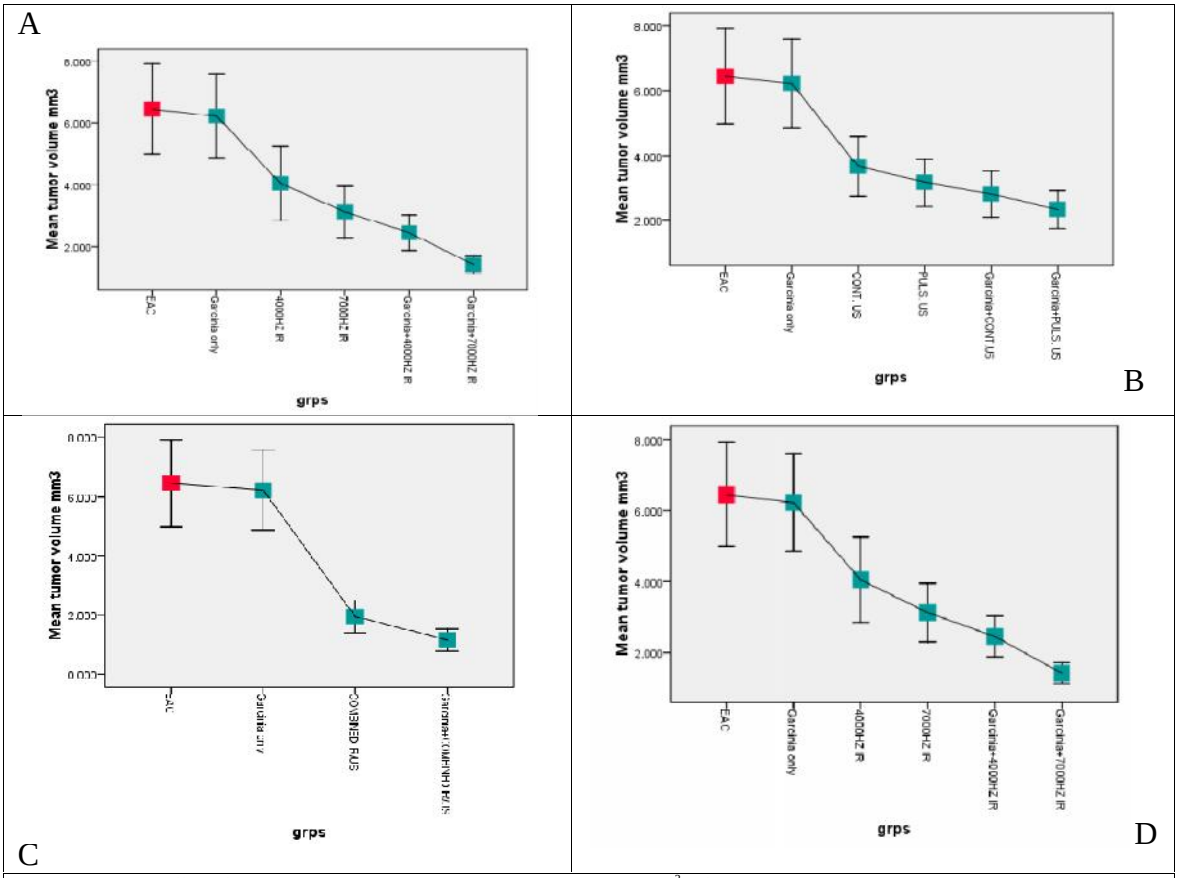

Figure (1): A. The effect of combined exposure (IRL and US) on tumor volume (mm^3) of untreated and garcinia treated groups.**B.**The average tumor volume (mm^3) of untreated and garcinia treated combined exposure (IRL and US) groups. **C.**The effect of IRL at different frequencies on the tumor volume (mm^3) of untreated and garcinia treated groups.**D.** The effect of continuous and pulsed ultrasound waves on tumor volume (mm^3) of untreated and garcinia treated groups.

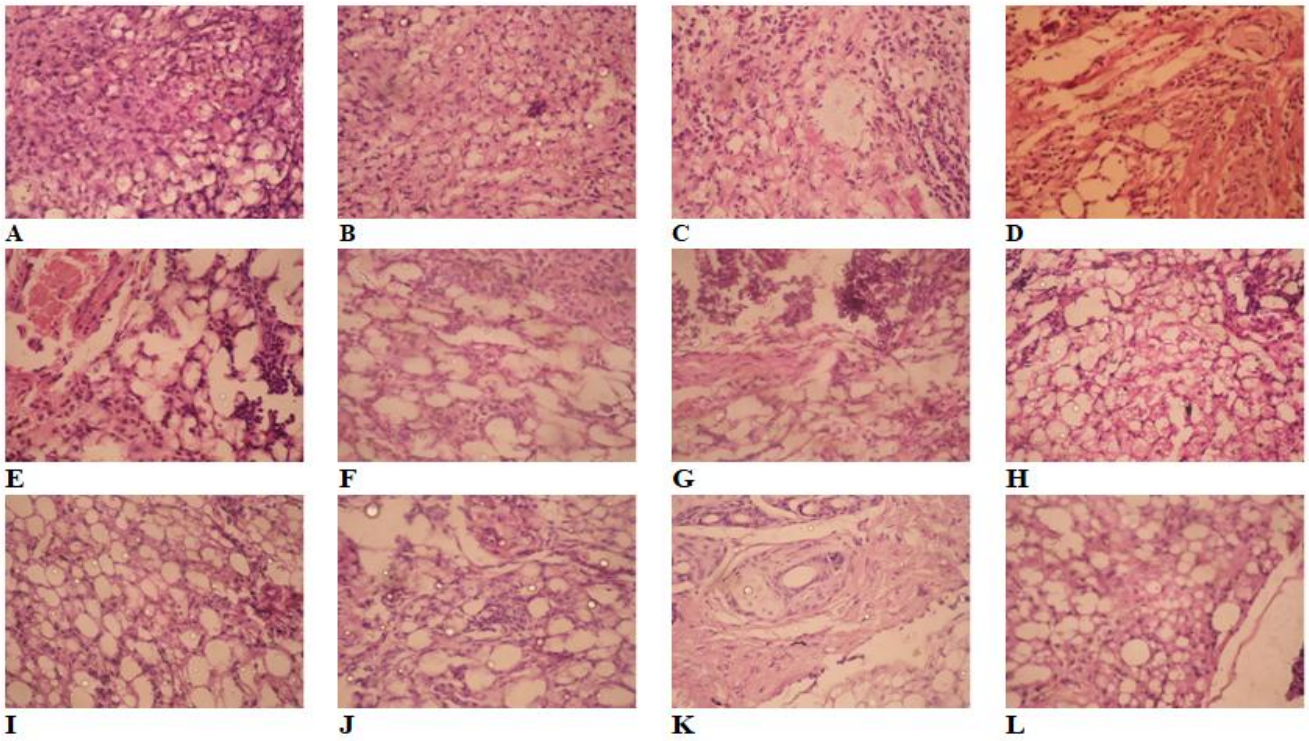

Figure (2): A: Group of EAC bearing mice without treatment as a control group: (poorly differentiated malignant tumor (necrosis; 0-5%). B: Group of mice tumor bearing mice treated with *G. cambogia* extract as sonophotosensitizer only: (poorly differentiated malignant tumor (necrosis; 10-15%). C: Group of mice treated with 4000 Hz IRL for 3 minutes: (poorly differentiated malignant tumor (necrosis; 30-45%). D: Group of mice treated with 7000Hz IRL for 3 minutes: (Poorly differentiated malignant tumor (necrosis; 45-60%). E: Group of mice injected i.p. with *G. cambogia* extract and irradiated with 4000 Hz IRL: (poorly differentiated malignant tumor (necrosis; 50-65%). F: Group of mice injected i.p. with *G. cambogia* extract and irradiated with 7000 Hz IRL: (poorly differentiated malignant tumor (necrosis; 60-75%). G: Group of mice treated with continuous US for 3 minutes: (poorly differentiated malignant tumor (necrosis; 45-60%). H: Group of mice treated with pulsed US for 3 minutes: (poorly differentiated malignant tumor (necrosis; 50-65%). I: Group of mice injected i.p. with *G. cambogia* extract and irradiated with continuous US: (poorly differentiated malignant tumor (necrosis; 60-75%). J: Group of mice injected i.p. with *G. cambogia* extract and irradiated with pulsed US: (poorly differentiated malignant tumor (necrosis; 65-80%). K: Group of mice irradiated to 7000Hz IRL, followed by pulsed US for 3 minutes: (poorly differentiated malignant tumor (necrosis; 70-80%). L: Group of mice injected i.p. with *G. cambogia* extract and irradiated to 7000Hz IRL, followed by pulsed US for 3 minutes:(poorly differentiated malignant tumor (necrosis; 75-85%)

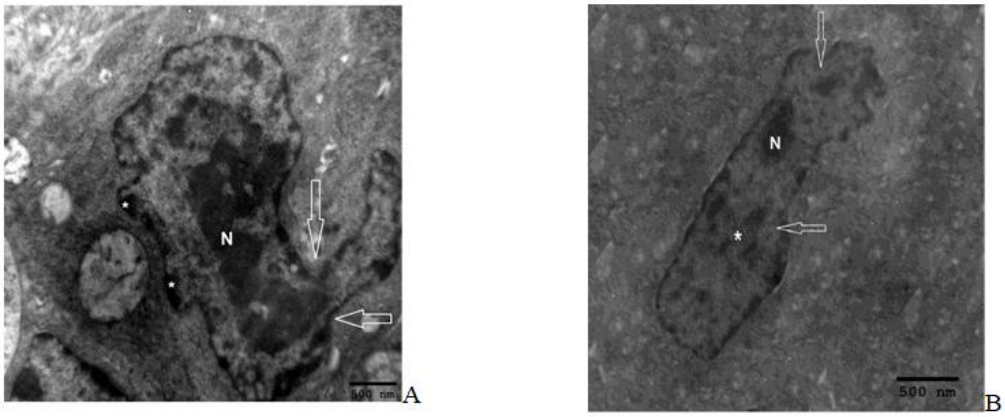

Figure 3. A: EAC untreated cancerous group; showed irregular serrated nuclear membrane with deep indentations (arrows), having polypoid large nuclei (N), and coarsely clumped dense heterochromatin situated on the nuclear membrane (*). B: *G. cambogia* extract, 7000Hz IRL and pulsed US treated group; showed massive areas of tumor dead cell seen as ghosts of pale cells without nuclei (arrow), cells having swollen ruptured cytoplasmic organelles (*)

showed significant areas of necrosis (60-65%, respectively). The Group of mice injected i.p. with *G. cambogia* extract, then the EAC site was irradiated to pulsed or continuous US the areas of necrosis (70-75%, respectively), when compared with only EAC group. While in case of two combination groups, 7000 Hz followed by pulsed US, and group of mice injected i.p. with *G. cambogia* extract then tumor site was irradiated to 7000 Hz, followed by pulsed US. Large foci of necrosis areas (80-85%, respectively) were present which were distinctly appeared. Figure (3) showed the ultrastructure examination of studied mice groups using TEM; histological evaluation revealed that EAC without treatment as a control group was highly malignancy with 5 % necrosis while in case of two combination groups injected i.p. with *G. cambogia* extract, tumors showed large foci of necrosis areas (85%). In this study, *G. cambogia* extract as sensitizer for IRL-PDT and US-SDT were accomplished through investigation alone or combined manner in EAC bearing swiss albino mice. The *G. cambogia* extract could be safely administered, provide an increased local carcinoma cytotoxic response and represents a potential cancer therapy approach.

The first description of the EAC was in 1932 by Loewenthal and Jahn [34]. It is a spontaneous murine mammary adenocarcinoma [35] adapted to ascites form and carried in outbred mice by serial i.p. passages. It is a transplantable, poorly differentiated malignant tumor and one of the experimental breast neoplasia. Its cells fill the peritoneal cavity by rapid division of cells, accumulation of a fluid named ascitic fluid and animal dies 17 - 18 days following EAC transplantation [36]. It has been reported that EAC cells lack H-2 histocompatibility antigens [37], which apparently is the reason for their rapid proliferation in almost any mouse host [38]. EAC is used as ascites or a solid form due to these purposes, if the ascites fluid contains the tumor cells that injected i.p., the ascites form is obtained, but if it is injected s.c., a solid form is obtained [39,40]. The quantity of TV and TW are used in a wide range of studies therapeutic effectiveness by expressing TVGR% and TMIR%. It could therefore be inferred that significantly enhanced decrease of TVGR%, meanwhile, increase of TMIR% are considered as antitumor activity through natural product in solid EAC bearing mice study models [41,42] were crossmatched with our results in the current study. The EAC induced ROS in mice regarded to several studies [43,44]. Over production of ROS has been implicated in the pathogenesis of breast cancer [45,46]. ROS are contributed to all carcinogenesis phases from initiation to malignant conversion. This is true for all kinds of cancers [47] including breast carcinoma. Also, it result in LPx and subsequent increase in MDA and other thiobarbituric acid reactive substances (TBARS) levels which lead to degradation of cellular macromolecules [48]. MDA can be produced from free radical attack on polyunsaturated fatty acids [49]. The probable reason for the elevated level of serum lipid peroxide in breast cancer patients may be due to defective antioxidant system which leads to the accumulation of lipid peroxides in cancer tissue followed by release into the blood circulation [50]. MDA constitutes a highly cytotoxic major aldehyde final peroxyl radical product of LPx. It is claimed to be an inhibitor to protective enzymes. Hence, it could have both mutagenic and carcinogenic effects [51]. Also, the histopathology of this group illustrated poorly differentiated malignant tumor with necrosis.

On other hand, PDT uses a combination of light, photosensitizer and O_2 to kill cancerous cells [52]. Typically, a light source is used to excite an electron in the photosensitizer from the ground state to the first excited singlet state [53]. The $^1O_2$ is a ROS that ultimately leads to cell death [54]. In an oxygenated medium, $^1O_2$ largely mediates photosensitization, but the supplemental role of H_2O_2, hydroxyl group ($OH^{\bullet}$) and superoxide anion ($O_2^{\bullet-}$) is also relevant. Therefore, only substrates situated very close to the places of ROS generation will be firstly affected by the PDT [55]. The damage of the plasma membrane can be observed within minutes after light exposure. This type of damage is manifested as LPx [56]. Light induced membrane alteration involves inflammatory cellular damage. Photooxidative lesions of membrane lipids prompt a rapid activation of membranous phospholipases [57] leading to accelerated phospholipid degradation with a massive release of lipid fragments and metabolites of arachidonic acid [58,59]. Also, increased macrophage activity was demonstrated after PDT *in vitro* and *in vivo* [60–62]. The data represented the mechanism of PDT cytotoxicity *in vivo* is TNF-mediated tumor necrosis. It reported that macrophages release TNF-α following PDT

treatment [63] and preferentially destroy PDT treated tumor cell targets [64]. This increase could be due to a rise in macrophage produced ROS [65], that induces or upregulates TNF-α [63]. In the same concept of PDT, the SDT relies on the generation of ROS through the simultaneous combination of low intensity US, O_2 and a sonosensitizer [13]. SDT offers significant advantages over PDT because US can penetrate in soft tissue up to several tens of centimeters depending on the frequency used [14]. SDT induces apoptosis characteristic features such as mitochondrial transmembrane potential disturbances, loss of phosphatidylserine asymmetry, morphological changes and DNA fragmentation [66–68]. This revealed when US treatment leaded to activated caspase-3 induced apoptosis [66]. Evidence against $^1O_2$ formation during SDT [69] that induced apoptosis was suggested before [66]. Moreover, after a successful US treatment *caspase-3* gene mRNA level was significantly increased in another study. This is a further supporting evidence of US-induced apoptosis [70] through caspase-3 mechanism.

Cells are also equipped with antioxidant system that contribute a crucial role in the elimination of free radicals. SOD and CAT, GST, and glutathione peroxidase (GPx) are involved in the clearance of $O_2^{\bullet-}$ and hydrogen peroxide (H_2O_2). SOD catalyzes the conversion of $O_2^{\bullet-}$into H_2O_2, which must be eliminated by GPx and/ or CAT [71]. GST catalyze the nucleophilic addition of the thiol of reduced glutathione (GSH) to a variety of electrophiles [72]. While, GR converts oxidized glutathione (GSSG) back to GSH uses NADPH [73]. In EAC bearing mice, the depletion of the CAT, SOD [74,75], GST [44,76], TAC [77], GSH [78] GPx as well as GR levels significantly because of increased LPx [79]. Our result showed *G. cambogia* extract enhanced antioxidants such as SOD, GSH and CAT significantly [80]. In contrary, the antioxidants were decreased in EAC group than that in either *G. cambogia* extract treated groups or control group.

The photosensitization property of benzophenone nucleus was reported when exposed to ultraviolet [81], laser [82] and US [83] that produces $^1O_2$. Many previous studies suggested the antitumor, proapoptotic and antiangiogenic impacts of benzophenone analogues. Benzophenones showed significant antitumor activity in both *in vivo* and *in vitro* against many cancer cell lines [84–87]. Also, the inhibitory role of benzophenone analogues against tumor growth was mediated through a direct effect on tumor cells that resulted in cell cycle arrest, differentiation or apoptosis [88,89]. Their analogues also were reported as antiangiogenic agents [90,91]. Therefore, it could explain the improvement in TV and TW of EAC groups that exposed to either IRL-PDT, US-SDT or SPDT beside received *G. cambogia* extract that contains benzophenone based compounds such as garcinol, isogarcinol [22], guttiferones I, N, J, K and M as well as the polyisoprenylated benzophenones [24,25]. These results were compatible with that *G. cambogia* extract alone had minimal inhibitory effect on the different groups that showed highest TV and TW. Also, the groups that expose to IRL alone at either 4000 Hz or 7000 Hz, revealed lower growth rate than that of groups that exposed to US either pulsed or continuous modalities. The group that exposed to IRL at 4000 Hz and 7000 Hz combined with *G. cambogia* extract showed synergistic antitumor inhibitory impact than US with *G. cambogia* extract. The combination of IRL at 7000 Hz, pulsed wave US and *G. cambogia* extract showed high suppression of growth rate. Additionally, the results of the present work illustrated the impacts of tumor mass in different modalities of treatment after IRL-PDT or US-SDT in the presence of *G. cambogia* extract were reduced compared with that after IRL-PDT or US-SDT alone. Moreover, the hematoxylin and eosin histopathological examination showed that all tumors from the EAC bearing mice group were included highly malignant cells. EAC masses were excised from animals that received *G. cambogia* extract. They showed significant areas of necrosis in comparison to groups did not receive the extract. In the group of mice subjected to combination of both IRL-PDT and US-SDT in the presences of *G. cambogia* extract, large foci distinct necrosis areas were observed. These were crossmatched with tumor tissues ultrastructure that unveiled the EAC untreated cancerous group; showed irregular serrated nuclear membrane with deep indentations, having polypoid large nuclei, and coarsely clumped dense heterochromatin situated on the nuclear membrane. Besides *G. cambogia* extract, 7000Hz IRL and pulsed US treated group; showed massive areas of tumor dead cell seen as ghosts of pale cells without nuclei, cells having swollen ruptured cytoplasmic organelles.

Furthermore, hepatocytes are severely damaged in the animals with EAC mass [92]. This could show an increase in the levels of ALT, AST and GGT in the serum of EAC bearing mice [93,94]. Hepatic and renal damages associated with significant elevations in serum Cr, Ur, ALT, AST and GGT confirms the involvement of EAC-induced hepatorenal toxicity [95,96].The elevation of liver and kidney markers is an index of impaired hepatorenal functions due to cancer as observed in EAC mice groups. It was found that a rise in MDA level which was correlated with impaired hepatorenal injuries [97] in EAC mice groups. The inhibitory effect of *G. cambogia* extract on the LPx damage induced by its antioxidant property. After treatment, the levels of serum ALT and AST were found to be normal [98]. Reduced level of these hepatic enzymes in serum is one of the indications of the antitumor potentiality [99]. In our results, the diminished hepatic, and renal markers in either *G. cambogia* extract treated group or control group were less than that in EAC groups. This was suggested that phenolic constituents present in the *G. cambogia* extract might be responsible for the activity [100].

In conclusion, the effect of exposing the tumor to IR laser as a photodynamic therapy increased with increasing the laser energy resulted in decreasing the tumor volume, tumor growth rate and increasing tumor volume inhibition ratio. These effects were observed either on using infrared laser alone (with its two frequencies) or in the presence of the garcinia. According to IR results, the inhibition in the tumor volume has maximum value on using 7000 Hz IR laser in the presence of the garcinia. The effect of exposure to pulsed ultrasound wave was more than that in case of using continuous ultrasound wave. Similar variations occurred in case of using ultrasound exposure only or in the presence of the garcinia, with maximum effect occurred on using ultrasound in the presence of the garcinia. Combined treatment of IR laser at 7000 Hz and pulsed ultrasound wave in the presence of garcinia was more effective than either IR laser or ultrasound alone. While, in the group of mice carrying the tumor only, a significant increase in the levels of MDA as compared to the control group of animals. The combination of photodynamic and sonodynamic therapy in the presences of

garcinia significantly decreased the levels of MDA. In all Ehrlich bearing mice groups decreased activities of SOD, CAT, GR, GST, and TAC in comparison with normal group were observed. A significant increase in the enzymatic and non-enzymatic antioxidant guard was observed in the groups subjected to combination of photodynamic and sonodynamic therapy in the presences of garcinia. It was observed that treatment with garcinia ameliorated the levels of serum Cr and Ur which is an indication of renal protection. This also confirms the protective role of garcinia against renal toxicity that unveiled by Ur and Cr concentrations. Also, treatment with garcinia protected against increase in serum activities of ALT, AST, and GGT, which is an indication of hepatoprotection by garcinia. This also confirms the protective role of garcinia against hepatotoxicity. The histological evaluation revealed that all tumors from the group of mice bearing the tumor included highly malignant cells. Tumors excised from animals receiving treatment garcinia showed significant areas of necrosis compared to groups without garcinia. In the group of animals subjected to combination of photodynamic and sonodynamic therapy in the presences of garcinia, large foci distinct necrosis areas were appeared.

REFERENCES

1. Sijabat TWS. *World Health Organiztion Statistics 2018*.; 2018. http://e-journal.uajy.ac.id/14649/1/JURNAL.pdf
2. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2018;68(6):394-424. doi:10.3322/caac.21492
3. Boulos S, Gadallah M, Neguib S, et al. Breast screening in the emerging world: High prevalence of breast cancer in Cairo. *Breast*. 2005;14(5):340-346. doi:10.1016/j.breast.2005.07.002
4. Elatar I. Cancer registration, NCI Egypt 2001. *Natl Cancer Inst*. Published online 2002.
5. Ibrahim AS, Khaled HM, Mikhail NNH, Baraka H, Kamel H. Cancer Incidence in Egypt : Results of the National Population-Based Cancer Cancer Incidence in Egypt : Results of the National Population-Based Cancer Registry Program. 2015;2014(January). doi:10.1155/2014/437971
6. Registry DC, Registry MC. World Health Organization. Population fact sheets. Egypt 2018. 2018;399:19-20. http://gco.iarc.fr/today/data/factsheets/populations/818-egypt-fact-sheets.pdf
7. LIPSON RL , BALDES EJ OA. A New Aid for Endoscopic Detection of Malignant Disease. *Hematop Deriv a new aid Endosc Detect Malig Dis Abstr Citations Relat Artic Data BioEntities Extern Links LIPSON RL , BALDES EJ , OLSEN AM J Thorac Cardiovasc Surg*. 1961;42:623-629. https://europepmc.org/abstract/med/14465760
8. Epstein J. Phototoxicity and photoallergy. *Semin Cutan Med Surg*. 1999;18:274 – 284.
9. Dimofte A, Zhu TC, Hahn SM, Lustig RA. In vivo light dosimetry for motexafin lutetium-mediated PDT of recurrent breast cancer. *Lasers Surg Med*. 2002;31(5):305-312. doi:10.1002/lsm.10115
10. Dougherty TJ, Lawrence G, Kenneth J, Weishaupt R, Goldfarb A. Photoradiation in the treatment of recurrent breast carcinoma. *J Natl Cancer Inst*. 1979;62(2):231-237. doi:10.1093/jnci/62.2.231
11. Mang TS, Allison R, Hewson G, Snider W, Moskowitz R. A phase II/III clinical study of tin ethyl etiopurpurin (Purlytin)-induced photodynamic therapy for the treatment of recurrent cutaneous metastatic breast cancer. *Cancer J Sci Am*. 1998;4(6):378—384. http://europepmc.org/abstract/MED/9853137
12. Kremkau FW, Kaufmann JS, Walker MM, Burch PG, Spurr CL. Ultrasonic enhancement of nitrogen mustard cytotoxicity in mouse leukemia. *Cancer*. 1976;37(4):1643-1647. doi:https://doi.org/10.1002/1097-0142(197604)37:4<1643::AID-CNCR2820370404>3.0.CO;2-L
13. Shibaguchi H, Tsuru H, Kuroki M, Kuroki M. Sonodynamic cancer therapy: A non-invasive and repeatable approach using low-intensity ultrasound with a sonosensitizer. *Anticancer Res*. 2011;31(7):2425-2430.
14. Bailey MR, Khokhlova VA, Sapozhnikov OA, Kargl SG, Crum LA. Physical mechanisms of the therapeutic effect of ultrasound (a review). *Acoust Phys*. Published online 2003. doi:10.1134/1.1591291
15. Wang X, Zhang W, Xu Z, Luo Y, Mitchell D, Moss RW. Sonodynamic and Photodynamic Therapy in Advanced Breast Carcinoma: A Report of 3 Cases. *Integr Cancer Ther*. 2009;8(3):283-287. doi:10.1177/1534735409343693
16. McCaughan B, Rouanet C, Fowley C, et al. Enhanced ROS production and cell death through combined photo- and sono-activation of conventional photosensitising drugs. *Bioorganic Med Chem Lett*. 2011;21(19):5750-5752. doi:10.1016/j.bmcl.2011.08.015
17. Bellnier DA, Ho Y -K, Pandey RK, ert JR, Dougherty TJ. Distribution and Elimination of Photofrin Ii in Mice. *Photochem Photobiol*. 1989;50(2):221-228. doi:10.1111/j.1751-1097.1989.tb04152.x
18. Umemura S -i, Yumita N, Nishigaki R, Umemura K. Mechanism of Cell Damage by Ultrasound in Combination with Hematoporphyrin. *Japanese J Cancer Res*. 1990;81(9):962-966. doi:10.1111/j.1349-7006.1990.tb02674.x
19. ROY S, RINK C, KHANNA S, BAGCHI DPC, BAGCHI M, K SC. Body weight and abdominal fat gene expression profile in response to a novel hydroxycitric acid-based dietary supplement. *Gene Expr*. 2003;11(5-6):251-262. http://www.embase.com/search/results?subaction=viewrecord&from=export&id=L38744499%0Ahttp://bj7rx7bn7b.search.serialssolutions.com?sid=EMBASE&issn=10522166&id=doi:&atitle=Body+weight+and+abdominal+fat+gene+expression+profile+in+response+to+a+novel+hydroxy
20. Tharachand. S, Avadhani M. Medicinal properties of malabar tamarind [Garcinia cambogia (Gaertn) DESR]. *Int J Pharm Sci Rev Res*. 2013;19(2):101-107.
21. Y SL, Neelakantan S. (–)-Hydroxycitric acid-the principal acid in the fruits of Garcinia Cambogia Desr. 1965;4(1959):620-625.
22. Iinuma M, Ito T, Miyake R, Tosa H, Tanaka T, Chelladurai V. A xanthone from Garcinia cambogia. *Phytochemistry*. 1998;47(6):1169-1170. doi:10.1016/S0031-9422(98)80096-4
23. Kumar S, Sharma S, Chattopadhyay SK. High-performance liquid chromatography and LC-ESI-MS method for identification and quantification of two isomeric polyisoprenylated benzophenones isoxanthochymol and camboginol in different extracts of Garcinia species.

Biomed Chromatogr. 2009;23(8):888-907. doi:10.1002/bmc.1202
24. Masullo M, Bassarello C, Bifulco G, Piacente S. Polyisoprenylated benzophenone derivatives from the fruits of Garcinia cambogia and their absolute configuration by quantum chemical circular dichroism calculations. *Tetrahedron*. 2010;66(1):139-145. doi:10.1016/j.tet.2009.11.034
25. Masullo M, Bassarello C, Suzuki H, Pizza C, Piacente S. Polyisoprenylated benzophenones and an unusual polyisoprenylated tetracyclic xanthone from the fruits of Garcinia cambogia. *J Agric Food Chem*. 2008;56(13):5205-5210. doi:10.1021/jf800416j
26. KIM K-Y, LEE HN, KIM YJ, PARK T. Garcinia cambogia Extract Ameliorates Visceral Adiposity in C57BL/6J Mice Fed on a High-Fat Diet . *Biosci Biotechnol Biochem*. 2008;72(7):1772-1780. doi:10.1271/bbb.80072
27. Altiner A, Ates A, Gursel FE, Bilal T. Effect of the antiobesity agent garcinia cambogia extract on serum lipoprotein (a), apolipoproteins a1 and b, and total cholesterol levels in female rats fed atherogenic diet. *J Anim Plant Sci*. 2012;22(4):872-877.
28. Elizabeth A. Mazzio, A. SKF. In Vitro Screening for the Tumoricidal Properties of International Medicinal Herbs. *Phyther Res*. 2009;23:385-398. doi:10.1002/ptr
29. Saadat N, Gupta S V. Potential Role of Garcinol as an Anticancer Agent. *J Oncol*. 2012;2012:1-8. doi:10.1155/2012/647206
30. Ong CY, Ling SK, Ali RM, et al. Systematic analysis of in vitro photo-cytotoxic activity in extracts from terrestrial plants in Peninsula Malaysia for photodynamic therapy. *J Photochem Photobiol B Biol*. 2009;96(3):216-222. doi:10.1016/j.jphotobiol.2009.06.009
31. Faustino-Rocha A, Oliveira PA, Pinho-Oliveira J, et al. Estimation of rat mammary tumor volume using caliper and ultrasonography measurements. *Lab Anim (NY)*. 2013;42(6):217-224. doi:10.1038/laban.254
32. Hather G, Donelan J, Liu R, et al. Growth Rate Analysis and Efficient Experimental Design for Tumor Xenograft Studies. *Cancer Inform*. 2014;13(Suppl 4):65. doi:10.4137/CIN.S13974
33. Zheng SG, Xu HX, Guo LH, Liu LN, Lu F. The safety and treatment response of combination therapy of radioimmunotherapy and radiofrequency ablation for solid tumor: A study in vivo. *PLoS One*. 2014;9(5). doi:10.1371/journal.pone.0096539
34. Loewenthal H, Jahn G. Übertragunsversuche mit carcinomatöser Mäuse-Ascitesflüssigkeit und ihr Verhalten gegen physikalische und chemische Einwirkungen. *Z Krebsforsch*. 1932;37(1):439-447. doi:10.1007/BF01618550
35. Hanafi N, Mansour SZ, Plaza A, et al. Beobachtungen uber maligne mausentumoren. *J Biol Sci*. 2005;10(6):594-603.
36. Ulakoglu G, Altun S. The effects of epirubicin on proliferation and DNA synthesis of Ehrlich ascites carcinoma cells in vitro and in vivo. *Biol Bratislava*. 2004;59(6):727-734.
37. Chen L, Watkins JF. Evidence against the presence of H2 histocompatibility antigens in Ehrlich ascites tumour cells. *Nature*. 1970;225(5234):734.
38. Patt HM, Straube RL. Measurement and nature of ascites tumor growth. *Ann N Y Acad Sci*. 1956;63(5):728-737.
39. Okay HG. Deneysel EAT Oluşturulan Fare Karaciğer Plazmasında Nitrik Oksit Metabolizmasının İncelenmesi. Published online 1998.
40. Zeybek ŞÜ. En Uygun Ehrlich Ascites Tümör Modellerinin Farklı Soy ve Cinsiyetteki Farelerde Gösterilmesi. İstanbul Üniversitesi Sağlık Bilimleri Enstitüsü Deney Hayvanları Biyolojisi ve Biyomedikal Uygulama Teknikleri Anabilim Dalı. Published online 1996.
41. Aldubayan MA, Elgharabawy RM, Ahmed AS, Tousson E. Antineoplastic Activity and Curative Role of Avenanthramides against the Growth of Ehrlich Solid Tumors in Mice. *Oxid Med Cell Longev*. 2019;2019:1-12. doi:10.1155/2019/5162687
42. Kumar RS, Rajkapoor B, Perumal P, Dhanasekaran T, Jose MA, Jothimanivannan C. Antitumor Activity of Prosopis glandulosa Torr. on Ehrlich Ascites Carcinoma (EAC) Tumor Bearing Mice. *Iran J Pharm Res*. 2011;10(3):505-510.
43. Gupta M, Mazumder U, Kumar RS, Kumar TS. Antitumor activity and antioxidant role of Bauhinia racemosa against Ehrlich ascites carcinoma in Swiss albino mice. *Acta Pharmacol Sin*. 2004;25:1070-1076.
44. Gupta M, Mazumder UK, Kumar RS, Sivakumar T, Vamsi MLM. Antitumor Activity and Antioxidant Status of Caesalpinia bonducella Against Ehrlich Ascites Carcinoma in Swiss Albino Mice. *J Pharmacol Sci*. 2004;94(2):177-184. doi:10.1254/jphs.94.177
45. Lee YJ, Galoforo SS, Berns CM, et al. Glucose deprivation-induced cytotoxicity and alterations in mitogen- activated protein kinase activation are mediated by oxidative stress in multidrug-resistant human breast carcinoma cells. *J Biol Chem*. 1998;273(9):5294-5299. doi:10.1074/jbc.273.9.5294
46. Wiseman H, Halliwell B. Damage to DNA by reactive oxygen and nitrogen species: role in inflammatory disease and progression to cancer. *Biochem J*. 1996;313(Pt 1):17.
47. Hanimoglu H, Tanriverdi T, Kacira T, et al. Relationship between DNA damage and total antioxidant capacity in patients with transitional meningioma. *Clin Neurol Neurosurg*. 2007;109(7):561-566.
48. Yoshikawa T, Tanaka H, Yoshida N, Seto O, Sugino N, Kondo M. Adjuvant arthritis and lipid peroxide protection by superoxide dismutase. *Lipid Peroxide Res*. 1983;7:108-110.
49. Ayala A, Muñoz MF, Argüelles S. Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. *Oxid Med Cell Longev*. 2014;2014.
50. Wang M, Dhingra K, Hittelman WN, Liehr JG, De Andrade M, Li D. Lipid peroxidation-induced putative malondialdehyde-DNA adducts in human breast tissues. *Cancer Epidemiol Prev Biomarkers*. 1996;5(9):705-710.
51. Niedernhofer LJ, Daniels JS, Rouzer CA, Greene RE, Marnett LJ. Malondialdehyde, a product of lipid peroxidation, is mutagenic in human cells. *J Biol Chem*. 2003;278(33):31426-31433.
52. Diamond I, Mcdonagh A, Wilson C, Granelli S, Nielsen S, Jaenicke R. PHOTODYNAMIC THERAPY OF MALIGNANT TUMOURS. *Lancet*. 1972;300(7788):1175-1177. doi:10.1016/S0140-6736(72)92596-2
53. Dougherty TJ. Photosensitizers: therapy and detection of malignant tumors. *Photochem Photobiol*. 1987;45:879-889.

54. Ramaiah D, Eckert I, Arun KT, Weidenfeller L, Epe B. Squaraine Dyes for Photodynamic Therapy: Mechanism of Cytotoxicity and DNA Damage Induced by Halogenated Squaraine Dyes Plus Light (> 600 nm)¶. *Photochem Photobiol*. 2004;79(1):99-104.
55. Moan J, BERG K. The photodegradation of porphyrins in cells can be used to estimate the lifetime of singlet oxygen. *Photochem Photobiol*. 1991;53(4):549-553.
56. Thomas JP, Girotti AW. Role of lipid peroxidation in hematoporphyrin derivative-sensitized photokilling of tumor cells: protective effects of glutathione peroxidase. *Cancer Res*. 1989;49(7):1682-1686.
57. Agarwal ML, Larkin HE, Zaidi SIA, Mukhtar H, Oleinick NL. Phospholipase activation triggers apoptosis in photosensitized mouse lymphoma cells. *Cancer Res*. 1993;53(24):5897-5902.
58. Korbelik M. Induction of tumor immunity by photodynamic therapy. *J Clin Laser Med Surg*. 1996;14(5):329-334.
59. Ochsner M. Photophysical and photobiological processes in the photodynamic therapy of tumours. *J Photochem Photobiol B Biol*. 1997;39(1):1-18.
60. Korbelik M, Naraparaju VR, Yamamoto N. Macrophage-directed immunotherapy as adjuvant to photodynamic therapy of cancer. *Br J Cancer*. 1997;75(2):202-207. doi:10.1038/bjc.1997.34
61. Yamamoto N, Hoober JK, Yamamoto N, Yamamoto S. Tumoricidal capacities of macrophages photodynamically activated with hematoporphyrin derivative. *Photochem Photobiol*. 1992;56(2):245-250.
62. Krosl G, Korbelik M, Dougherty GJ. Induction of immune cell infiltration into murine SCCVII tumour by photofrin-based photodynamic therapy. *Br J Cancer*. 1995;71(3):549.
63. Evans S, Matthews W, Perry R, Fraker D, Norton J, Pass HI. Effect of photodynamic therapy on tumor necrosis factor production by murine macrophages. *JNCI J Natl Cancer Inst*. 1990;82(1):34-39.
64. Korbelik M, Krosl G. Enhanced macrophage cytotoxicity against tumor cells treated with photodynamic therapy. *Photochem Photobiol*. 1994;60(5):497-502.
65. Hoek JB, Pastorino JG. Ethanol, oxidative stress, and cytokine-induced liver cell injury. *Alcohol*. 2002;27(1):63-68.
66. Lagneaux L, De Meulenaer EC, Delforge A, et al. Ultrasonic low-energy treatment: A novel approach to induce apoptosis in human leukemic cells. *Exp Hematol*. 2002;30(11):1293-1301. doi:10.1016/S0301-472X(02)00920-7
67. Ashush H, Rozenszajn LA, Blass M, et al. Apoptosis induction of human myeloid leukemic cells by ultrasound exposure. *Cancer Res*. 2000;60(4):1014-1020.
68. Firestein F, Rozenszajn LA, Shemesh-Darvish L, Elimelech R, Radnay J, Rosenschein U. Induction of Apoptosis by Ultrasound Application in Human Malignant Lymphoid Cells: Role of Mitochondria-Caspase Pathway Activation. *Ann N Y Acad Sci*. 2003;1010(1):163-166.
69. Miyoshi N, Igarashi T, Riesz P. Evidence against singlet oxygen formation by sonolysis of aqueous oxygen-saturated solutions of hematoporphyrin and rose bengal: the mechanism of sonodynamic therapy. *Ultrason Sonochem*. 2000;7(3):121-124.
70. Su X, Wang P, Wang X, Cao B, Li L, Liu Q. Apoptosis of U937 cells induced by hematoporphyrin monomethyl ether-mediated sonodynamic action. *Cancer Biother Radiopharm*. 2013;28(3):207-217.
71. Zhan C, Sindhu RK, Pang J, Ehdaie A, Vaziri ND. Superoxide dismutase , catalase and glutathione peroxidase in the spontaneously hypertensive rat kidney : effect of antioxidant-rich diet. *J Hypertens*. 2004;22:2025-2033.
72. Jakoby WB. The glutathione S-transferases: a group of multifunctional detoxification proteins. In: *Adv Enzymol Relat Areas Mol Biol*. Vol 46. ; 1978:383-414.
73. Boveris A, Cadenas E. No Title. *FEBS Lett*. 1975;54:311-314.
74. Kanti P, Bhattacharya S, Bala A, Kar B. Chemopreventive role of Indigofera aspalathoides against 20-methylcholanthrene-induced carcinogenesis in mouse. *Toxicol Environ Chem*. 2010;92(9):1749-1763. doi:10.1080/02772241003783703
75. Dolai N, Karmakar I, Kumar RBS, Kar B, Bala A, Kanti P. Evaluation of antitumor activity and in vivo antioxidant status of Anthocephalus cadamba on Ehrlich ascites carcinoma treated mice. *J Ethnopharmacol*. 2012;142(3):865-870. doi:10.1016/j.jep.2012.05.050
76. Haldar PK, Kar B, Bala A, Bhattacharya S, Kanti U. Antitumor activity of Sansevieria roxburghiana - rhizome against Ehrlich ascites carcinoma in mice. *Pharm Biol*. 2010;48(12):1337-1343. doi:10.3109/13880201003792592
77. Donia TIK, Gerges MN, Mohamed TM. Amelioration effect of Egyptian sweet orange hesperidin on Ehrlich ascites carcinoma (EAC) bearing mice. *Chem Biol Interact*. 2018;285:76-84. doi:10.1016/j.cbi.2018.02.029
78. Bhattacharya S, Prasanna A, Majumdar P, et al. Antitumor efficacy and amelioration of oxidative stress by Trichosanthes dioica root against Ehrlich ascites carcinoma in mice Antitumor efficacy and amelioration of oxidative stress by Trichosanthes dioica root against Ehrlich ascites carcinoma in mice. *Pharm Biol*. 2011;49(9):927–935. doi:10.3109/13880209.2011.557080
79. Khan MR, Zehra H. Amelioration of CCl 4 -induced nephrotoxicity by Oxalis corniculata in rat. *Exp Toxicol Pathol*. 2013;65(3):327-334. doi:10.1016/j.etp.2011.11.007
80. Pamidiboina V, Chikkaboraiah SH, Razdan R, Krishnamurthy PT. Evaluation of antihypercholesterolemic activity of antichol against cholesterol cocktail induced hypercholesterolemia in rats. *Pharmacologyonline*. 2009;3:470-478.
81. Cuquerella MC, Lhiaubet-Vallet V, Cadet J, Miranda MA. Benzophenone photosensitized DNA damage. *Acc Chem Res*. 2012;45(9):1558-1570. doi:10.1021/ar300054e
82. Boscá F, Miranda MA. Photosensitizing drugs containing the benzophenone chromophore. *J Photochem Photobiol B Biol*. 1998;43(1):1-26. doi:10.1016/S1011-1344(98)00062-1
83. Gaplovsky A, Gaplovsky M, Toma S, Luche JL. Ultrasound effects on the photopinacolization of benzophenone. *J Org Chem*. 2000;65(25):8444-8447. doi:10.1021/jo000611+
84. Burns DT, Tungkananuruk N, Thuwasin S. Spectrophotometric determination of bismuth after extraction of tetrabutylammonium tetraiodobismuthate (III) with microcrystalline benzophenone. *Anal Chim Acta*. 2000;419(1):41-44.
85. Kumazawa E, Hirotani K, Burford SC, et al. Synthesis and antitumor activity of novel benzophenone derivatives. *Chem Pharm Bull*. 1997;45(9):1470-1474.

86. Tanaka T, Kohno H, Shimada R, et al. Prevention of colonic aberrant crypt foci by dietary feeding of garcinol in male F344 rats. *Carcinogenesis*. 2000;21(6):1183-1189.
87. Hsieh H-P, Liou J-P, Lin Y-T, et al. Structure–activity and crystallographic analysis of benzophenone derivatives—the potential anticancer agents. *Bioorg Med Chem Lett*. 2003;13(1):101-105.
88. Ito C, Itoigawa M, Miyamoto Y, et al. Polyprenylated Benzophenones from Garcinia a ssigu and Their Potential Cancer Chemopreventive Activities. *J Nat Prod*. 2003;66(2):206-209.
89. Matsumoto K, Akao Y, Kobayashi E, et al. Cytotoxic benzophenone derivatives from Garcinia species display a strong apoptosis-inducing effect against human leukemia cell lines. *Biol Pharm Bull*. 2003;26(4):569-571.
90. Liou J-P, Chang C-W, Song J-S, et al. Synthesis and structure− activity relationship of 2-aminobenzophenone derivatives as antimitotic agents. *J Med Chem*. 2002;45(12):2556-2562.
91. Prabhakar BT, Khanum SA, Shashikanth S, Salimath BP. Antiangiogenic effect of 2-benzoyl-phenoxy acetamide in EAT cell is mediated by HIF-1α and down regulation of VEGF of in-vivo. *Invest New Drugs*. 2006;24(6):471-478. doi:10.1007/s10637-006-6587-0
92. Chakraborty T, Bhuniya D, Chatterjee M, et al. Acanthus ilicifolius plant extract prevents DNA alterations in a transplantable Ehrlich ascites carcinoma-bearing murine model. *World J Gastroenterol*. 2007;13(48):6538-6548. doi:10.3748/wjg.13.6538
93. Kathiriya A, Das K, Kumar EP, Mathai KB. Evaluation of Antitumor and Antioxidant Activity of Oxalis Corniculata Linn. against Ehrlich Ascites Carcinoma on Mice. *Iran J cancer Prev*. 2010;3(4).
94. Al Abdan M. Alfa-lipoic acid controls tumor growth and modulates hepatic redox state in ehrlich-ascites-carcinoma-bearing mice. *Sci World J*. 2012;2012. doi:10.1100/2012/509838
95. Özkol H, Musa D, Tuluce Y, Koyuncu I. Ameliorative influence of Urtica dioica L against cisplatin-induced toxicity in mice bearing Ehrlich ascites carcinoma. *Drug Chem Toxicol*. 2012;35(3):251-257. doi:10.3109/01480545.2011.598531
96. Al Abdan M. Alfa-lipoic acid controls tumor growth and modulates hepatic redox state in ehrlich-ascites-carcinoma-bearing mice. *Sci World J*. 2012;2012. doi:10.1100/2012/509838
97. Mansour SZ, Anis LM. Possible effect of 5, 6-dimethyl-4 isothiocyanate thieno [2, 3-d] pyrimidine and I or irradiation on Ehrlich carcinoma in mice. *J Radic Res Appl Sci*. 2010;3:599-618.
98. Mahendran P, Devi CSS. The modulating effect of Garcinia cambogia extract on ethanol induced peroxidative damage in rats. *Indian J Pharmacol*. 2001;33(2):87-91.
99. Sundaram M, Patra S, Maniarasu G. Antitumor activity of ethanol extract of Gracilaria edulis (Gmelin) Silva on Ehrlich ascites carcinoma-bearing mice. *Zhong xi yi jie he xue bao= J Chinese Integr Med*. 2012;10(4):430-435.
100. S Shivakumar, S Sandhiya, N Subhasree AA. In vitro assessment of antibacterial and antioxidant activities of fruit rind extracts of Garcinia cambogia L. *Int J Pharm Pharm Sci*. 2013;5:254-257.

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